NOVEMBER 2024 Volume 10, Issue 10

CELL & GENE THERAPY INSIGHTS

SPOTLIGHT ON
Cell therapy downstream processing and analytics

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EXPERT INSIGHT

Interpreting the new FDA draft potency guidance: an RNA cell therapy perspective

Damian Marshall and Kayleigh Thirlwell

The FDA's new draft guidance on *Potency Assurance for Cellular and Gene Therapy Products* offers a structured framework for developing a robust potency assurance plan to ensure that therapies consistently achieve their intended biological effects. As with all guidance documents, its effectiveness will depend, at least in part, on how it is interpreted and applied—an undertaking with unique challenges in the complex and diverse cell and gene therapy sector. This article examines the application of the new draft guidance, exploring how its key principles can be practically implemented using a novel gene-modified cell therapy as a case study.

Cell & Gene Therapy Insights 2024; 10(10), 1433–1442

DOI: 10.18609/cgti.2024.164

The development of reliable potency assays for product lot release testing and stability evaluation remains a significant challenge for the cell and gene therapy field. This can be for many reasons ranging from product complexity or poor assay performance, through to the ability to define the precise mechanism(s) of action (MoA) from which to derive a robust potency assurance strategy. These challenges are not limited to therapies in early phase clinical development. Indeed, products that have received marketing approval from regulatory authorities can still have poorly understood MoA and/or difficulty correlating potency readouts to efficacy in the patient [1].

In recognition of these challenges, the FDA has released draft guidance on *Potency*

Assurance for Cellular and Gene Therapy Products which is designed to help developers navigate the complexities of potency assay development [2]. This guidance aims to provide more clarity on the requirements for implementing a potency assurance plan to ensure that products maintain consistent and adequate potency throughout their lifecycle. This plan is designed to integrate elements of product development, manufacturing, and quality control processes to ensure that the therapeutic product consistently meets predefined potency criteria. This document, once finalized, will supersede the 2011 guidance on potency tests for cell and gene therapy products [3].

While the new draft guidance offers a framework for developing a potency assay strategy, its applicability for the diverse and complex range of therapies in the cell and gene therapy sector has yet to be evaluated. In this article, we will examine the implications of this guidance and explore how its key elements can be applied using a novel gene-modified cell therapy product as a case study (Box 1).

CONSIDERATIONS WHEN DEVELOPING A POTENCY ASSURANCE PLAN

Potency assurance: it all starts with the mechanism(s) of action

The new FDA draft guidance emphasizes the importance of designing potency assays that are closely tied to the product's MoA. This is crucial to ensure that potency related critical quality attributes (CQAs) are identified and suitable control strategies implemented. However, defining a products MoA, particularly prior to clinical evaluation is complex.

This complexity arises because the MoA is a biochemical process by which the drug achieves its pharmacological effect, while potency is a measure of the drugs biological activity. During early development it is hoped that an assay to measure product potency will be linked to product efficacy and can be correlated in a meaningful way. However, this is often not the case [1]. Cell therapies also often have multiple MoAs which may be dynamic, dependent upon disease state. Nevertheless, it is important to generate data which can inform a presumptive MoA early in the research and discovery process through extensive product characterization and pre-clinical evaluation.

For the pro-regenerative macrophage therapy used as a case study in this article the MoA will be linked to the innate biological properties of the cells alongside the functional enhancements engendered by the transfected mRNA. An understanding of the MoA related to macrophage biology was established based on scientific literature in combination with in-house non-clinical studies and product characterization. This collective knowledge demonstrates that pro-regenerative macrophages are efficacious in pre-clinical models of liver disease through anti-inflammatory and anti-fibrotic effects. The MoA is considered attributable to four key processes: 1) phagocytosis; 2) monocyte recruitment and polarization; 3) fibrosis breakdown; and 4) fibrogenesis suppression [4,6] (Figure 1). Using these four processes as a starting point an initial suite of assays to measure potency related CQAs linked to pro-regenerative macrophage function can be developed. These assays, which will be discussed further in the following sections, help establish an initial strategy for potency assessment which can be applied for product lot release testing or extended characterization to increase overall product knowledge.

Given the importance of defining the product MoA to the overall potency assurance strategy, more detail to support developers would have been welcome in the draft guidance. For example. When considering product characterization the only recommendation is to "assess a broad range of product attributes to understand the properties of the product more completely".

BOX 1

Case study: a novel gene-modified cell therapy.

Resolution Therapeutics have developed an autologous mRNA engineered monocyte derived macrophage therapy for the treatment of patients with end stage liver disease [4]. The product, called RTX001, is in the early stages of clinical evaluation and is manufactured as follows:

1. Patient leukapheresis is obtained at the clinical site and the cells are transported to the GMP manufacturing facility.
2. Monocytes are extracted and purified from the leukapheresis and are differentiated into macrophages.
3. The monocyte derived macrophages are transfected with mRNA containing the oligonucleotide sequences for two separate transgenes.
4. The macrophages are formulated into multiple drug product doses and cryopreserved ready for lot release testing.
5. Following lot release, individual doses of the engineered macrophages are transported to the clinical site where they are thawed are administered to the patient by intravenous infusion.

Mechanism of action: the pro-regenerative macrophages home to the liver where they elicit an anti-inflammatory and anti-fibrotic effect, leading to hepatocyte regeneration and liver remodeling. This effect is enhanced through the secretion of proteins produced from the transfected mRNA.

FIGURE 1

Presumed mechanism of action pro-regenerative macrophages.

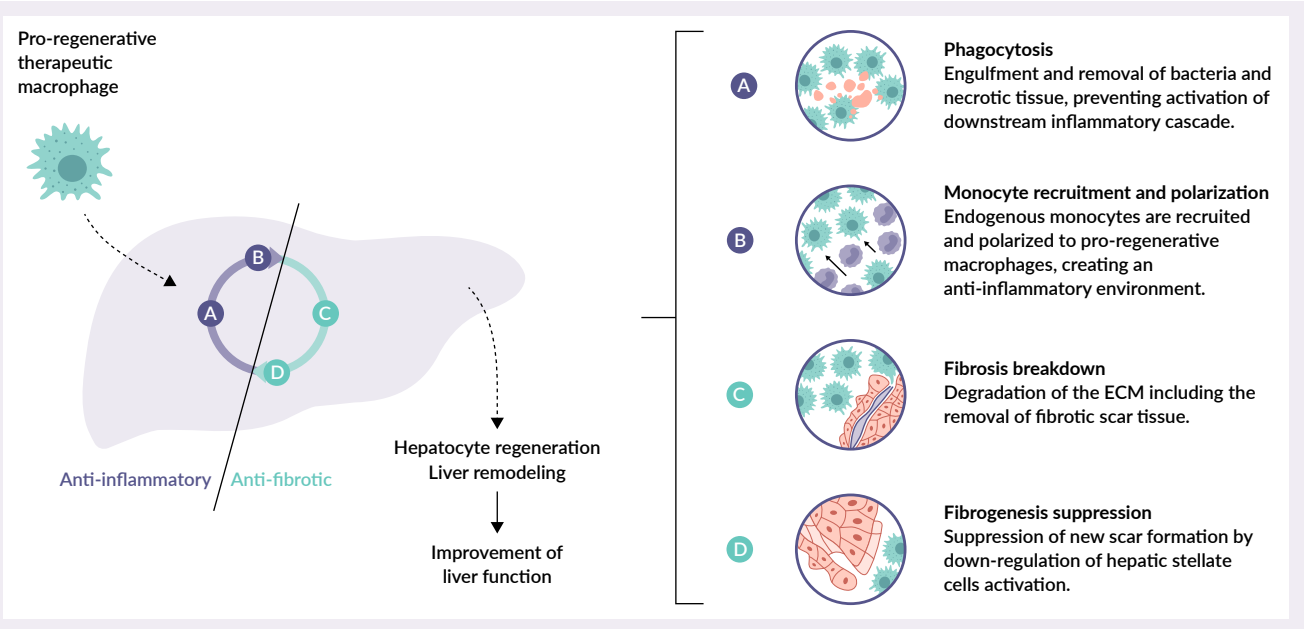


Figure redrawn with permission from Resolution Therapeutics.

For non-clinical studies, cell therapy developers may also have benefitted from additional detail around the impact of the 2022 FDA modernization Act 2.0 [5]. This states that *in vivo* models are no longer a requirement for development of an IND package. This presents a potential opportunity for increased application of *in vitro* models as alternatives. Guidance would have been welcome on considerations and acceptability of *in vitro* and *in vivo* studies when establishing product MoA, particularly given the well-established challenges posed by animal models.

Potency consideration for gene modified cell therapies

The draft FDA document provides no specific guidance for cell therapies that incorporate a gene modification step to enhance or alter product efficacy (viral transduction, DNA/mRNA transfection, etc.). The only recommendation is to ensure "your strategy for assuring potency of the cellular DP should include not only a potency assay and quantitative acceptance criterion for DP lot release, but also a bioassay and quantitative acceptance criterion for release of each vector lot". However, it is important to demonstrate control of the engineering process and its related impact on potency. Where multiple transgenes are engineered into a product, the potency of each transgene usually needs to be demonstrated separately. Examples may include products containing transgenes for two or more therapeutic proteins that act independently, as per the case study in this article, or CAR-T cells targeting multiple antigens or incorporating a cytokine transgene to increase CAR activity [7].

For guidance in this area, therapy developers need to look at other documents such as the recent 2024 guidance for industry on the *Considerations for the Development of Chimeric Antigen Receptor (CAR) T Cell Products* [7]. This provides information on CAR-T cell potency approaches which could be translated to other gene modified cell therapy products but unsurprisingly is focused on potency characteristics relating to viral vector-based transduction. For mRNA-based gene modification it is left to the individual therapy developers to interpret this guidance and incorporate elements into their potency assurance strategy.

The authors opinion of some of the key considerations for demonstrating product potency relating to mRNA-based gene modified cell therapies are shown in Table 1.

Other considerations that can be linked to product potency but may not form part of potency release testing include:

1. The integrity and stability of the mRNA before and after transfection. The mRNA must be stable enough to be effectively transfected into cells without significant degradation. This can be confirmed through long-term and accelerated stability studies together with forced degradation to demonstrate stability prior to implementation in the GMP process. The mRNA must also be stable enough post transfection to resist cellular processes designed to control gene expression through mRNA degradation and be resistant to product cryopreservation.

2. Intracellular persistence. mRNA transfection typically results in transient expression of the transgene protein. Therefore, the mRNA must persist within the cells and be translated for a timeframe sufficient to maintain therapeutic potency and achieve clinical efficacy.

What makes a good potency assay

The identification, development and implementation of potency assays which can support products throughout their lifecycle is a critical component of the potency assurance plan in the new FDA draft guidance. Accordingly, there are numerous criteria highlighted that need to be considered when

TABLE 1

Potency consideration for mRNA-based cell engineered products.

Product attribute	Definition	Role in product potency	Example analytical techniques	Application
Transfection efficiency	The percentage of cells successfully transfected with the mRNA	A consistent and controlled transfection efficiency is necessary to ensure that a sufficient proportion of the cells express the desired protein, which is required for the therapeutic effect	Flow cytometry	Applicable for both intracellular and surface marker assessment of transfection efficiency
			Secretion assay	Applicable where the transgene protein(s) are secreted by the cells; requires an antibody conjugate which can simultaneously bind to the cell surface and the target antigen
Transfection level	The number of mRNA transcripts present in the dose for administration	A controlled transfection process should result in a defined range of mRNA transcripts being present in the final drug product; this should ensure that sufficient transgene protein is produced to elicit the therapeutic effect	RT-qPCR	Can be used to allow highly targeted quantification of the transfected mRNA
Transgene protein expression	Quantification of the amount of protein expression (and duration where applicable) following mRNA transfection	Efficacy often depends on achieving sufficient levels of expression for a specific duration; mRNA transfection typically results in transient expression, so the timing and magnitude must align with the therapeutic application	ELISA	Applicable were transgene protein(s) are secreted by the cells; the assay provides a quantitative readout which can be normalised to cell number to give a kinetic measure of protein production
			Flow cytometry	Flow cytometry can be applied to give a semi-quantitative measure of transgene protein expression based on mean/median fluorescent intensity; can be applied for transgene proteins expressed intracellularly or on the cell surface
Transgene protein activity	Quantification of the specific activity of the transgene protein	Efficacy depends on the production of active transgene proteins which have correct 3D folding and post-translational modifications	Cell based bioassays	Cell based bioassays can include permissive cell lines that elicit a specific response to the transgene protein or engineered cell lines which activate a colorimetric or fluorescent output in response to the transgene protein; cell based assays require careful optimisation to ensure robustness

deciding what makes a 'good' potency assay. It should provide a quantitative measure of the biological activity of the cell therapy product and be predictive of clinical efficacy. It must be sensitive, specific, and capable of detecting changes in potency that could affect patient outcomes and should be relevant for use in the assessment of product stability.

Another consideration for what makes a good potency assay is its amenability to validation. This is essential to demonstrate

that it consistently produces accurate and reproducible results across different batches of the product. In-line with guidance from the international council for harmonization, potency assays should have suitable precision and accuracy to measure the intended biological activity. They must be specific to the relevant biological function, have a suitable range for their intended application and be robust enough to produce reliable results under varying conditions [8]. In this respect the new draft FDA potency assurance guidance provides additional details around the regulatory expectations. This includes qualification of potency assays prior to initiation of clinical evaluation for performance characteristics such as accuracy, precision, specificity, and sensitivity. This is then followed by a full validation study to appropriate pre-specified acceptance criteria prior to submitting a biologics license application.

For the analytical techniques highlighted in Table 1, ELISA and RT-qPCR based assays are generally more amenable to qualification or validation with guidance available on the parameters to control and considerations for study design [9,10]. For flow cytometry assays it may not always be possible to perform validation for parameters such as linearity, range or accuracy. For example, when determining the percentage of T cells expressing a transgene protein within a population of transfected cells, the assay measures the transfected cells ($CD3^+$, $Transgene^+$) relative to the T cell population ($CD3^+$). In this case, since there is no reference standard containing a known number of T cells and the assay readout (% transgene positive T cells) is proportional to the sample (number of T cell), it is not possible to determine accuracy. Guidance on the validation of flow cytometry assays for advanced therapies has recently been published by the *British Pharmacopoeia* [11].

In comparison, bioassays can be more challenging to qualify and validate. Bioassays are designed to measure the biological function of the cell therapy product in a relevant biological system (such as a cell line), making them a powerful tool for assessing potency. These assays can provide direct insights into the therapeutic activity of the product and are often considered the gold standard in potency testing. However, as outlined in the draft FDA guidance, bioassays can be difficult to standardize and have higher levels of variability due to the complexity of the assays and the responses of the cell lines. It can be difficult to achieve high levels of sensitivity and specificity, and data interpretation can be more complex than conventional assays. Despite this, bioassays are covered more predominantly in the new draft FDA guidance with a recommendation that "lot release testing for most CGT products should include at least one bioassay that measures a biological activity related to the intended therapeutic effect of the product".

Establishing a potency assurance strategy for early-phase clinical trials

In early-phase clinical trials, the product CQAs that are linked to the mechanism of action may be speculative or poorly defined. In recognition of this the draft FDA potency guidance recommends "developing multiple assays that measure known or potential potency-related CQAs and evaluate the utility of these assays in parallel during early clinical investigations. Assays that are redundant may be discontinued later in development". For gene modified cell therapies this would include potency assays relating to the MoA of the cells as well as the potency attributes associated with the engineered transgenes. This would form what is often referred to as a potency assay matrix.

Using this approach a potency assurance strategy for early-stage clinical products may contain a larger number of potency assays which are performed with a view to being progressively refined as product knowledge increases. For the case study used in this article, the initial potency assurance matrix

could include assays for macrophage MoA linked to their anti-inflammatory and anti-fibrotic attributes. This would be in addition to assays for potency related characteristics associated with the mRNA engineering process. This could lead to a potency assay matrix which is made up of a large number of potential assays (Figure 2). In this type of scenario, the initial potency assurance strategy may include assays that are selected for product release testing based on an assessment of the most critical potency enabling CQA's and assays that are used for extended characterization to increase overall product understanding.

Potency assurance strategy for pivotal trials and commercialization

As product knowledge increases through clinical evaluation it is anticipated that the potency assurance strategy will be refined. The draft FDA guidance outlines several ways this could be achieved:

1. **Minimizing assay redundancy.** Where components of product potency can be demonstrated to be dependent upon a stepwise chain of biological events then direct testing of each component may not be necessary. For example, an assay which adequately controls potency relating to the later step in the chain will typically be sufficient for product release, removing the need for potency assays to measure the earlier steps.

2. **Improving process control.** If developers can demonstrate that their process control strategy is sufficient to ensure that a potency related CQA remains within acceptable limits, then a lot release assay for that CQA may not be needed.

3. **Streamlining the potency assay matrix.** As developers generate a more in-depth understanding of the relationship between product potency, mechanism of action and clinical efficacy it may be possible to remove assays which are no longer considered to be measuring potency-related CQAs.

Another area for consideration in the potency assurance strategy for later phase clinical trials is assay scalability. In some instances, assays used to measure potency related CQAs in early phase trials may not be appropriate to support large scale manufacturing. Under these circumstances it may be possible to implement an alternative strategy using surrogate assay(s) if it can be demonstrated that they achieve at least the same degree of control of the potency-related attribute as the original assay. Implementation of surrogate assays requires sufficient data to demonstrate the correlative relationship between the surrogate assay and the biological activity of the product. This needs to take into account the relevance of the correlation being made, the amount of product information accumulated, how well the biological activity is understood and how well the surrogate measurement reflects the biological activity [3]. The use of surrogate assays may be particularly attractive as an alternative to bioassays, which as highlighted previously can be more variable than physicochemical assays and more complex to perform, often taking several days or weeks to run.

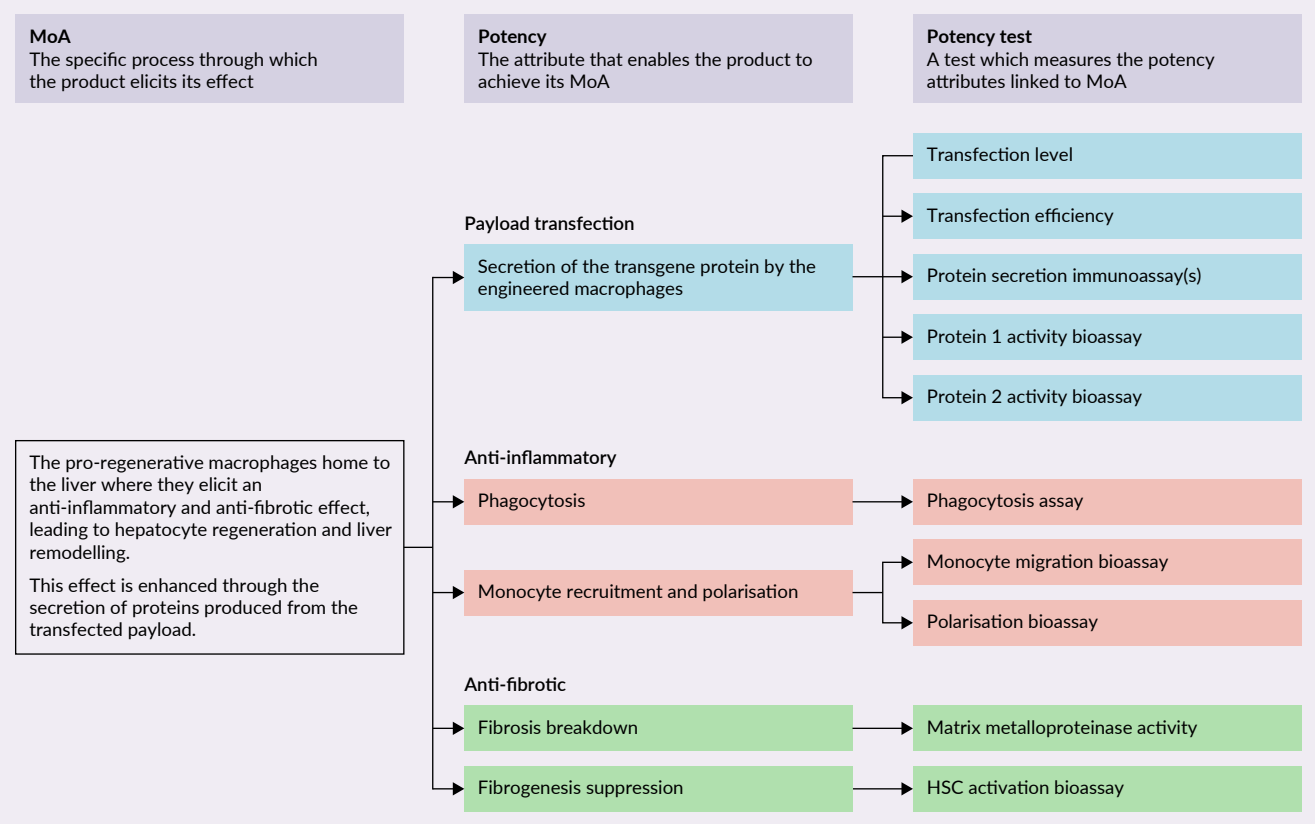
Looking beyond traditional potency assays

Traditional potency release tests rely on direct measurements. However, as cell and gene manufacturing advances, there may be an opportunity to apply inferential measurements to complement and, in some cases, replace direct testing.

One of the most significant applications of inferential measurements in drug release testing is within the framework of Process Analytical Technology (PAT) [12]. PAT involves the use of in-line, on-line, or at-line

FIGURE 2

Potential potency assay matrix for a pro-regenerative macrophage therapy.



Approach adapted from Simon *et al* [1].

sensors to monitor and control the manufacturing process. Data from these sensors could be analyzed using multivariate statistical models to infer the quality of the final product, reducing or even replacing the need for extensive end-product testing. Application of PAT has been demonstrated using Raman spectroscopy for adaptive process control during cell therapy manufacture [13] and a framework for incorporating PAT as part of a quality-by-design approach has recently been published [14].

Inferential measurements are also a cornerstone of real-time release testing (RTRT), a regulatory strategy where products are released based on real-time in-process data [15]. In this context, RTRT can significantly reduce production cycle times and improve product availability. While the concept of RTRT may still be some way off for cell and gene therapies the principles which could support its development such as continual process verification to maintain a state of control which assures product potency are discussed in the draft guidance. If implementable, in theory, RTRT could allow the immediate release of the batch at the end of the manufacturing process without the need to wait for traditional product release testing.

FINAL THOUGHTS

The FDA's new draft guidance on potency assurance for cellular and gene therapy products sets a higher standard for the development and implementation of potency assays and provides much-needed direction for the field. It also addresses several areas not fully covered in the 2011 FDA potency

guidance document that it will supersede. These include, managing products with complex and multi-functional MoA and continuous refinement of potency assays as product understanding progressively increases. It also includes guidance on the use of nonclinical data and provides more detailed strategies for products with incomplete MoA understanding. These updates reflect the growing complexity of the cell and gene therapy landscape, providing more nuanced and flexible approaches to ensure potency across various stages of development. The challenge once the draft document is finalized will be in its interpretation and application. Hopefully, it will help therapy developers avoid some of the pitfalls that pioneering therapy developers have had to overcome.

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

Acknowledgements: None.

Disclosure and potential conflicts of interest: The authors are Resolution Therapeutics employees and hold vested shares.

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

ARTICLE & COPYRIGHT INFORMATION

Article source: Invited; externally peer reviewed.

Submitted for peer review: Oct 8, 2024; **Revised manuscript received:** Nov 15, 2024;
Publication date: Dec 2, 2024.

CELL & GENE THERAPY INSIGHTS

CELL THERAPY DOWNSTREAM PROCESSING AND ANALYTICS

SPOTLIGHT

REVIEW

Phase-appropriate analytical control of cell therapy manufacture in early development stages

Vaibhav Patel

Phase-appropriate analytical controls are critical to ensure the safety, efficacy, and quality of cell therapy products throughout the drug development lifecycle. In the early stages of development, analytical strategies focus on essential attributes that define the product while maintaining the flexibility to adapt to evolving regulatory requirements. This article examines the key analytical methodologies employed in the early phases of cell therapy manufacturing and highlights their significance in maintaining product quality. It discusses the evolving nature of analytical controls as development progresses toward clinical trials and commercialization, providing insights into regulatory expectations. Key elements such as identity, purity, potency, and safety are discussed in the context of phase-appropriate controls, along with the challenges faced by manufacturers in early-stage development. Case studies and real-world examples of cell-based therapies, such as CAR-T cell therapies, were included to illustrate the practical implementation of these analytical strategies.

Cell & Gene Therapy Insights 2024; 10(10), 1397–1405

DOI: 10.18609/cgti.2024.160

Cell therapies represent a transformative approach to the treatment of various diseases, particularly in oncology, immunology, and regenerative medicine. Unlike traditional pharmaceuticals, cell therapies are living products that are complex and variable.

Development of these therapies requires a robust analytical framework to ensure that the product is safe, effective, and reproducible at each stage of development.

Early-stage development is a critical phase where fundamental analytical controls are established. These controls ensure that the product meets basic quality standards such as identity, purity, potency, and safety. However, unlike later stages of development, where regulatory requirements are more rigid, early-stage development allows for greater flexibility in analytical methods. This flexibility is essential because of the evolving nature of the product and limited availability of materials for testing.

This article explores the concept of phase-appropriate analytical control, focusing on the challenges and strategies employed during the early stages of cell therapy manufacture. We delve into key analytical techniques, the evolving regulatory landscape, and real-world examples to illustrate the importance of analytical control in ensuring the success of cell therapy products.

PHASE-APPROPRIATE ANALYTICAL CONTROL: AN OVERVIEW

Phase-appropriate analytical control refers to the implementation of testing strategies tailored to the specific stage of the development of a cell therapy product. In the early phases of development, these strategies prioritize critical quality attributes (CQAs) that are essential for ensuring patient safety and product quality while allowing for flexibility to accommodate evolving processes and product understanding.

At this stage, the goal is not to finalize the analytical methods, but to develop assays that can provide meaningful data on the product's identity, purity, potency, and safety. As the product moves toward later stages of development, these methods are refined, validated, and standardized to meet more stringent regulatory requirements.

Key analytical control attributes

In the early stages of cell therapy development, the focus is on four primary analytical attributes: identity, purity, potency, and safety. Each of these attributes plays a critical role in defining the quality of the product and ensuring that it meets the necessary standards for use in clinical trials. A summary of analytical control attributes in early-stage development is shown in Table 1.

- **Identity testing:** ensures that the cells being produced are of the correct type and express the intended markers. This is especially important in cell therapies, where the therapeutic effect is often dependent on the specific characteristics of the cells. For example, in CAR-T cell therapy, identity testing ensures that the engineered T cells express the CAR that is necessary for targeting cancer cells.

- **Purity testing:** detects any contaminants or impurities that could affect the safety or efficacy of the product. In early-stage development, purity testing may be less stringent because of the evolving nature of the manufacturing process. However, it is still essential to identify potential contaminants from the manufacturing process, such as residual host cells or by-products.

- **Potency testing:** verifies that the cells are functioning as intended and are capable of eliciting the desired therapeutic effect. In CAR-T cell therapies, potency assessment often involves evaluating cytolytic activity, a major function by which CAR-T cells destroy target cancer cells. Other mechanisms of action (MoAs) may also be assessed depending on the design of the CAR-T therapy. For example, some CAR-T therapies may be engineered with additional features, such as 'armored CARs' or co-stimulatory domains, which enhance the CAR-T cells' ability to persist,

proliferate, and resist immune suppression in the tumor microenvironment. Potency assays at the early stages are typically exploratory, assessing *in vitro* activities such as proliferation, differentiation, secretion of cytokines, and cytotoxicity against target cells.

- **Safety testing:** ensures that the product is free from harmful agents such as viruses, bacteria, or endotoxins. Safety testing is a critical requirement for any cell therapy product to enter clinical trials, as the risk of introducing harmful contaminants to patients must be minimized. Mycoplasma and sterility testing are common methods used to confirm the absence of microbial contamination.

These attributes are chosen based on a combination of regulatory guidance, risk assessment, and product-specific knowledge.

- **Regulatory guidance:** regulatory agencies, including the US FDA and EMA, emphasize the importance of monitoring CQAs to meet baseline standards for cell therapies. Early-stage requirements focus on foundational attributes to ensure product quality and patient safety. For instance, the FDA's *Guidance for Industry: CMC Information for Human Gene Therapy INDs* outlines expectations for characterizing identity, purity, potency, and safety, even in initial clinical phases.

- **Risk assessment:** risk assessment is crucial for identifying which attributes pose the highest risk to safety and efficacy. By prioritizing high-risk attributes, manufacturers can mitigate potential hazards in early development. For example, focusing on mycoplasma contamination (safety) and cell marker expression (identity) addresses critical risks associated with cell-based therapies.

- **Product-specific knowledge:** each therapy has unique characteristics that influence the choice of attributes. CAR-T cell therapies, for instance, require thorough identity testing to confirm CAR expression, as therapeutic efficacy depends on accurately targeting cancer cells. Gene-edited products, meanwhile, may require additional genomic integrity testing to detect off-target effects. Leveraging detailed product knowledge enables manufacturers to tailor their analytical strategies to the specific requirements and risks of the therapy.

This combination of regulatory requirements, risk assessment, and product-specific considerations forms the basis for selecting analytical control attributes in early development.

TABLE 1

Summary of analytical control attributes in early-stage development.

Analytical attribute	Purpose	Testing methodology	Early-stage focus
Identity	Verifies cell type	Flow cytometry, molecular assays	Flexibility due to variable expression profiles
Purity	Detects contaminants	PCR, ELISA, cell count	Less stringent due to process evolution
Potency	Confirms functionality	In vitro assays (e.g., cytotoxicity, cytokine production)	Exploratory assays; assessing MoAs like cytolytic activity and additional CAR-T features
Safety	Ensures absence of harmful agents	Mycoplasma testing, sterility tests	Prioritized for clinical trial readiness

EARLY-STAGE DEVELOPMENT: ANALYTICAL CHALLENGES

The early stages of cell therapy development present several challenges for manufacturers, particularly for the implementation of analytical controls. These challenges arise from the inherent variability of living cells, limited availability of samples for testing, and evolving nature of the manufacturing process.

Variability in cell source and product

One of the primary challenges in cell therapy manufacturing is managing the variability in both the source material and the final product. Unlike traditional small-molecule drugs, which can be synthesized with a high degree of consistency, cell therapies depend on living cells, which naturally exhibit significant variability. This variability can arise from differences in donor characteristics, cell culture conditions, and manufacturing processes, each potentially impacting the quality and functionality of the final therapeutic product.

In autologous cell therapies, where cells are derived from individual patients, the quality and characteristics of the starting material can vary greatly from one patient to another. This variability affects the outcomes of identity and potency assays, making it challenging to establish standardized analytical methods. Early-stage development thus requires flexible and adaptable analytical methods that can account for this variability while still producing reliable results.

To accurately distinguish variability derived from the analytical method itself (method variability) from variability introduced by the donor or manufacturing process (donor variability), several approaches are employed to ensure method performance:

- **Standardized controls and reference materials:** using standardized controls or reference materials can help assess method variability by providing a consistent baseline. These controls enable analysts to monitor assay performance independently of donor or product-specific factors, helping identify fluctuations due to the method rather than biological differences.
- **Analytical method validation and robustness testing:** robustness testing assesses the method's resilience under varying conditions, which is essential for understanding method performance. Early-stage development involves optimizing key parameters in methods such as flow cytometry, where gating strategies and marker selection may need adjustments to accommodate product characteristics. Regular validation ensures the method's capacity to deliver reliable results, even when donor characteristics vary.
- **Replicate testing and statistical analysis:** conducting replicate tests on samples from multiple donors enables analysts to separate donor variability from method-related variability. Statistical approaches, such as variance component analysis, are used to quantify and distinguish the contributions of method, donor, and process variability.
- **Use of process controls:** in-process controls that monitor specific parameters during cell culture or processing stages can provide additional data points, helping identify whether observed variability is due to the manufacturing process rather than the method itself.

Together, these strategies enable manufacturers to ensure that analytical methods are both accurate and reliable, providing insights into how donor or process variability impacts the final product.

Limited availability of samples

The availability of samples for testing is often limited to the early stages of cell therapy. This

is particularly true for autologous therapies, where each batch is derived from a single patient and there may be limited material available for analytical testing. Additionally, the use of patient-derived samples is typically bound by informed consent agreements, which outline the specific uses for these samples and may restrict certain types of testing. These consent requirements can further limit the availability and scope of testing that can be performed on each sample batch. This constraint can pose challenges for performing extensive analytical assays, particularly when multiple tests are required to assess the product's identity, purity, potency, and safety.

To overcome this challenge, manufacturers may prioritize certain tests in the early stages of development, focusing on those that are most critical for ensuring product quality. For example, safety testing for contaminants such as mycoplasma and endotoxins may take precedence over more exploratory potency assays.

Evolving nature of the manufacturing process

The manufacturing process for cell therapies is often dynamic and subject to refinement as the product progresses through development. Early-stage cell therapy manufacturing is characterized by ongoing adjustments to optimize processes, address variability, and incorporate new insights about the product. This evolution can lead to changes in cell culture conditions, handling protocols, and even the materials used in production, all of which can impact product consistency and quality.

Due to this evolving nature, analytical methods must also be adaptable to reflect process modifications. Method re-validation or adjustments may be necessary when significant changes are made to the manufacturing process. Such adaptability is crucial to maintaining quality control and ensuring that each batch of the product meets predefined CQAs, despite ongoing process refinements.

ANALYTICAL TECHNIQUES IN EARLY DEVELOPMENT

Several analytical techniques are commonly employed during the early stages of cell therapy development to assess product quality (Table 2). These techniques are designed to provide meaningful data on the product's identity, purity, potency, and safety, while allowing for flexibility as the manufacturing process evolves.

Flow cytometry for identity testing

Flow cytometry is one of the most widely used techniques for identity testing in cell therapy manufacture. This technique allows for the analysis of specific surface markers on cells, providing a detailed profile of the cell population. In early-stage development, flow cytometry is often used to confirm that the cells being produced are of the correct type and express the intended markers.

For example, in the manufacture of CAR-T cell therapies, flow cytometry is used to confirm that T cells express the CAR on their surface. This is a critical step in ensuring that cells have been successfully engineered and are capable of targeting cancer cells.

Potency assays

Potency assays are designed to demonstrate that the cell therapy product is functional and capable of eliciting the desired therapeutic effect. In early-stage development, these assays are often exploratory and may involve *in vitro* functional tests that assess the ability of cells to proliferate, differentiate, or secrete therapeutic molecules.

One example of a potency assay used in early-stage cell therapy development is the measurement of cytokine production by engineered T cells. Cytokines are signaling molecules that play a key role in the immune response, and their production can serve as an indicator of cell functionality. By measuring the levels of specific cytokines *in vitro*, manufacturers can

TABLE 2

Analytical techniques in early development.

Analytical technique	Purpose	Common methods
Flow cytometry	Identity testing	Analysis of surface markers (e.g., CAR expression on T cells)
Potency assays	Functional testing	*In vitro* functional assays, cytokine production, cytolytic activity
Mycoplasma testing	Contamination detection	PCR-based detection methods for rapid and sensitive analysis
Sterility testing	Safety assurance	Growth-based sterility assays
Genomic integrity testing (for gene-edited products)	Detecting off-target effects and ensuring genome stability	Next-generation sequencing, digital droplet PCR

assess whether engineered cells are capable of exerting the desired therapeutic effect.

Safety testing: mycoplasma and sterility

Safety testing is a critical component of the analytical control process, particularly in the early stages of development when the product is being prepared for clinical trials. To ensure patient safety, sterility, and safety assays should be validated from the start of clinical trials, providing reliable and consistent results across testing phases.

Mycoplasma contamination is a significant concern in cell therapy manufacturing, as these microorganisms can remain undetected in cell cultures and pose a risk to patient safety. Mycoplasma testing is typically performed using PCR-based methods, which allow for rapid and sensitive detection of mycoplasma DNA in cell cultures.

Sterility testing is another essential safety test that ensures that cell therapy products are free from bacterial and fungal contamination. This test is typically performed using growth-based sterility assays, where samples of the product are incubated in nutrient-rich media to detect the presence of viable microorganisms.

Gene-edited CAR-T cell therapies, which involve precise modifications at the genetic level, may require additional and more stringent safety studies. These advanced products raise unique safety concerns, such as the potential for off-target genetic modifications, insertional mutagenesis, or unexpected immune responses. Therefore, safety testing for gene-edited CAR-T products may include additional assays to monitor genome integrity and ensure the stability of genetic modifications over time.

For gene-edited CAR-T cells, the safety testing scope may extend beyond standard mycoplasma and sterility assays to incorporate in-depth genetic analysis. This may include methods such as next-generation sequencing (NGS) to detect off-target edits and assess the integrity of the edited genome. Enhanced safety testing protocols for gene-edited products help ensure that any unintended genetic changes or safety risks are detected before clinical administration.

REGULATORY PERSPECTIVE

The regulatory landscape for cell therapy products is constantly evolving, with agencies, such as the FDA and EMA, providing guidance on the appropriate level of analytical control required at each stage of development. In the early phases of development, regulatory agencies recognize the need for flexibility in analytical methods, allowing manufacturers to adapt their testing strategies as they learn more about the product.

Early-stage regulatory flexibility

Regulatory agencies, such as the FDA and EMA, provide some flexibility in the level of

analytical control required during early-stage development. This flexibility is necessary because of the evolving nature of the product and the limited availability of samples for testing. However, certain minimum requirements must be met, particularly in terms of identity testing, purity testing, potency resting and safety testing to ensure product safety, quality and consistency.

For example, the FDA's guidance on cell therapy development emphasizes the importance of phase-appropriate control, allowing for a gradual increase in the stringency of analytical methods as the product moves through the development pipeline. In the early phases, manufacturers are expected to establish basic assays for identity, purity, and safety, with the understanding that these methods may need to be refined as the product progresses to clinical trials.

Regulatory considerations for cell therapy manufacture

Regulatory agencies have outlined specific guidelines for the manufacture and testing of cell-therapy products. These guidelines emphasize the importance of CQAs that must be monitored throughout a product's life cycle. During early-stage development, manufacturers must identify and establish controls for CQAs, such as identity, purity, potency, and safety.

Although the regulatory requirements for early-stage cell therapy products may be less stringent than those for later-stage products, manufacturers are expected to implement a robust and scientifically sound analytical control strategy. This strategy must include appropriate testing methods that are fit for purpose at early stages to ensure product safety and quality for use in clinical trials.

As the understanding of CAR-T therapies and their mechanisms evolves, regulatory agencies, along with sponsors, are actively updating guidance to reflect new insights and address emerging challenges. Recently, there has been an increase in updated guidance documents related to CAR-T cell therapy, focusing on areas such as safety testing, genomic stability for gene-edited products, and enhanced potency assays. These evolving guidelines highlight the need for flexibility and adaptation in manufacturing practices as new CAR-T cell therapies are developed. Manufacturers must stay informed and responsive to these regulatory changes to maintain compliance and align with the latest safety and quality standards.

TRANSLATION TO LATER STAGES

As cell therapy products progress through the development pipeline, analytical controls become more stringent, and the methods used in the early stages are refined and validated for use in clinical trials and commercialization.

From early to late development: evolving analytical controls

In the early stages of development, manufacturers often work with limited data on product characteristics and behavior. As a result, the analytical methods used in early-stage development may be more exploratory in nature, with a focus on identifying key attributes that need to be monitored throughout the product's lifecycle.

As the product moves toward later stages of development, these methods are refined and validated to meet regulatory requirements. In particular, potency assays have become more defined, with a greater focus on demonstrating the correlation between *in vitro* results and clinical outcomes.

Case study: a CAR-T therapy example

CAR-T therapies have emerged as one of the most promising cell-based therapies for the treatment of cancer. These therapies involve genetic modification of a patient's T cells to express a CAR that allows the cells to target and kill cancer cells.

During the early stages of CAR-T therapy development, manufacturers faced significant challenges in implementing robust analytical controls. The variability of the starting material (T cells from the patient), combined with the complexity of the genetic modification process, made it difficult to establish consistent assays for identity, purity, and potency.

In the early stages of CAR-T therapy development, identity testing was focused on confirming the expression of the CAR on the surface of the T cells. Flow cytometry was used to assess the percentage of T cells that had been successfully engineered to express CAR. Potency assays were exploratory, with *in vitro* assays used to assess the ability of T cells to recognize and kill cancer cells.

As CAR-T therapies progressed to later stages of development, analytical controls became more defined. Potency assays were refined to include more sophisticated *in vitro* functional tests, and regulatory agencies began to require manufacturers to demonstrate a clear correlation between *in vitro* results and clinical outcomes.

CONCLUSION

Phase-appropriate analytical control is essential in the early stages of cell therapy development to ensure that the product meets the basic quality and safety standards. Flexibility in analytical methods is crucial at this stage, allowing for the exploration of various testing methodologies while focusing on key quality attributes, such as identity, purity, potency, and safety. As development progresses, these controls become more stringent and standardized, ultimately ensuring that the product is ready for clinical trials and commercialization.

Through a combination of flow cytometry, potency assays, safety testing, and regulatory oversight, manufacturers can navigate the challenges of early-stage development and set the stage for the successful translation of cell therapies from the laboratory to the clinic.

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AUTHORSHIP & CONFLICT OF INTEREST

Contributions: The named author takes responsibility for the integrity of the work as a whole, and has given their approval for this version to be published.
Acknowledgements: None.
Disclosure and potential conflicts of interest: The author has no conflicts of interest.
Funding declaration: The author received no financial support for the research, authorship and/or publication of this article.

ARTICLE & COPYRIGHT INFORMATION

Article source: Invited; externally peer reviewed.
Submitted for peer review: Oct 1, 2024; **Revised manuscript received:** Nov 6, 2024;
Publication date: Nov 11, 2024.

Cell therapy ANALYTICS

Cell therapy analytics is challenging due to variability in the starting material (cells and patients), complex mechanisms of action (MoAs), and the diversity and complexity of the final product. Cell therapy manufacturers looking to avoid some of these complexities should implement analytical processes early that can scale during the product lifecycle to ensure continuity and compliance through to commercialization.

COMMERCIAL SOLUTIONS to simplify your cell therapy workflow

COLLECTION

From healthy donors (allogeneic) or patient (autologous)

Integrated solutions that scale with you help manage costs and get you to market faster

Working with a reliable partner ensures you are supported from discovery through to commercialization.

Global cold chain logistics services

Partnering with Thermo Fisher Scientific grants access to:

- Global infrastructure
- Supply and cold chain logistics
- Documentation and chain of custody

ANALYTICS

Validate your starting material process and testing strategy early in development to ensure continuity and consistency, helping to ease the process of gaining regulatory approval.

CELL ISOLATION & ACTIVATION (T CELLS)

Closed, modular, integrated cell therapy manufacturing

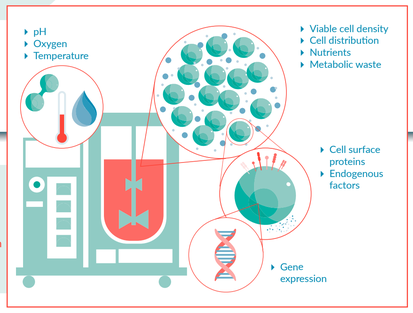
- Automated systems are cost-effective – saving time and labor, and ensuring processes are efficient, accurate and reproducible
- Closed systems offer improved consistency, purity, and safety while helping to lower overall manufacturing costs

Choosing instruments that scale from research and discovery to commercialization ensures an accelerated speed to clinic.

In-process analytics are key to successful cell therapy manufacturing, and provide essential information on critical quality attributes (CQAs) throughout the manufacturing process.

- Cell proliferation (viability and density)
- T-cell and impurity profiling (presence of contaminating cell types)
- Physicochemical parameters (pH, dissolved O2, temperature, nutrients and metabolites)

If you have open manipulations consider implementing rapid sterility testing to mitigate the risk of contamination.



CELL ENGINEERING

Viral and non-viral delivery tools, and gene-editing solutions

- Solutions that span the entire production workflow—from plasmid production through vector production and purification to analytical testing—can help streamline operations

Lentiviral titer quantitation

Applied Biosystems™ ViralSEQ™ Lentivirus Titer Kits

- Optimized workflow from sample preparation to data analysis
- Seamless qPCR-to-qPCR comparison of genomic (total) and proviral (infectious) copy numbers
- More accurate, sensitive and robust than p24 ELISA

Digital integration tools can help achieve a more standardized manufacturing approach.

CELL EXPANSION & HARVEST

Solutions for all cell types across immunotherapy and stem cell workflows

- High-quality products and proper documentation and support are essential for a streamlined transition from research to the clinic
- Media, supplements, growth factors and equipment to ensure effective cell expansion

Precise control over the composition and source of components used in cell culture media helps enable compliant and consistent production.

Leveraging rapid mycoplasma and sterility detection techniques early on in your process can help provide confidence in the final product.

Mycoplasma detection

Applied Biosystems™ MycoSEQ™ Mycoplasma Detection Kits

- Rapid mycoplasma testing for in-process lot-release and cell banks
- Sensitive detection of ≥200 species without the use of live mycoplasma

Rapid sterility testing (collection)

Applied Biosystems™ SteriSEQ™ Rapid Sterility Testing Kit

- Leverages Applied Biosystems™ TaqMan™ chemistry for ultimate sensitivity and specificity
- Helps mitigate risk by delivering results in <5 hours

CHARACTERIZATION AND LOT RELEASE

Off-the-shelf analytical assay solutions can help drive efficiency, reproducibility and compliance

- Due to the fast-paced nature of the production process and the limited shelf-life of cell therapy products, rapid testing is critical to obtaining actionable insights
- Commercially available assays that have been thoroughly tested and validated to meet regulatory guidelines can help accelerate product release

Thermo Fisher Scientific offers a wide range of analytical platforms and assays to support cell therapy characterization

Analytical protocols should use the smallest sample volume necessary for accurate and reproducible testing, preserving valuable cells for therapeutic use.

Testing criteria for cell therapies

Safety

- Assays that test mainly for adventitious agents (e.g., sterility, mycoplasma, or endotoxin levels)
- Can also include other areas of concern (e.g., immunogenicity or tumorigenicity)

Purity

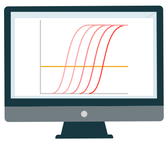
- Assays covering residual testing for materials used during the manufacturing process (e.g., isolation or activation beads, digestion enzymes, or genetic engineering reagents)
- Can refer to contaminating cell types that may have an adverse effect on final product safety and efficacy

Potency

- A measure of biological activity that demonstrates the capacity of a cell therapy product to affect a given result
- A matrix of assays is recommended because of difficulty selecting a single assay that assesses product quality and consistency while predicting clinical efficacy

Identity

- Assays that identify the product for proper labeling and will distinguish the product from other products manufactured in the same facility
- Examples include cell surface or intracellular markers, gene expression, secreted molecules, and peptide sequences



Software to quickly and accurately analyse your test results is crucial for rapid characterization and lot release.

Applied Biosystems™ SEQ™ solutions

Safety

- MycoSEQ™ Mycoplasma Detection System
- SteriSEQ™ Rapid Sterility Testing
- ViralSEQ™ virus detection and quantitation

Purity

- resDNASEQ™ residual host cell DNA Quantitation Kits (Available for qPCR and dPCR workflows)

Potency

- ViralSEQ™ Lentivirus Titer kits (Available for qPCR and dPCR workflows)

Identity

- MicroSEQ™ Microbial Identification system* (Not qPCR, employs sequencing technology)

Sample preparation

- PrepSEQ™ Sample Prep kits
- Supports both manual and automated solutions

Analysis

- AccuSEQ™ Real-Time PCR Data Analysis Software for biopharmaceutical analytical assays

Rapid sterility testing helps enable the discovery and resolution of potential contamination sources promptly, helping reduce the risk of product loss and unexpected production delays. This not only improves the overall efficiency of the workflow, but also preserves the integrity and effectiveness of the cell therapy product.

BENEFITS OF PARTNERING WITH THERMO FISHER SCIENTIFIC™

RAPID AND COST-EFFECTIVE ANALYTICS

- ✓ AccuSEQ software analyzes, interprets and provides reports eliminating complex manual calculations
- ✓ <5 hours from sample prep to data reporting
- ✓ Simple, streamlined workflows help reduce training needs
- ✓ Automated sample preparation for scalability
- ✓ Protocols that utilize the minimal volume for accurate and reproducible testing

ASSISTANCE MEETING REGULATORY REQUIREMENTS

- ✓ Implement compliant analytical strategies early in your process
- ✓ Support and advice from a team of experts on meeting regulatory requirements from FDA, WHO and EMA agencies
- ✓ cGMP manufactured and approved equipment
- ✓ Global network of cGMP, ISO certified facilities

SUPPORT AND SERVICES THROUGHOUT YOUR WORKFLOW

- ✓ One-stop-shop for custom media, scalable equipment, regulatory and training support
- ✓ Solutions for all cell types
- ✓ Supply chain reliability with over 30 years delivery and supply experience
- ✓ Pantheon™ Viral Vector Services provides clinical trial support

CELL & GENE THERAPY INSIGHTS

In partnership with ThermoFisher SCIENTIFIC

Manufacturing of CD19 CAR-T cells

CAR-T cell therapies have shown remarkable success within immuno-oncology in recent years, and the field is now looking at their promising potential in the treatment of non-oncology indications including autoimmune diseases. OmniaBio, a technology-focused cell and gene therapy CDMO, offers multiple CAR-T production processes that are tailorable to needs across preclinical to commercial scales. Integrating automated, closed, modular, and all-in-one systems.

This infographic explores three CD19 CAR-T cell manufacturing pathways using differing platforms: the G-Rex® System, the Cocoon® platform, and the CliniMACS Prodigy® System. Each of these all-in-one and unit-based manufacturing pathways is designed to enable therapeutic developers to meet their unique CAR-T cell production needs with consistent and efficient cell enrichment, transduction, expansion, and harvest. This experiment covers three systems to convey broad and deep CDMO expertise across various platform manufacturing approaches.

Manufacturing Process Overview

Automated T cell enrichment manufacturing process

DAY 0

ISOLATION

Apheresis from 3 healthy donors. Flow cytometry determined cellular composition before and after enrichment.

	Miltenyi Biotec CliniMACS Plus® System	Cytiva Sepax™ C-Pro	Miltenyi Biotec CliniMACS Prodigy® System
Viability	95.30% ± 3.08%	98.67% ± 0.15%	
Average recovery	55.91% ± 11.87%	60.46% ± 6.32%	
T cells in starting material	60.13% ± 9.32%	49.20% ± 23.77%	
Purity after enrichment	95.50% ± 1.71%	95.37% ± 1.43%	

DAY 0

ACTIVATION

100 million cells were activated with GMP TransACT™ T Cell Reagent.

G-Rex® System | Lonza Cocoon® Platform | Miltenyi Biotec CliniMACS Prodigy® System

DAY 1

VIRAL TRANSDUCTION EFFICIENCY AND VECTOR COPY NUMBER

Using second-generation CD19-CAR lentiviral vector.

	G-Rex® System and Fresenius Kabi Lovo® Cell Processing System	Lonza Cocoon® Platform and Gibco™ CTS™ Rotea™	Miltenyi Biotec CliniMACS Prodigy® System
CD19 CAR expression	43.8% ± 10.2	36.9% ± 11.9	31.9% ± 5.41
Yield of CD19-CAR cells	1.62e9 ± 2.2e8	1.07e9 ± 2.2e8	9.81e8 ± 2.6e7
CD19-CAR expression MFI	2608 ± 466	3416 ± 1216	2676 ± 370
TVCN/ transduced cells*	1.91 ± 0.3	1.8 ± 0.2	1.78 ± 0.07

DAY 2

72 hours post activation treatment with TransACT ™, T cells were observed to be in clusters.

DAY 3-13

EXPANSION

Cells from the proliferation chamber were sampled on days 6, 8, 10 and 13 after activation.

	G-Rex® 100M	Lonza Cocoon® proliferation chamber	Miltenyi Biotec CliniMACS Prodigy® proliferation chamber
Cell viability	97.5% ± 0.94%	94.9% ± 2.08%	97.4% ± 0.79%
Fold expansion	39.8 ± 3.2	27.9 ± 5.7	29.4 ± 4.5
Total CD19-CAR cells	1.62e9 ± 2.2e8	1.07e9 ± 2.2e8	9.81e8 ± 2.6e7

Cells were cultured in TexMACS™ serum-free GMP medium supplemented with 100 IU/mL rhIL-2. All the 3 donors tested showed similar expansion kinetics.

DAY 13

HARVEST

Buffer washout was determined by IL-2 ELISA using supernatants collected before and after harvest.
Uses the Gibco™ CTS™ Rotea™, CliniMACS Prodigy®, Lovo® Cell Processing System and CliniMACS Prodigy® System.

	G-Rex® System and Fresenius Kabi Lovo® Cell Processing System	Lonza Cocoon® Platform and Gibco™ CTS™ Rotea™	Miltenyi Biotec CliniMACS Prodigy® System
Cells per donor	4.34E9 ± 5.5e8	3.06E9 ± 7.7e8	3.14e9 ± 5.6e8
Average yield per donor	87.8% ± 6.6%	78.4% ± 7.5%	100.7% ± 7.2%
Viability pre-harvest	96.8% ± 0.9%	95.2% ± 1.6%	97.4% ± 0.8%
Viability post-harvest	91.6% ± 3.3%	95.1% ± 2.8%	98.5% ± 0.4%
Wash out Log reduction (IL2 wash out)	2.3 ± 0.9	2.8 ± 0.4	1.5 ± 0.7

DAY 13

FORMULATION

Using PlasmaLyte buffer supplemented with human serum albumin for formulation.
Final formulation of cryopreserved cells is 1 part Plasmalyte and 1 part human serum albumin and 2 parts CS10.

DAY 13

CRYOPRESERVATION

Preset programs were used on the CryoMed™ Controlled-Rate Freezers.
OmniaBio has expertise utilizing client-specific protocols.

Thermo Fisher™ CryoMed®

Cells were spun and resuspended in a freezing buffer for cryopreservation. Cells were frozen using a CryoMed™ Controlled-Rate Freezer. Preset-4 conditions were used to freeze bags and Preset-3 conditions were used for vials.

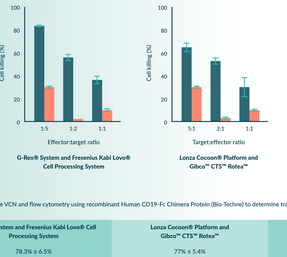
Analytics

T CELL FUNCTION

Determined using an in vitro luciferase-based killing assay. Untransduced cells were negative controls.

Target cells (T) were WIL-2-S-LUC2 | Effector cells (E) were CD19-CAR cells

CD19-CAR cells has a higher target cell killing compared to untransduced cells. Percentage of killing increased with increasing E:T ratio.



G-Rex® System and Fresenius Kabi Lovo® Cell Processing System | Lonza Cocoon® Platform and Gibco™ CTS™ Rotea™ | Miltenyi Biotec CliniMACS Prodigy® System

T CELL PHENOTYPE

Analyzed using digital droplet PCR (ddPCR) to measure the average VCN and flow cytometry using recombinant Human CD19-Fc Chimera Protein (Bio-Techne) to determine transduction efficiency.

	G-Rex® System and Fresenius Kabi Lovo® Cell Processing System	Lonza Cocoon® Platform and Gibco™ CTS™ Rotea™	Miltenyi Biotec CliniMACS Prodigy® System
CD8+	78.3% ± 6.5%	77% ± 5.4%	75.2% ± 8.5%
CD4+	18.7% ± 8.4%	21.7% ± 5.4%	22.9% ± 7.7%
Central memory state	77.5% ± 9.6%	63.9% ± 9.3%	67.8% ± 3.4%
Naïve memory state	11.7% ± 7.8%	20.6 ± 14.7%	11.7 ± 7.8%
Effector memory state	9.9% ± 13.0%	13.1% ± 17.8%	20.3% ± 12.0%
CD3+	98.8% ± 0.7%	99.1% ± 0.3%	97.2% ± 3.0%
Monocytes, B-cells, NK cells, and NK-T	0.7%	0.6%	2.2%

CELL & GENE THERAPY INSIGHTS

In partnership with OmniaBio

Cell & Gene Therapy Insights 2024; 10(10), 1473-DOI: 10.18609/cgti.2024.160

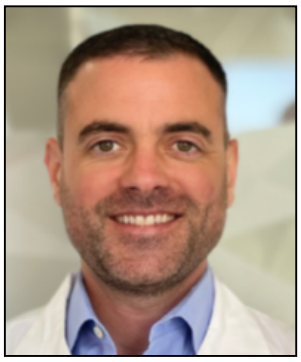
CELL & GENE THERAPY INSIGHTS

CELL THERAPY DOWNSTREAM PROCESSING AND ANALYTICS

SPOTLIGHT

INTERVIEW

Pioneering quality control in biomanufacturing of cell and gene therapies



Lauren Coyle, Commissioning Editor, *Cell & Gene Therapy Insights*, speaks with **Dhruv Sareen**, Executive Director at Cedars-Sinai Biomanufacturing Center, and **Jonathan Rodriguez**, Quality Control Manager at Cedars-Sinai Biomanufacturing Center, about the roles of in-process controls, method validation, risk management, and automation in biomanufacturing. They will highlight strategies to ensure product safety, consistency, and regulatory compliance for cell and gene therapy products.

Cell & Gene Therapy Insights 2024; 10(10), 1555–1564

DOI: 10.18609/cgti.2024.178

Q Can you briefly tell us about your careers and what you are currently working on?

JR: I currently serve as Quality Control Manager at Cedars-Sinai Biomanufacturing Center (CBC). My background is primarily in academia, starting a few years ago in France at the University of Lyon, where I completed my Bachelor's and Master's degrees in Cell Biology, Genetics, and Pathology. I later completed a PhD in Therapeutic Engineering. Largely, my expertise lies in human stem cells, molecular biology, and process development in preclinical studies within a CGMP environment—all of which are aimed at accelerating stem cell therapy.

DS: I am the Executive Director of the Biomanufacturing Center at Cedars-Sinai Medical Center, a role I have held for 15 years. I received a Bachelor's in Chemical Technology and Chemical Engineering from the University of Mumbai and my PhD in Biomolecular Chemistry from the University of Wisconsin-Madison. Shortly after, I moved to Cedars-Sinai Medical Center to establish a team focusing on induced pluripotent stem cell (iPSC) technology, disease modeling, and developing a biorepository.

At the Biomanufacturing Center, the team serves both academic and industry clients, providing contract manufacturing for cell and gene therapy in clinical trials. Additionally, they maintain an iPSC biorepository derived from patient-specific cells for drug discovery and disease modeling purposes.

Q How do you establish in-process controls and release specifications for specific intermediate cell banks, drug substances, and final drug products?

JR: The requirements for in-process and release testing are significantly different as they serve distinct purposes at various stages. Both are crucial to ensuring the quality and safety of the manufactured product. In-process controls are used to monitor ongoing manufacturing and ensure that critical process parameters (CPPs) remain within defined acceptance criteria. This aids in the detection of any deviations during cell expansion and allows for real-time adjustments to maintain product consistency.

At CBC, several in-process tests are carried out, such as cell morphology assessment, using a proprietary in-house ranking system. The iPSCs have a distinct morphology *in vitro,* and years of experience allow for the distinction of a good iPSC batch from a poor one simply by examining them under the microscope.

Another key in-process test is the residual reprogramming vector assay. The CBC proprietary iPSC reprogramming technology requires the use of multiple plasmids, which should

"...the primary goal is to confirm that the manufactured product meets predefined specification—this includes identity, purity, potency, and safety."

not be present in the final product cell banks. Therefore, clearance must be ensured during the expansion phase. Further, we have developed a highly sensitive in-house detection assay based on droplet digital PCR (ddPCR), capable of detecting as few as 0.004 copies of the reprogramming vector per cell. This serves as a go/no-go in-process control.

In addition to quality assurance, there is a business aspect to in-process controls, as time in a GMP environment is expensive. Detecting a batch that starts to deviate early allows for its termination so that the focus can remain with resources on compliant batches, therefore avoiding unnecessary expenses in the clean rooms.

On the other hand, final release QC testing is performed at the end of the manufacturing process. A distinction can be made between products that are fresh and those that are cryopreserved. In both cases, the primary goal is to confirm that the manufactured product meets predefined specification—this includes identity, purity, potency, and safety. These tests are mandatory for releasing the final product from the facility. They are specific to each type of final drug product and can vary depending on the materials used in manufacturing, the route of administration, and the mechanism of action.

DS: When it comes to defining in-process control and release specifications for different cell types at various stages, it is crucial to start by identifying the critical quality attributes (CQAs). This can be done through a variety of methods, considering the different cell types that we work with at CBC.

Next, risk assessment tools are employed, such as failure mode and effects analysis, to evaluate the risks associated with each attribute and prioritize them based on their potential impact on the cell bank or final drug product. Further, process mapping is performed, outlining each step in the manufacturing process and identifying parameters that could affect the defined quality attributes or CPPs.

Experiments are then conducted to determine the optimal ranges for those CPPs that would ensure the desired defined quality attribute. Once the experiments are completed and there are defined CQAs and CPPs for all stages, in-process controls are then established. Cell morphology is one example; however, we also measure cell viability at various passages, monitor growth rates, and track population doubling time. If any of these metrics fall outside acceptable ranges, it can be determined if the cell bank meets the go/no-go criteria.

For example, if iPSCs suddenly start dividing more rapidly, it may indicate a genetic abnormality, prompting genetic testing. Additionally, at certain points, potency testing is conducted to verify that the product, whether a cell or final drug product, delivers the intended therapeutic effect.

Q Why is in-process QC testing important for the development of cell banks such as iPSCs and final cell therapy products?

JR: In-process controls are crucial for real-time assessment of the manufacturing process. However, they require well-established procedures and trained personnel to be effective. Understanding and controlling CPPs is essential for manufacturing a final product that complies with predefined specifications, such as the CQAs.

The residual reprogramming material detection assay previously mentioned is vital for product safety. This reprogramming material could impact cells downstream in the process if it is not cleared during the expansion phase. From a regulatory perspective, monitoring for genetic instability that may occur *in vitro* is critical. This can be done by with traditional karyotype, which provides a high-level assessment but has a longer turnaround time. Alternatively, newer methods such as ICS ddPCR can be completed in just one day, focusing on well-documented instability loci in iPSCs. This quick turnaround makes it an effective go/no-go decision point for cell baking and final drug product.

DS: In addition to the parameters Johnathan mentioned, there are other specific aspects which are monitored to ensure safety, efficacy, and regulatory compliance. These include sterility testing and endotoxin testing, which are essential throughout the manufacturing process. Residual testing is another key factor—not only for the iPSC cell bank but also during the production of the final product—to detect any process-related impurities, such as leftover growth factors or cytokines.

We also conduct product identity testing to verify that the product has the correct cell composition, whether it is an iPSC bank or a final drug product. This ensures that the manufactured cell population has the anticipated mechanism of action or disease-modifying activity when administered to a patient. Additionally, cell viability is also monitored. All of these tests are carried out according to SOPs established prior to testing.

Q Can you explain the distinction between method qualification and method validation in the context of cell-based therapies? How does each contribute to ensuring product safety and efficacy?

JR: For any QC method, it is crucial to verify that this method is suitable at each stage of the drug product life cycle. This is typically performed by validating the method according to the International Conference of Harmonization (ICH) Q2 guideline. However, full validation is generally only required for late-phase and commercial stages.

During process development it is advisable to evaluate test methods for their reliability, specifically the pre-IND phase and early clinical trial phases. This is usually accomplished through a 'bridging' method validation, more commonly known as method qualification. Method

"In addition to precision, specificity, and linearity, a full validation requires testing for detection and quantitation limits, robustness, and accuracy of the assay."

qualification is based on ICH guidelines, but it is not as extensive as a full method validation. At CBC, factors such as repeatability, intermediate precision, and the limit of quantification for residual assays are examined. Additionally, specificity and linearity are assess, as defined in the ICH guidelines.

For full method validation, the process is much more demanding. It involves multiple operators using different lots of reagents, performing tests on various days—potentially in different lab locations—and utilizing several pieces of equipment. The goal is to ensure that the results are consistent across all variables. This full validation can be logistically challenging and cost-intensive.

DS: To frame it within the stages of cell therapy development, method qualification is typically used at earlier or intermediate stages, like Phase 1 or Phase 2 clinical trials. The aim at this stage is to demonstrate that the analytical QC method is suitable for its intended purpose and can reliably perform in a lab setting. During method qualification, parameters such as specificity, assay precision, and linearity are evaluated. This ensures that results are proportional to the concentration of the analyte being tested over a specific range.

Method validation, on the other hand, is a more formal and comprehensive process. It is meant to prove that the analytical method is fully acceptable for its intended use, particularly in later-stage development, such as Phase 3 or post-Biologics License Application (BLA). In addition to precision, specificity, and linearity, a full validation requires testing for detection and quantitation limits, robustness, and accuracy of the assay. These are the key parameters that go beyond what is assessed in a standard method qualifications.

The primary difference between method qualification and validation lie in the extent of testing and the resources required. A method validation, as Johnathan mentioned, adheres strictly to regulatory guidelines, involving a far more exhaustive evaluation to ensure product safety and efficacy at later stages.

Q What role does risk management play in the overall QC strategy for cell-based therapies and how are these integrated into the decision-making process?

JR: The regulatory bodies, including the US FDA place strong emphasis on a risk-based approach at every stage of a products life cycle. The ICH has developed a comprehensive guideline specifically for risk management, ICH Q9. It is crucial to have a thorough

understanding of the entire manufacturing process, to evaluate risks from a broader perspective, and to implement mitigation strategies early on.

In-process controls themselves are a form of risk mitigation as they allow for real-time monitoring of the manufacturing process. This enables for the anticipation of potential product failure. For example, personnel monitoring during manufacturing helps ensure aseptic processing, allowing any out-of-specification results to be quickly addressed.

One fundamental QC testing method is the potency assay, as Dhruv previously mentioned. The FDA recently released new draught guidance, recommending the development of a 'potency assurance strategy' to ensure that each manufacturing lot has the potency necessary for the intended therapeutic effect. This strategy is essentially a comprehensive approach to minimize risks that might affect potency by closely managing every aspect of the manufacturing process that could impact it.

It can be seen from this definition that risk management has a cross-functional aspect: the manufacturing and QC teams must collaborate closely to understand the manufacturing intricacies and respond accordingly, with support from the quality assurance team. Any changes in the manufacturing process during the early development phase could lead to changes in product potency. Therefore, it is crucial that these changes are evaluated and the resulting product scrutinized.

Another critical aspect of risk management is controlling the quality of materials used during manufacturing and QC. Some material attributes are essential to product quality, and these should be included in material specification. This includes reviewing supplier test results and verifying that each lot meets the acceptance criteria.

Preventive maintenance is another often-overlooked risk mitigation strategy. Ensuring that all equipment used in manufacturing or QC testing is well-maintained reduces the risk of equipment failure. This is also true for GMP standards, where staff training and competency assessments are themselves risk mitigations. Ensuring that personnel are properly trained minimizes risks related to human error.

DS: Cell-based therapies involve living cells and complex manufacturing processes with multiple steps, which can introduce numerous potential failure points. Given the novelty of the field and the limited historical data, effective risk prediction and management are essential. Techniques such as Failure Mode and Effects Analysis (FMEA), hazard analysis, and process mapping play a critical role in mitigating these risks.

One area where risk management is essential is raw material variability. Various cytokines are relied upon during different processes. For example, in one scenario, when transitioning from research-grade materials to GMP-grade cytokines, an unexpected outcome was observed where iPSCs differentiated into cardiac cells instead of the intended target immune cells. This highlights the importance of risk analysis during the transition from research to GMP material to prevent significant deviations and costly failures in cell manufacturing.

Another crucial area for risk management is transportation. Both fresh and cryopreserved cells need to be transported under specific conditions. If cryopreservation or shipping conditions are not validated, there is a risk of losing cell viability and potency by the time the

product reaches the patient. These are not typically measured in operating suites, so it is critical to deploy robust risk management strategies to safeguard the quality of the final cell product or cell bank.

Q Lastly, do you currently employ or plan to implement automated QC testing methods in your processes? If so, what advantages do you anticipate that these methods will bring to your QC strategy?

JR: Our QC department already utilizes several automated processes. For instance, we use various automated cell counters, each relying on different technologies. Additionally, we have an autosampler integrated with a flow cytometer, which allows for the analysis of up to 96 samples simultaneously. Traditional manual flow cytometry performance is a very time-consuming process, and automation has significantly streamlined this, increasing our throughput.

We also use automated equipment for DNA extraction, capable of handling 12 samples in under 40 minutes. This technology minimizes human intervention, which has a positive impact on reducing batch-to-batch variability and improving turnaround time. If the sample volume is high enough, automation can lead to significant cost savings due to greater consistency. Moreover, automation frees up personnel to focus in other essential lab tasks.

DS: In addition to automation benefits in QC labs, it also improves efficiency and enhances data management and traceability. With automated processes, we generate electronic records, which streamline compliance with regulatory requirements. This makes audit preparation much easier, whether for regulatory bodies or clients, as we have detailed electronic logs and standardized procedures.

Another major advantage is resource reallocation. As an executive director, automation allows me to strategically reassign skilled personnel to more complex assays that require more hands-on attention—particularly in the emerging fields of cell and gene therapies by automating standard tasks such as flow cytometry and DNA extraction, we can focus our expertise on the more intricate aspects of our work, which is a crucial advantage for QC labs in this rapidly growing field.

BIOGRAPHIES

DHRUV SAREEN is the founding Executive Director of the Cedars-Sinai Biomanufacturing Center (CBC), West Hollywood, CA, USA and the iPSC Core. He has extensive experience in iPSC-based disease models, GMP biomanufacturing, space medicine, and translating cell therapies to the clinic. The CBC specializes in iPSC and cell/gene therapy manufacturing, including a state-of-the-art cGMP facility for clinical-grade cell production. Dr Sareen established iPSC line and differentiation labs with automation for large-scale production and

curated a biorepository with over 1,200 iPSCs. His research focuses on stem cell differentiation into mature cells and building automation pipelines for scaling cell therapies. He holds patents and has published extensively.

JONATHAN RODRIGUEZ is the Manager of the Quality Control Department at the Cedars Sinai Biomanufacturing Center. He completed his Bachelor's and Master's degrees in Cell Biology, Genetics, and Pathology at the University of Lyon, Lyon, France. He conducted his doctorate studies and obtained his PhD in Therapeutic Engineering during which he worked on the role of mesenchymal stem cells from adipose tissue to ameliorate skin wound healing. He then decided to pursue his journey abroad and his post-doctoral work focused on limbal stem cells to treat patient suffering from limbal stem cells deficiency in the laboratory of Dr Sophie Deng at the University of California, Los Angeles (UCLA). Dr Rodriguez has extensive experience with human stem cells, molecular biology, process development during pre-clinical studies in a cGMP environment to accelerate stem cell therapies.

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AUTHORSHIP & CONFLICT OF INTEREST

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

Acknowledgements: None.

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

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

Article source: This article was based on a podcast recording, which can be found **here**.

Podcast recorded: Oct 17, 2024; **Revised manuscript received:** Nov 24, 2024;

Publication date: Dec 6, 2024.

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PIONEERING DIAGNOSTICS

Novel isolation platform with active-release technology for scalable cell therapy manufacturing

Ingrid Nyhus Moen, R&D Scientist—Cell Therapy, Thermo Fisher Scientific

Today, T cell therapy manufacturing challenges revolve around ensuring patient safety while reducing costs. This poster focuses on the second and third generations of the Gibco™ CTS™ Detachable Dynabeads™ platform, tailored for CD4+ and CD8+ cells, respectively. With the closed, automated Gibco™ CTS™ DynaCellect™ Magnetic Separation System, these new CTS Detachable Dynabeads deliver efficient, consistent, and optimal isolation and purity of target T cell populations while helping to address the issue of biological variability.

In a future where autologous and allogeneic cell therapies will coexist, flexible, automation-friendly cell processing platforms that can operate at a variety of different scales are required. The active release mechanism of the CTS Detachable Dynabeads platform allows users to actively detach Dynabeads from cells at any time during the process. The platform represents a new generation of isolation solutions that empower users with the ability to prioritize product quality and patient safety through enhanced process control.

PERFORMANCE DATA FOR CTS DETACHABLE DYNABEADS CD4

In a recent study conducted on the CTS DynaCellect Magnetic Separation System, CTS Detachable Dynabeads CD4 beads achieved an average purity of 95% CD4+ cells in the isolated material from starting material derived from three healthy donors (starting material CD4+ cell frequency was 43%). **Figure 1** demonstrates both a high average CD4+ cell recovery of 88% (**Figure 1A**) and an increase in cell viability (**Figure 1B**) for the isolated CD4+ cells compared to the starting material across the three samples tested.

Figure 1. Isolated CD4+ T cell recovery (**A**) and viability (**B**) data from three healthy donors.

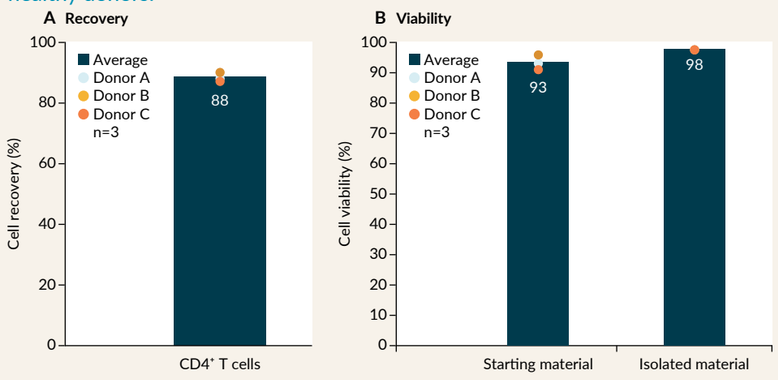
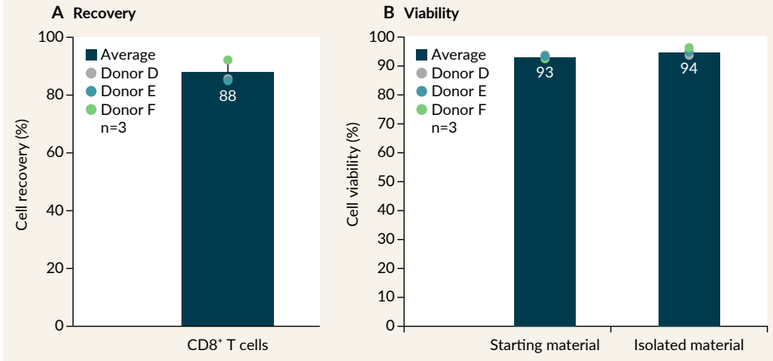


Figure 2. Isolated CD8+ T cell recovery (**A**) and viability (**B**) data from three healthy donors.



PERFORMANCE DATA FOR CTS DETACHABLE DYNABEADS CD8

Next, CD8+ T cell isolation performance was tested using the CTS Detachable Dynabeads CD8 beads, once more in tandem with the CTS DynaCellect Magnetic Separation System. In this study, an average purity of 89% CD8+ T cells was achieved in the isolated cells compared to an initial average purity of 26% in the starting material from three healthy donors. **Figure 2** shows that again, an average CD8+ T cell purity of 88% was achieved for the isolated cells (**Figure 2A**), with cell viability remaining high and in fact, increasingly slightly in the isolated cell population from 93% to 94% on average (**Figure 2B**).

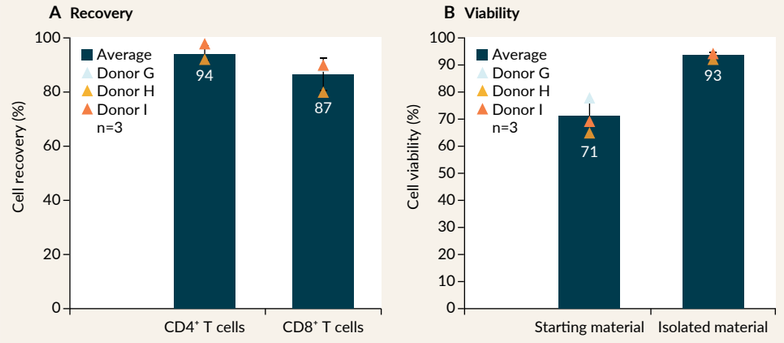
PERFORMANCE DATA FOR CTS DETACHABLE DYNABEADS CD4 AND CD8 IN COMBINATION

One of the benefits of the CTS Detachable Dynabeads platform is that the CD4 and CD8 beads can be used in combination – for instance, when there is no need to target and obtain separate populations of the two T cell subsets. In a third study, both CTS Detachable Dynabeads CD4 and CD8 beads were tested together in a combined isolation protocol. The study parameters mirrored that of the first two studies. A final average purity of 99% CD4+/CD8+ cells was achieved for the isolated cells across three healthy donor samples. **Figure 3** depicts the average CD4+/CD8+ T cell recovery (**Figure 3A**) and viability (**Figure 3B**) across the samples. Strong recovery was observed for both cell subsets, with a slightly higher average recovery of 94% achieved for the CD4+ cells. Cell viability increased significantly from an average of 71% CD4+/CD8+ T cells in the starting material to 93% for the isolated cells. Furthermore, the ratio of CD4+:CD8+ cells remained consistent, with a range of 1.5–1.7 observed across the three donor starting material samples, and a range of 1.7–1.8 in the isolated cells.

SUMMARY

The next-generation CTS Detachable Dynabeads platform delivers process flexibility, scalability, and consistent performance for cell therapy manufacturing. The new CD4 and CD8 beads—both individually and in combination—have demonstrated the ability to deliver high purity of the target cell population isolated from healthy donor samples, as well as both high recovery and viability rates for the isolated cells.

Figure 3. Isolated combined CD4+/CD8+ T cell recovery (**A**) and viability (**B**) data from three healthy donors.



Cell & Gene Therapy Insights 2024; 10(10), 1525; DOI: 10.18609/cgti.2024.174

CELL & GENE THERAPY INSIGHTS

CELL THERAPY DOWNSTREAM PROCESSING AND ANALYTICS

SPOTLIGHT

INTERVIEW

Troubleshooting process and analytical tool changes in an approved ATMP process

This article is part of our 'Rising Stars' series, giving a platform to the emerging leaders of the sector. In this series, we share the perspectives of fledgling thought-leaders, chosen by our Editorial Advisory Board members as future stars in their field. Pilar Redondo, Site Head, Takeda Madrid, Cell Therapy Technology Center, had this to say about her Rising Star nominees:

> "Marta and Maitane are two young talents who make us very proud of their commitment and dedication. They inspire all of us at Takeda by the way they collaborate transversally throughout the company, and they represent our knowledgeable team very well."

RISING STARS

With the continuing rapid pace of process and analytical tools innovation in the cell and gene therapy field, manufacturers of approved products must embrace improved new technologies while ensuring their regulatory requirements are fulfilled. **David McCall** (Senior Editor, BioInsights) talks to Takeda's **Maitane Ortiz Virumbrales** (Associate Director) and **Marta Malo de Molina** (Process Engineer Lead) about key considerations and success factors gleaned from their experiences with Alofisel®.

Cell & Gene Therapy Insights 2024; 10(10), 1546–1552

DOI: 10.18609/cgti.2024.176

Q What are you working on right now?

MMM: My role is focused on ensuring that facilities, utilities, and equipment are designed and optimized in a manner that meets the requirements of both Takeda and the regulatory bodies. I act as a project manager for these initiatives, defining project scope, identifying the potential solutions, assigning budget and resources, and following the project's development through to completion.

MOV: I work in the Cell Therapy Sciences team, focusing on R&D. I am concerned with building our understanding of the mechanism of action behind the products we are developing. A key part of this work is better understanding which specific mechanisms are relevant to be translated into bioactivity assays. Additionally, I work on providing solutions that may improve the manufacturing process of cell-based products, including the development of new automated solutions, so that we can make it more sustainable in the long term.

Q You have both been involved in the process of transitioning your group from a pure R&D focus to a lifecycle management focus for an approved ATMP. Can you talk us through the challenges and considerations in undertaking this transition, and how cross-functional collaborations have helped you on the journey?

MOV: I came from a pure academic research background prior to joining the pharmaceutical industry. The challenge for me was developing a comprehensive understanding of the strategy for an ATMP, because sometimes as a researcher you don't have the holistic vision that the company has for a given product—what specific needs the manufacturing process has, for instance, or what solutions or developments could or should be introduced. For me, doing this collaboration cross-functionally with other departments such as finance, manufacturing, quality, and regulatory affairs gave me that holistic view of the business and the strategy. I now have a better understanding of which new developments are actually needed and how feasible they will be to implement.

MMM: In my case, when I joined Takeda Madrid CTTC, it was 2020 and we were already manufacturing an approved and commercialized product in Europe. However, the site was still in the transition from a purely R&D facility that provided clinical supplies to a fully commercial manufacturing facility, in this manner, there were opportunities to optimize

"One of the things that has been really helpful to us is to make sure the development process is very comprehensive, whether it be for a new process tool or an analytical solution..."

the production and laboratories areas as well as to improve the adherence to regulatory requirements for commercial ATMPs manufacturing. This included adapting the requisite quality standards to Takeda policies, which as a global big pharma manufacturer are very robust.

As per the regulatory requirements for a newly commercialized product and the expected sales forecast, we worked on a project to expand the facilities and improve adherence to EU GMP Annex 1 for the manufacture of sterile products. We built a production area with new large clean rooms, and both an architectural design and equipment that met the requirements for aseptic product manufacturing.

As part of this transition to a fully GMP facility for a commercial product, to stay state of the art and to ensure the compliance with the highest quality standards, we enhanced the material transfer process to transfer the materials from the lower classification areas to the new clean room environment by introducing vaporized hydrogen peroxide surface biodecontamination pass-throughs. We also introduced isolators into the microbiology laboratory for performing sterility testing of the final drug product and we implemented new document management systems. All the achievements were the result of a stretch and multidisciplinary collaboration.

Q Can you expand on the keys to ensuring success with such cross-functional collaborations—for instance, to ensure that the latest process and analytical tools and technological innovations can be leveraged to the benefit of Alofisel®?

MOV: For me, a key aspect is to have a deep understanding of the manufacturing process flow—to know the pain-points of the process and the needs of the manufacturing, quality and analytical departments. That is the first thing: to identify the needs that we have to solve with our developments from the Cell Therapy Sciences department. Then, once we have identified a gap, we can develop a new solution.

One of the things that has been really helpful to us is to make sure the development process is very comprehensive, whether it be for a new process tool or an analytical solution such as a novel cell counter. This greatly assists the next steps when we transfer the solution to the manufacturing sciences or analytical sciences team. For example, we try to cover as wide a range of aspects of these tools as possible, so that we can facilitate the transition towards their validation. This will also facilitate and accelerate the eventual implementation of these new solutions.

Staying with the example of a novel cell counter, we implemented a new equipment to address gaps caused by the previous method we were utilizing. We sought to ensure compliance

with 21 CFR part 11 on data integrity whilst also improving the process by making it more robust and reproducible. Finally, we wanted to simplify the operations associated to cell counting, increasing the efficiency of this activity. The tight collaboration between my group and global manufacturing, global quality, and the analytical sciences team was very important to the success of this particular project.

MMM: I believe that a key success factor here is to have a robust roadmap, a well stablished site strategy defined by the leadership team, and a good scale-down approach to the rest of the personnel onsite that is aligned with the magnitude of the given project. I believe that committing sufficient resources and creating a cross-functional workforce that is fully dedicated to the projects at hand are both essential. Establishing plausible goals and ensuring detailed planning are key to keeping the team motivated and focused on the upcoming results. It is important to make sure they can see and understand the benefits of the projects they are working on.

A good example of a successful cross-functional collaboration that recently went live at the Madrid site was the implementation of the Manufacturing Execution System (MES). This is software designed to optimize and increase robustness in terms of the quality of the manufacturing process batch records by tracking materials, documents and controlling the entire production lifecycle. Different departments including Data, Digital, & Technology (DD&T), Manufacturing, and Business Excellence, as well as MES experts, were involved and together they did a fantastic job. As I mentioned earlier, it was very important that the overall site strategy was aligned with this project.

Q You mentioned implementing a new cell counter—are there any other examples you can share that illustrate the challenges in integrating novel technologies into the manufacturing process and QC/release testing regimen for an approved, non-engineered cell therapy product such as Alofisel®?

MOV: One example is the improvement of the freeze-thaw process to make it automated and more robust. In this case, the solution we required was not available on the market, so we had to develop it from scratch with a technology partner. This is one of the main challenges with advanced therapies: sometimes the solution that you need for your process simply doesn't yet exist. So, you have to invest time and money in developing a new solution together with the engineers from the external company with which you collaborate. It takes somewhat longer but at the end of the day, you have a personalized solution that you can apply to your process. This means you improve and advance not just your own therapy, but the field in general, because you become a pioneer for the particular type of technology in question.

The main challenge for an already-approved therapy is the regulatory aspect. Any change that you make to your manufacturing process, whether you are improving an analytical tool or

"From the operational point of view, batch size, staff retraining, new equipment adherence to regulations...must be taken into consideration when integrating new technologies for drug production processes."

implementing a new process technology, is going to have a regulatory impact. You must define what is the regulatory strategy behind this change—for example, do you just need to inform the authorities, or will you need to file a variation of the process or, if the change is considerable, even conduct a new clinical trial? This is one of the main constraints. You already have a very specific battery of release tests with which the therapy must comply, and once you have implemented a new technology or a change in the process, the new product must prove to be comparable with the old one.

You also will already be very specifically constrained in terms of the Critical Quality Attributes (CQAs) that your product must have. In this sense, you cannot really be too creative and innovative when it comes to an already-approved cell therapy. You need to adhere to these constraints and be really careful about the regulatory strategy. I think this is one of the main concerns: how to balance the need for innovation and improvement with avoiding having such a great impact on the regulatory aspects that the sustainability of your product is affected?

MMM: There are also challenges from the operational and facilities point of view. Here, the issues relate to the scale of impact from the integration of novel technologies. From the facilities standpoint, a redesign would be required as pharma automated processes more often encompasses closed systems while cell therapies processes commonly take place in highly controlled opened environment. From the operational point of view, batch size, staff retraining, new equipment adherence to regulations such as GMP Annex 1 or 21 CFR part 11 must be taken into consideration when integrating new technologies for drug production processes.

To deep further into the facilities matter, manual cell therapies processes are performed in an opened Grade A environment classification for which, according to GMP Annex 1 requirements for sterile products manufacturing, a Grade B background is required. However, when integrating closed-system novel technologies, the background classification can be downgraded to Grade C or even Grade D where room air requirements are less strict as product is protected from the background. This change in classification requirements, together with the new equipment introduction, imply a complete production area redesign, including the heating, ventilation, and air conditioning system modifications to meet air quality requirements. In addition, some of the new technologies for cell culturing require the installation and commissioning of utilities, such us different gases, that are new to the manufacturing facility.

For all the previously mentioned considerations, it is essential to develop and follow a detailed project plan to continue serving products to patients while modifications of the area and posterior verification and qualification activities are taking place to implement the new processes.

Finally, returning to batch size, this must be taken into consideration because for industrial pharma companies, a batch size typically measures in the thousands or hundreds of thousands of units. However, the batch size for cell-based products can be in the hundreds of units at most. For this reason, as Maitane mentioned, it is important to consider carefully what equipment to employ or develop in tandem with a third party optimized for the concrete batch size.

Q Turning to the automation and digitization of the manufacturing process for an approved ATMP, what are the keys to successful integration there?

MMM: As we have already discussed, commercial ATMP processes are still relatively rare in the pharma industry, which means the status of automation within the field remains relatively immature. It is difficult to achieve all the regulatory requirements and business requirements using the existing solutions on the market.

It is frequently the case that the pieces of equipment to acquire need to be specially customized for us, requiring close collaboration with the supplier. For example, in the standardized, automated world of pharma manufacturing, a User Requirement Specifications (URS) document would be firstly issued and provided to the equipment provider to cross check with them if their product can comply with all the requirements. But in this particular case, before issuing a detailed URS document, discussions regarding the different possibilities with the technology innovation supplier need to happen first in order to be able to develop a detailed assessment, because the equipment and the systems that are about to be designed are somewhat new to both supplier and manufacturer. Consequently, it is extremely important to consider the fact that the sharing of highly sensitive proprietary information with the technology supplier is needed in order to be able to develop a customized automated or digital solution with them.

In this regard, we need to synchronize with the supplier beforehand through either a Confidentiality and Non-Disclosure Agreement (CDA), or a Master Service Agreement (MSA). Ultimately, the key success factor here is to ensure you have a great definition of the URS to begin with.

MOV: For me, the key to success in implementing any new technology in your already-approved manufacturing process is to have a very robust and well-thought-out regulatory strategy. You may have different regulatory requirements or 'asks' from different regulatory bodies. For example, EMA (Europe) and PMDA (Japan) have different regulatory pathways for implementing a new technology. You therefore need to put a global strategy in place—for instance, sometimes you might want to do a stepwise implementation of the technology in different territories.

One of the things you can do to ensure a successful implementation is to engage regulatory bodies early on during the development process. By doing so, you will already have a vision of what the regulatory agencies may ask once you have implemented a new technology—for

example, what kind of comparability package they will require. You can check to see if the comparability strategy you are proposing would be acceptable, or if you may need to conduct a clinical trial. You can start working on the comparability assays that you may have to create and implement. Comparability remains one of the chief challenges in the cell therapy field, so your regulatory strategy has to be very carefully planned.

Q Lastly, can you each sum up some key goals or priorities that you have for your work over the foreseeable future?

MMM: I would like to see our current process automation projects implemented, resulting in robust processes that considerably outperform our current processes in terms of timeframe, quality, and cost. In addition, it would be very fulfilling to work on the development of new solutions that will not only be part of current products, but of many more commercial cell therapy products to come.

MOV: I agree with Marta—it would be really satisfying to see some of these process improvement initiatives that we are developing materialize and bring benefits to the current processes.

Also, from the R&D aspect, it would be really interesting to continue working with the translational team so that we can improve the efficacy of the therapies. I would also like to continue working in product biomarker discovery, because I think this could be a pioneering effort that is applicable to other cell therapy products.

BIOGRAPHIES

MARTA MALO DE MOLINA is a pharmacist and experienced professional in the pharmaceutical industry with over a decade of experience. Since joining Takeda Madrid, Spain in August 2020, Marta has progressed from Validation Specialist to her current position as Process Engineer Lead. Her work is marked by a strong commitment to innovative solutions and a keen interest in sterile pharmaceutical processes. Marta's career includes diverse roles in quality assurance and compliance, validations, process development, and process engineering. Her commitment to staying at the forefront of industry advancements is reflected in her active involvement in the International Society of Pharmaceutical Engineering (ISPE) since 2017. Beginning as an Emerging Leader at the Spanish affiliate, Marta later served as chair of the ISPE EL Spanish affiliate and is now a member of the ISPE Spanish board. Driven by a continuous desire to learn and grow, Marta is focused on contributing to the future of pharmaceutical innovation.

MAITANE ORTIZ VIRUMBRALES is currently an Associate Director at Takeda Cell Therapy Sciences department, Madrid, Spain. She has been contributing to the advancement of cell therapy products in industry since 2017. Prior to this, her PhD in Immunology at CNB-CSIC, Madrid, Spain and her postdoc at Mount Sinai Hospital and NYSCF, New York, NY, USA granted her ample experience in diverse scientific disciplines, including stem cell work,

immunology, and neurodegeneration. Her position at Takeda covers diverse roles, from in depth understanding of the mechanism of action of licensed cell therapy products to the development of manufacturing process improvements, where automation optimizes process operations and makes processes more sustainable. Maitane's goal is to put her skills to the service of the patients. Her ample vision and varied experience together with her creative and innovative nature make her a key team player to move forward the cell therapy field.

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

Acknowledgements: None.

Disclosure and potential conflicts of interest: The authors are Takeda employees.

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

ARTICLE & COPYRIGHT INFORMATION

Article source: Invited.

Interview conducted: Sep 30, 2024; **Revised manuscript received:** Nov 20, 2024;

Publication date: Dec 3, 2024.

CELL & GENE THERAPY INSIGHTS

CELL THERAPY DOWNSTREAM PROCESSING AND ANALYTICS

SPOTLIGHT

INTERVIEW

Exploring innovations in CAR-T therapy for solid tumors: the role of automated systems, analytical testing, and AI

Abi Pinchbeck, Editor, *Cell and Gene Therapy Insights*, speaks to **Arindam Mitra**, Director, CMC, Leucid Bio, exploring the development of CAR-T therapies for solid tumors, innovations in automated manufacturing, and the need for cost-effective, scalable solutions. They also discuss the complexities of phase-appropriate analytical testing and the evolving role of artificial intelligence (AI) for data analysis in cell and gene therapy manufacturing.

Cell & Gene Therapy Insights 2024; 10(10), 1195–1200

DOI: 10.18609/cgti.2024.136

Q What are you working on right now?

AM: We are currently developing Leucid Bio's first pipeline product, which is a CAR-T cell therapy targeting multiple solid tumors by focusing on ligands that are commonly found across various subtypes of these tumors. This program dubbed as LEU011 has received the

"One major issue remains quality control, particularly sterility testing, which is the most time-consuming assay, impacting the time to release the product."

MHRA's approval to initiate Phase 1 and 2A trials, which are expected to begin shortly including an innovative passport designation through the Innovative licensing and access pathway.

Q Where, for you, do the key issues in cell therapy downstream processing remain?

AM: There are plenty of challenges throughout the entire manufacturing process, not only during downstream processing. Firstly, there is some variability in the final product because the starting material differs with each patient (in an autologous setting). Secondly, scaling up the production of CAR-T therapies poses additional challenges, considering different bioreactor systems might be used, whether single or modular. This is especially challenging when scaling up across multiple centers and products. Finally, while quality release testing has made significant progress, there are still key areas to improve to reduce vein-to-vein time. Overall, while some new products are entering the market (e.g., CD19 CAR-T), implementing them in trials and setting the standards remains challenging.

Q Can you explore any recent technological innovations and initiatives looking to solve these aforementioned challenges?

AM: There is currently a wide variety of bioreactors on the market that perform several functions well, but a 'one-touch' system has yet to be achieved. The systems available today still rely upon upstream or downstream manipulations outside the bioreactor itself, often involving open manipulations. While the current systems prepare the product in a way that makes some steps easier, additional off-the-shelf products are still required. A 'fire-and-forget' device for full automation has not yet been developed.

One major issue remains quality control, particularly sterility testing, which is the most time-consuming assay, impacting the time to release the product. There are now assays that can deliver the results within 3–4 hours, but they require validation and wider adoption within early phase trials, both in academia and industry. There are also novel systems coming up for product characterization. For example, flow cytometry systems with cassettes are essentially a fire-and-forget device, reducing manual, labor-intensive processes and minimizing subjectivity. The market is evolving, but the manufacturing of these therapies remains complex and requires

an intricate skillset. The goal in the future is to make the processes simpler and less manual thereby achieving standardization.

Q Can you tell me more about Leucid Bio's interleukin 4 (IL-4)-based selective CAR-T cell expansion approach?

AM: The IL-4 expansion system was used to develop a therapy called T4, which primarily targets head and neck cancer. The system utilizes the principles of designing a viral vector construct so that not only the CAR is expressed, but an IL-4-based signaling component is also linked to commonly found cell cytokine receptors. During manufacturing, adding IL-4 allows for the selective enrichment of the transduced cells.

A common issue with viral vectors is that the T cell product may not always end up becoming enriched with CARs at the end of manufacturing. Typically, the final product may contain 60–70% CAR-positive T cells, which is still considered good. However, with the IL-4 system, the cells that grow in culture are predominantly the transduced ones due to the all-in-one vector construct. So far, we have treated 19 patients with late-stage head and neck cancer using this technology, as shown in recently published data [1].

Q Turning to the wider field, what does phase-appropriate analytical control of cell therapy manufacture look like, particularly in the early stages of development?

AM: Analytical testing for more traditional biologics, such as monoclonal antibodies and small molecules, is already well-established and has clear standards as these materials are not very variable. Since these products are not sourced from a highly heterogeneous population of cells, the final product is highly homogeneous, meaning testing methods can be relatively robust. The product simply needs to meet specific targets of different sets of standards to pass.

In cell therapy, however, the challenge is that the product is highly variable, especially in the context of autologous cell therapies. In the allogeneic space, it may be slightly different, although there may be some donor-to-donor variation. Overall, the variability of the starting material makes it difficult to establish a fixed set of standards, especially in the early stages of clinical trials.

Regarding phase-appropriate analytical control of cell therapy manufacturing, the standards and ranges must be continuously developed and adjusted as the product moves through clinical trials and late-stage developments, instead of having one 'go-to' set of standards. Additionally, cost is a significant factor—finding an assay that provides meaningful data, is easy to run, and is cost-effective can be challenging in the early stages, especially without knowing what the

"...the role of AI extends beyond data processing—it can also help organize data sets for complex trials, such as basket trials, where patients with different diseases/indications are treated."

inherent variabilities in different patient population is like. The variability is also compounded by the disease status. Therefore, phase appropriateness involves continuously validating the assay throughout the cell therapy development process.

It is crucial to recognize that you may not have set ranges for analytics, and strict validation principles, such as those in ICH guidelines, may not always apply in the early phases when your data is still growing and changing somewhat. Therefore, it is important to shift the mindset and trust the qualified assay to provide meaningful data instead of relying on traditional validation standards and strict standard controls.

Q How can we move further towards the automation of data analysis in cell therapy manufacturing?

AM: When discussing data analysis and automation, it is important to consider data collection and curation, and interrogate whether it is automated, and if the data feed directly into a repository during manufacturing. If the answer is yes, then automating data analysis becomes slightly easier. However, in many early phase clinical trials, especially those run by academic institutions, or smaller industrial organizations, much of the data is still paper-based. These data need to be manually entered into the repository before it can be used, which adds complexity.

AI is a powerful tool in this space due to its ability to process large data sets and use proximity data testing to identify and interpret patterns. AI, coupled with statistics, could help us 'train' data and better understand data variability, helping to find ways to use it more efficiently. Furthermore, the role of AI extends beyond data processing—it can also help organize data sets for complex trials, such as basket trials, where patients with different diseases/indications are treated. AI can be utilized to group and analyze data based on disease type, disease status, starting cellular characteristics, or specific phenotypical cell populations.

Q How are regulatory CMC compliance strategies and analytical toolkit innovation evolving to address the ever-increasing complexity of engineered cell therapy products?

AM: When I started working in this field 14 years ago, engineering cell therapy products was almost like alchemy. We have come a long way since then, and regulators have also

evolved. Considering many more products entered early-phase trials, with some being marketed as therapeutics already, it has expanded the regulators' knowledge and field of vision. Nowadays, we see a lot more pragmatism, especially in the UK, the EU, and the US FDA, and there are more bidirectional conversations between drug developers and regulators when starting to develop product pipelines at an early stage of the development process, rolling reviews have also played a part in late-stage marketing authorization filings.

Regarding analytics, there have also been significant advances, including biochemical testing, bulk sequencing, as well as rapid sterility testing for endotoxins and mycoplasma. More laboratories now offer these services, and cost competitiveness has come into play as well. In the past, we relied heavily on large vendors, whereas nowadays, more small vendors are achieving regulatory compliance for analytical testing. They can also provide bespoke development and qualification services. This has led to a domino effect on CMC, helping reduce costs per batch, even for autologous therapies.

Q What are your key goals and priorities, both in your own work and for Leucid Bio as a whole, over the next 12–24 months?

AM: At Leucid Bio, we focus on CAR-T cell therapies for solid tumors. Our goal is to make an impact by addressing patient populations that are hard to treat with conventional therapies. Currently, there are very few CAR-T therapies targeting solid tumors, and none that are marketed yet. With our first pipeline product, we have seen great *in vivo* and *in vitro* data, which will hopefully translate into meaningful clinical outcomes.

The goal of the manufacturing group is to develop cost-effective, simple, and non-invasive methods for producing these therapies. We want to leverage the latest technologies including robust regulatory CMC strategies, not necessarily the most expensive off-the-shelf solutions, but systems that provide us with deep data insights, allowing us to have a robust compliant process in later phase trials. In essence, we want a 'plug and play' approach, where we can swap out the CAR to establish the next pipeline product without overhauling the manufacturing process. While we operate in a niche area, we are focused on 'tightening the screw' on the latest technologies to streamline the manufacturing process.

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BIOGRAPHY

ARINDAM MITRA is an biotechnology professional with over 13 years of experience in lean manufacturing strategies including CMC regulatory strategies focused on early market access. Further, he has professional experience encompassing manufacture process development, analytical process development, and routine manufacture for clinical delivery for first-in-human trials of cell and gene therapy products. He has developed campaign manufacture strategies and cost-effective manufacture models and has an interest in developing sustainable carbon neutral processes. He has worked in Europe's first double-blinded cell therapy study and developed over 13 cell and gene therapies from pre-clinical concepts, including cell therapeutics for long COVID patients featured on the BBC. He has held several leadership positions in organizations such as Cell Medica Ltd and King's College London Guy's Hospital BRC, and currently serves as the Director, CMC Operations at Leucid Bio, London, UK.

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Contributions: The named author takes responsibility for the integrity of the work as a whole, and has given their approval for this version to be published.

Acknowledgements: None.

Disclosure and potential conflicts of interest: The author is a Leucid Bio employee and share options holder.

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

ARTICLE & COPYRIGHT INFORMATION

Article source: Invited.

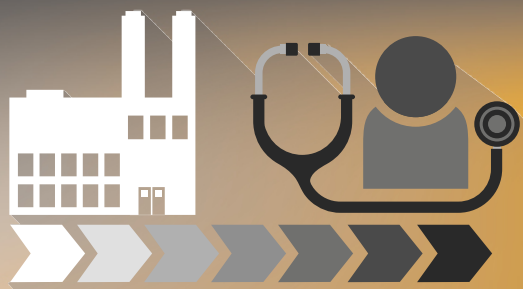
Interview conducted: Sep 2, 2024; **Revised manuscript received:** Sep 23, 2024;

Publication date: Oct 2, 2024.

CELL & GENE THERAPY INSIGHTS

SUPPLY CHAIN CHANNEL EDITION

Securing the supply chain



NOVEMBER 2024
Volume 10, Issue 10

CELL & GENE THERAPY INSIGHTS

SECURING THE SUPPLY CHAIN

CHANNEL CONTENT

EXPERT INSIGHT

Operations strategy for a scalable CGT supply chain: key CXO insights

Shesh Sharma, Tim Sirichoke, and Edward Ballesteros†

The cell and gene therapy (CGT) sector is rapidly evolving, with many early-stage companies developing innovative therapies for rare and serious diseases, particularly those preparing for commercial readiness. A robust and scalable supply chain is essential for successfully filing Biological License Applications (BLA) and launching CGT products on time. This article examines the unique complexities of CGT supply chain operations and highlights the importance of early strategic planning to align manufacturing, technology, and talent resources. We propose a three-phased roadmap for establishing a commercially ready supply chain, focusing on foundational infrastructure, preparation for scale, and readiness for launch. Additionally, we identify key success factors that are essential for building a resilient supply chain capable of supporting high-volume clinical manufacturing. By strategically addressing these critical elements, organizations can successfully navigate the complexities of CGT, enhancing patient access to innovative therapies.

Cell & Gene Therapy Insights 2024; 10(10), 1455–1462

DOI: 10.18609/cgti.2024.166

The CGT field continues to evolve, with many early-stage companies exploring novel and innovative technologies to develop therapies for patients with rare and serious diseases. To date, 37 CGT products in the USA [1,2] have been approved, with several others in late-stage development that have the potential to be launched as drug products. Regarding trends in clinical development, there are currently 103 ongoing late-stage clinical trials (Phase 2/3, and Phase 3) in the USA, accounting for about 79% of the total

† Edward Ballesteros sadly passed away on August 2, 2024

BOX

Operations strategy

Operations strategy is a strategic plan that aligns with the overall corporate strategy, outlining how an organization will leverage its resources, technologies, and processes to scale production, optimize supply chain operations, and maintain quality control. The goal is to ensure operational efficiency while supporting the organization's overall objectives, driving growth, and achieving long-term success [9].

130 trials worldwide. The remaining trials are distributed among Europe, Asia Pacific, and the rest of the world [3].

The global landscape is further enriched by recent scientific advancements, fueled by positive outcomes in the discovery phase. These successes have encouraged companies to explore cutting-edge technologies such as natural killer (NK) cells, induced pluripotent stem cells (iPSCs), T cell receptors (TCRs), tumor infiltrating lymphocytes (TILs), mRNA, and radiopharmaceuticals, targeting both oncology and non-oncology indications. These clinical developments have attracted significant interest from major pharmaceutical companies, besides venture investors, leading to strategic partnerships, investments, and alliances with many early-stage clinical-phase companies.

As companies advance into late-stage clinical development, it is crucial to shift from a science-driven focus to an operationally focused company for a seamless transition from clinical trials to commercial readiness. A well-structured and scalable supply chain is essential for both the successful filing of a BLA and the timely, efficient launch of CGT products. Without a robust supply chain, even the most promising therapies face risk of delays in product launch, manufacturing disruptions, and regulatory setbacks, which could ultimately compromise patient access and overall market success.

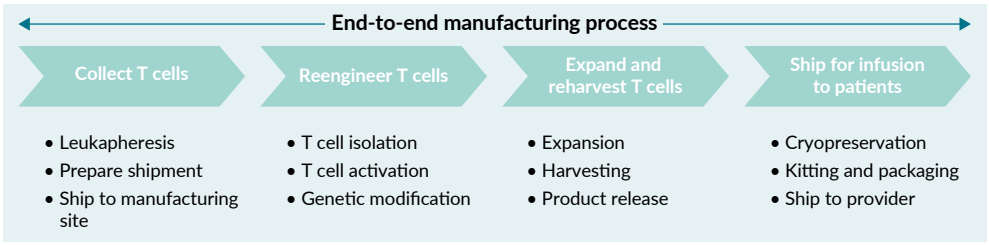
OPERATIONAL COMPLEXITIES IN CGT: AN OVERVIEW

Beyond CGT, the industry has seen the emergence of pioneering therapies such as mRNA, gene therapy, and, more recently, radiopharmaceutical-based therapies. It is important to underscore that each of these modalities presents unique complexities and supply chain challenges. However, this article will limit the discussion to supply chain complexities involved in launching CAR-T cell products, to ensure a focused discussion while leveraging our collective experience in this area. CAR-T products typically involve intricate manufacturing processes, complex logistics, challenges in raw material sourcing, stringent regulatory oversight, and significant sensitivity to environmental conditions—factors that must be carefully managed to ensure both product quality and patient safety.

In the case of CAR-T therapies, operational complexities are further influenced by the portfolio size, product type—whether autologous or allogeneic (see Figure 1), the phase of clinical development, manufacturing capacity constraints, and resource availability—both in terms of capital and human talent. The degree of complexity also varies based on a company's stage of evolution. i.e., early-stage or late-stage or a Big Pharma company which is commercializing its own products or a partner's products. While this article focuses on how early- to late-stage CAR-T companies can overcome operational complexities to commercialize their products, many of the approaches discussed are also applicable to mature, larger companies, as well as other emerging technologies mentioned above, providing valuable insights for broader industry application. Figure 1 illustrates the T cell manufacturing process, with the starting material being a patient's cells for an autologous therapy versus healthy donor cells for an allogenic therapy.

FIGURE 1

T cell manufacturing process.



Autologous CAR-T therapy is a highly individualized or a batch process that begins with leukapheresis, where the patient's T cells are collected. These cells are then shipped in a temperature-controlled container (e.g., NanoCool™) to the manufacturing center. The CAR-T manufacturing process involves several complex steps, including T cell isolation, activation, and genetic modification using a viral vector (e.g., lentivirus or retrovirus). This is followed by cell expansion, harvesting, and culminates in the cryopreservation of the final drug product. Once the drug product is released, it is shipped back to the healthcare provider in a temperature-controlled container (e.g., Dewar) for patient-specific administration [4,5].

In contrast, allogeneic therapies, which use T cells from healthy donors, follow a similar manufacturing process but allow for mass production. However, they present distinct challenges, such as donor selection, regulatory compliance, inventory management, and logistics.

It is important to note that a growing number of early-stage companies are exploring innovative therapies, such as the directed differentiation of iPSCs into NK cells, macrophages, microglia, and cardiomyocytes. These emerging therapies often leverage gene editing—sometimes involving multiple modifications—and typically require lengthy processes of differentiation, expansion, and harvesting, adding significant complexity to manufacturing, fill and finish processes, and logistics.

Regardless of the therapy type, whether autologous or allogeneic, gaining supply chain visibility and managing end-to-end supply chain risks introduces another layer of operational complexity. The supply chain risks can be categorized as internal or external. Internal risks stem from factors such as portfolio size, differing processes for each product, and the development phase of each product. External risks include economic challenges, environmental threats, political instability, and cybersecurity issues—common across industries and mitigated using established strategies. Additionally, CGT companies face specific risks, including disruptions due to fluctuating patient demand, reliance on single-source suppliers, supplier quality issues, and challenges related to cold chain logistics.

Additionally, strict adherence to GMP and Good Distribution Practice (GDP) standards requires seamless coordination among leukapheresis centers, manufacturing facilities, couriers, and clinical sites to ensure product integrity, timely delivery, and compliance with regulatory requirements. As a result, these requirements introduce significant operational challenges for the planning, sourcing, manufacturing, storage, and distribution functions within the supply chain [6].

Managing the chain of identity and chain of custody, as well as ensuring visibility into inventory management and the entire supply chain, requires the selection and implementation of both enterprise-wide technologies and track-and-trace systems. These technologies are crucial for ensuring regulatory compliance, maintaining quality standards, and enabling smoother operational scale-up.

However, implementing these systems requires significant planning and investment

in resources, making it costly and time-consuming. This adds complexity to managing scale-up operations effectively. Additionally, selecting the right technology at the right time adds to the operational complexity, requiring effective coordination, resource management, and integration with existing systems and processes.

Given these complexities, organizations regardless of their stage of development, face the daunting task of navigating demand uncertainty, overcoming manufacturing bottlenecks, dealing with unforeseen supply chain disruptions, designing a fit-for-purpose organization with the right skills and competencies, and managing more with limited resources—all while racing against tight timelines for regulatory filings and product launches. In the face of these competing constraints, companies are under immense pressure to scale efficiently, optimize costs, and remain agile and effective.

However, building an agile and experienced supply chain organization is often more complex than anticipated. For instance, finding and recruiting specialized CGT talent is a time-consuming and resource-intensive process, as it requires securing individuals with the right expertise. This challenge significantly adds to the operational complexity of scaling quickly and effectively. Many companies underestimate the intricacies involved in developing a fast-paced, nimble, and skilled supply chain organization capable of achieving operational readiness for commercial-scale production.

TRANSFORMING TO A COMMERCIAL SUPPLY CHAIN ORGANIZATION

The timing and roadmap for scaling an organization are primarily driven by scientific progress, beginning with promising pre-clinical results. Once positive data on safety, toxicity, and efficacy are established in clinical trials, the focus shifts to scaling up operations to produce the final product at larger volumes. To support this growth, a comprehensive supply chain strategy must address the unique challenges and complexities, as any operational lapse can compromise product quality, efficacy, and patient safety.

A common approach is to first assess the current operational readiness to establish a baseline to highlight strengths and weaknesses across the organization, including manufacturing and team capabilities, systems, processes, and execution risks. This baseline can then be mapped to what is required for commercial readiness. The outcome should result in a set of long-term, sustainable supply chain strategies that ensures the organization remains flexible and agile, able to adapt to unforeseen events such as portfolio changes, fluctuations in manufacturing demand, resource shortages, supply chain disruptions, and delays in patient enrollment or provider onboarding.

In support of a robust strategy, we have identified a three-phased approach to develop and implement a commercial ready supply chain organization as shown in Figure 2.

1. **Assess current state:** identify operational gaps affecting portfolio delivery and establish a baseline across all functions. Assess organizational flexibility to adapt to changes in portfolio, regulatory, manufacturing, and market dynamics. Evaluate the skills and competencies of current resources and identify those needed for scaling. Focus on potential failure points in scalability readiness, including risks in planning, sourcing, manufacturing, and logistics. Develop risk mitigation strategies aligned with business objectives to strengthen operational resilience.

2. **Plan and build:** scale operations to manage larger volumes and more complex logistics by expanding facilities, securing specialized equipment, sourcing critical raw materials, and implementing efficient inventory management strategies. Focus on scaling manufacturing and cold chain

logistics to meet growing demand, while ensuring compliance and maintaining strict quality control. Implement technology solutions to automate and streamline operations enhancing efficiency and supporting scalability. Recruit resources with the appropriate experience and skill sets to support the expanded operations. Additionally, establish plans to optimize the cost of goods manufactured as the company moves into subsequent phases.

3. **Prepare for launch:** involves finalizing commercial manufacturing capabilities and establishing robust quality control measures. It also includes solidifying the end-to-end supply chain systems integration, forming strategic partnerships to secure critical raw materials, and ensuring reliable cold chain distribution. Additionally, it is important to establish contingency plans to address potential risks across the supply chain, ensuring smooth operations from manufacturing to delivery.

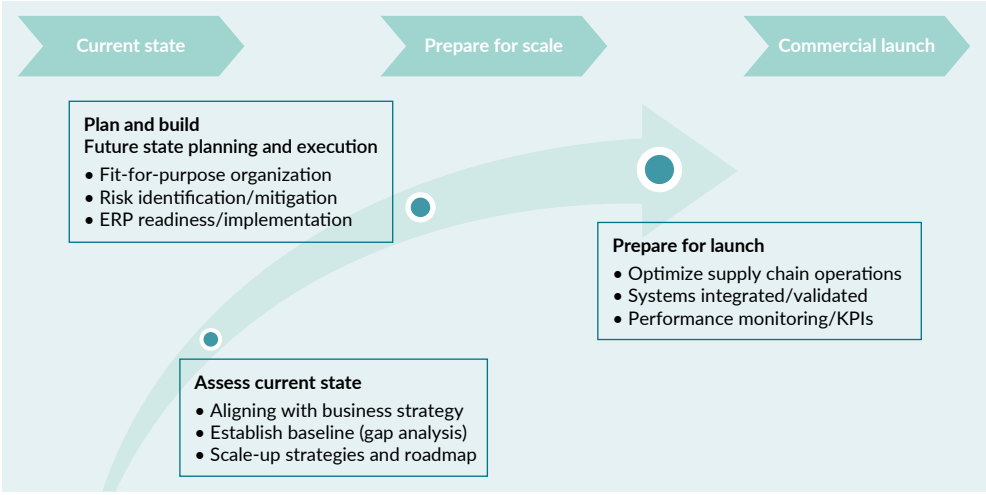
A key best practice for successfully transitioning to commercial-scale readiness is aligning the strategy with key stakeholders and the C-suite early on, while also starting the operations strategy and planning process proactively. This ensures that technical operations, commercial, finance, and technology functions are well-coordinated, helping to avoid delays and disruptions. Implementing supply chain strategies early provides enough time to manage or reduce program execution risks.

Once the strategy is aligned, the next step is to prioritize the timely execution of critical initiatives—such as facility expansion, organizational development, manufacturing decisions, technology selection, and long-term supplier commitments. This should be supported by a robust, risk-managed approach across the Plan, Source, Make, and Deliver functions. Additionally, demand planning should be integrated to align production with provider needs, optimize inventory, and minimize risks of supply shortages. Technology should be leveraged to enhance visibility, traceability, and control throughout the process, helping to manage inventory, improve manufacturing efficiency, ensure product integrity, and enable timely delivery of the final product. To execute these initiatives successfully, hiring experienced resources with the appropriate skill set is crucial to ensure operational readiness and effectively navigate the complexities of scaling.

Achieving operational readiness requires strong leadership, rooted in the ability to

FIGURE 2

Roadmap to commercialization.



thrive in unstructured start-up environments where processes are still evolving, and teams may have limited experience in launching CGT products. These leaders are not only comfortable in a start-up ecosystem but also have the expertise to transform a science-focused start-up into a commercially ready organization. They anticipate execution risks, implement mitigation strategies quickly, and avoid costly financial trade-offs by applying a blend of transformational, transactional, and situational leadership styles to drive success [7,8].

Equally important, leaders must foster an innovative and problem-solving culture that promotes transparency and collaboration as the company scales. This culture is essential for maintaining momentum and overcoming challenges in a rapidly evolving organization. The foundation of a resilient, patient-centric, and commercially ready supply chain rests on leaders' ability to make timely, decisive decisions across the Plan, Source, Make, and Deliver functions. By setting clear decision-making processes and empowering teams to act quickly, leaders create an environment that fosters an efficient and adaptable supply chain organization.

As the company moves into the later stages of development, improving operational efficiency and optimizing the cost of goods manufactured becomes critical. This can be achieved through automating manufacturing processes, reducing overhead, negotiating better prices for raw materials, and leveraging technology to enhance productivity. A cost-conscious approach frees up resources that can be used elsewhere in the business, while also instilling discipline throughout the organization.

To build on these perspectives, several key questions must be addressed to ensure phase-appropriate operational readiness. First, what is the optimal timing for organizational expansion? Expanding too early can lead to excessive cash burn, while delaying expansion may jeopardize the launch timeline. Second, how do we achieve end-to-end visibility across the value chain to ensure product integrity? Third, what is the best manufacturing strategy—should the company pursue in-house manufacturing, outsource to a CDMO, form strategic partnerships, or adopt a hybrid model? Fourth, what is the most effective approach to minimize supply assurance risks and ensure the availability of critical materials? Lastly, which technology should be implemented for traceability and when? This includes deciding between an ERP system, a custom-built solution, or other options, and determining the best timing for deployment.

There are no one-size-fits-all answers to these questions. Instead, the emphasis should be on evaluating and integrating these decisions strategically at the right time in the development life cycle, considering their impact on the organization's strategy, operations, and finances. By adopting these strategies, organizations can build a resilient, patient-centric supply chain that supports large-scale commercial launches while maintaining high quality and regulatory standards.

WHAT ARE SOME KEY SUCCESS FACTORS

There are several factors that drive the successful transformation and lay a robust foundation for a resilient supply chain that supports every phase of clinical manufacturing. Based on our experience, we have distilled these factors into six major levers as shown in Figure 3.

1. Leadership and vision: providing clear direction and strategic guidance
2. Culture: fostering innovation, transparency and collaboration with all stakeholders
3. Organization capability: developing skills and competencies to support the strategic vision
4. Operations excellence: streamlining processes to optimize costs and performance

FIGURE 3

Key success factors.

5. Leveraging technology: utilizing digital tools for seamless, efficient operations

6. Performance management: monitor progress through KPIs (key performance indicators)

Each of the above levers play a crucial role in fostering a resilient supply chain capable of supporting large scale clinical manufacturing from start to finish. This approach provides visibility into the entire supply chain, from apheresis collection and shipment to manufacturing, through internal coordination, and final product delivery to the healthcare provider. Such visibility is paramount for optimal outcomes. A supply chain focused on operational excellence ensures on-time product delivery and aligns with the overarching goal of improving patient outcomes.

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

Acknowledgements: This paper is dedicated to our dear friend and colleague, the late Edward Ballesteros, former Head of Supply Chain at Arsenal Bio. Many of the thought leadership ideas are credited to Edward, whose insights were instrumental in inspiring us to write this paper.

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

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

Article source: Invited; externally peer reviewed.

Submitted for peer review: Oct 10, 2024; **Revised manuscript received:** Nov 15, 2024; **Publication date:** Dec 2, 2024.

CELL & GENE THERAPY INSIGHTS

SECURING THE SUPPLY CHAIN

CHANNEL CONTENT

INTERVIEW

Supply chain sourcing and sustainability strategies for cell therapy and advanced biomaterial products

Abi Pinchbeck, Editor, *Cell & Gene Therapy Insights*, spoke to **Raj Joshi**, Head of Supply Chain and Strategic Sourcing, Celularity. As demand for human placental tissue and cord blood rises, Raj emphasizes the need for ethical donor engagement, fostering strong relationships with tissue providers, and implementing risk mitigation strategies. Other critical supply chain bottlenecks are explored in the discussion, including shortages of raw and starting materials and logistical challenges in maintaining temperature-sensitive products at cryogenic temperatures.

Cell & Gene Therapy Insights 2024; 10(10), 1371–1376

DOI: 10.18609/cgti.2024.157

Q What are you working on right now?

RJ: As the Head of Supply Chain and Strategic Sourcing at Celularity, I am responsible for all sourcing and supply chain activities for the company. This includes supporting our advanced biomaterial products derived from human placental tissues, biobanking, and cell

therapy products. We currently have four commercialized advanced biomaterials products: BIOVANCE®, BIOVANCE® 3L, Interfyl®, and CentaFlex™. I work closely with our manufacturing and commercial teams to support the supply chain activities for these products. On the cell therapy side, my team continues to work with R&D, process development (PD), and manufacturing teams to support supply chain and material needs.

Q What are the current key challenges that you face in the sourcing of raw and starting materials for cell therapies?

RJ: Cord blood is a key starting material for the cell therapies we work on, and is collected from placental tissue after birth. Placental tissue is normally discarded, so we partner with providers to encourage mothers to instead donate this tissue to be used for medical benefits. This is done through a process focused on education and consent of the donor mothers.

In the last 12 months, the demand for placental tissue has increased significantly, which has been a large challenge for us. Although there are increasing numbers of placental tissue donors, over the last year, the demand has far exceeded the supply, leading to a shortage. Other materials are experiencing similar shortages where demand outweighs supply, including cell culture media, particularly those sourced from cattle. Occasionally, we also see issues with reagents, and processing/freezing bags, and other materials, which require close management.

Q What considerations are involved in the management of cellular starting material donors & donations for allogeneic therapies?

RJ: There are several factors involved in sourcing the cord blood. We need to ensure the material is ethically sourced and complies with the Association for the Advancement of Blood and Biotherapies (AABB) guidelines. We only collect cord blood from US donors, ensuring that applicable state and federal laws are followed. The key criteria we require includes an informed consent form. The donor mother is educated about the program and the benefits of donating the placental tissue and cord blood. Donors are also required to complete a donor medical history questionnaire and relevant medical records are collected. Further criteria include that the donor must be over 18 years of age, the pregnancy cannot involve surrogacy or egg/sperm donor, and the pregnancy results in a single birth. Finally, we have serology testing performed on blood sample.

Once this data is collected, our medical director performs the final eligibility assessment to ensure that all criteria are met and the cord blood is safe for further processing. We have a specially designed kit to collect the placental tissue and cord blood. This is highly time-sensitive; it is critical to ensure that the sample is delivered to Celularity within a certain time frame.

"When selecting a cord blood supplier, a few questions must be addressed: do they follow AABB guidelines? Do they have strong process, procedures, and systems in place to collect and store data?"

Q Similarly, what are your key considerations when selecting and qualifying an external materials supplier?

RJ: When selecting a cord blood supplier, a few questions must be addressed: do they follow AABB guidelines? Do they have strong process, procedures, and systems in place to collect and store data? Do they have a good network of hospitals from which to collect the placental tissue and cord blood? Our quality assurance team performs an audit involving a questionnaire, and if needed, an on-site quality audit is performed. Final approval is given by our medical director.

For other raw materials, for example, media or reagents, our key aim is to ensure all technical requirements are met. We work closely with our R&D, PD and Manufacturing teams to understand their current and future needs and implement strategies to manage the supply chain. Our quality team will review and approve any new supplier, making sure that they have strong processes and procedures in place. From a sourcing perspective, we want to ensure that we have the right pricing structure in place. We negotiate long-term supply agreements to make sure that our current and future needs are met while minimizing costs.

Q What are your key pieces of advice for those looking to secure their sourcing and procurement strategies for cell therapy development?

RJ: For those looking identify a new supplier to source cord blood, I advise them to ensure the supplier have strong processes, standard operating procedures (SOPs), and controls in place; they are following AABB guidelines and all applicable state and federal laws; and having a strong network of hospitals from which they can collect cord blood to support a program.

For the other materials, it is critical to qualify alternate sources for key materials, manage the supplier relationships closely, gather market intelligence and have risk mitigation strategies in place. During the period of the COVID-19 pandemic, there was a severe shortage of many raw and processing materials in the cell therapy space. We were able to navigate that period by working closely with our key suppliers. We held frequent meetings to communicate our short-term and medium-term needs, discuss the supplier situation, capacity and lead times and developed supply plans to ensure our needs are met. We were also able to identify

"...it is key to work closely with your internal stakeholders, including manufacturing, technical operations, and process development, to understand their current and future needs."

alternate sources for some of the materials, which helped us manage any supply issues as they arose.

Q Looking at the entire cell therapy supply chain, where do the key bottlenecks lie in your view? And how can these be overcome?

RJ: The bottlenecks fall into two key categories. First, the limitation of material sourcing. This includes the overall high demand for human placental tissue and cord blood as mentioned, as well as limitations around cell culture media. The global cell culture media market is growing fast, estimated to be worth US$6.2 billion in 2023 and expected to grow to US$13 billion in 2028 (a compound annual growth rate of 16% year on year) [1]. We also see some shortages of processing and freezing bags.

To overcome this, it is key to work closely with your internal stakeholders, including manufacturing, technical operations, and PD, to understand their current and future needs. Partner and communicate clearly with your key suppliers and ensure there is mutual understanding of both your needs and their capacity and lead times. This means that if any issues arise, you will be notified quickly and can partner with your stakeholders and find solutions. In addition, build your sourcing and supply chain strategies to meet both short-term and medium-term needs.

The other bottleneck is logistics, particularly because cell therapy has an extremely temperature-sensitive, and global supply chain. We have labs located across the USA and in Europe, and sometimes international events can impact transport links. As these are extremely temperature sensitive materials, any delays may cause loss of material and disrupt the supply chain. It is important to choose the right logistics partners that have experience and expertise and can provide customized solutions for your needs. Qualifying multiple logistics suppliers is one way to help manage these challenges, as it provides a back-up source to support your needs.

Q What are your key goals and priorities for your work, and for Celularity, over the next 12–24 months?

RJ: My priorities are to manage the supply chain, minimize any disruption to our manufacturing, and support the development of new products. There are many exciting things

happening at Celularity right now. For example, on our advanced biomaterials side, we are developing three new products. One is the Celularity Tendon Wrap, which is a scaffold composed of collagen and other native proteins derived from decellularized human placental tissue to be used in the management and protection of tendon injuries. We are also developing a Celularity bone void filler and a Celularity placental matrix.

On the cell therapy side, we are continuing to develop T cells and natural killer (NK)-cell products at the IND-enabling study stage to target oncology, autoimmune, and aging-related diseases. We have also developed a novel approach to addressing age-related conditions by using healthy young NK cells to attack and destroy senescent cells using the established mechanism of attacking stress ligand-expressing cells. Data on our preclinical cells and ablation study has been submitted for presentation at the American Society of Gene and Cell Therapy. We also continue to advance our preclinical autoimmune candidates, modified NK cells, and T cells in systemic lupus erythematosus (SLE), scleroderma, and multiple sclerosis. We are also exploring the opportunity to investigate APPL001, our genetically modified placental-derived mesenchymal-like adherent stem cells. To learn more about our products and pipeline, visit **our website**.

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RAJ JOSHI is currently working as the Head of Strategic Sourcing and Supply Chain at Celularity, Florham Park, NJ, USA. With over 20 years of global experience in strategic sourcing, supply chain, and procurement, Raj has previously worked at Amgen, Allergan, Purdue Pharma, and Cosette Pharma. He has an MBA in Supply Chain Management and a BS in Mechanical Engineering.

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Contributions: The named author takes responsibility for the integrity of the work as a whole, and has given their approval for this version to be published.

Acknowledgements: None.

Disclosure and potential conflicts of interest: The author has stock options and RSUs from Celularity.

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

ARTICLE & COPYRIGHT INFORMATION

Article source: Invited.

Interview conducted: Sep 23, 2024; **Revised manuscript received:** Oct 29, 2024;

Publication date: Nov 4, 2024.

NOVEMBER 2024
Volume 10, Issue 10

CELL & GENE THERAPY INSIGHTS

LATEST ARTICLES

CELL & GENE THERAPY INSIGHTS

INNOVATOR INSIGHT

Next-generation risk mitigation in the temperature-controlled supply chain for advanced therapies with ISO 21973 compliance

Edward Grimley and Leanne Kodsmann

The rapid growth of the cell and gene therapy market, projected to reach nearly US$37 billion by 2028, has highlighted the critical need for scalable, reliable logistics solutions to address the complex challenges of transporting temperature-sensitive biologics. This article evaluates the implementation of risk mitigation strategies within the cell and gene therapy supply chain, focusing on compliance with ISO 21973, a standard that provides comprehensive guidelines for the safe transportation of therapeutic cells. Cryoport Systems' innovations, including the Veri-Clean® validated cleaning protocol and the Chain of Compliance® traceability framework, are examined for their role in safeguarding therapy integrity. Advanced tools like the Smartpak II® monitoring system and the Cryoportal® logistics management system are also discussed for their contributions to near real-time tracking, risk mitigation, and regulatory adherence. Together, these strategies demonstrate how next-generation technologies ensure the quality, safety, and efficacy of cell and gene therapies throughout their supply chain journey, ultimately supporting the commercialization and scaling of advanced therapies.

Cell & Gene Therapy Insights 2024; 10(10), 1505–1514

DOI: 10.18609/cgti.2024.172

The cell and gene therapy (CGT) market is experiencing unprecedented growth with projections indicating a compound annual growth rate (CAGR) of 46%, reaching nearly US$37 billion by 2028 [1]. This rapid expansion underscores the critical need for scalable, reliable logistics solutions to meet the increasing demand for these therapies. As

the market grows, so does the complexity of the supply chain with products requiring specialized handling and transportation to maintain their efficacy [2]. ISO 21973: general requirements for transportation of cells for therapeutic use was developed to address these challenges and provides a framework for developing a risk-mitigating supply chain for the life sciences.

BACKGROUND

In the evolving landscape of advanced therapies, maintaining the integrity of temperature-sensitive biologics throughout the supply chain is paramount. Advanced therapies, such as CGTs, as seen in Figure 1, represent the forefront of medical innovation. These offer potential cures for previously untreatable conditions [3], as further illustrated in Figure 2 [4].

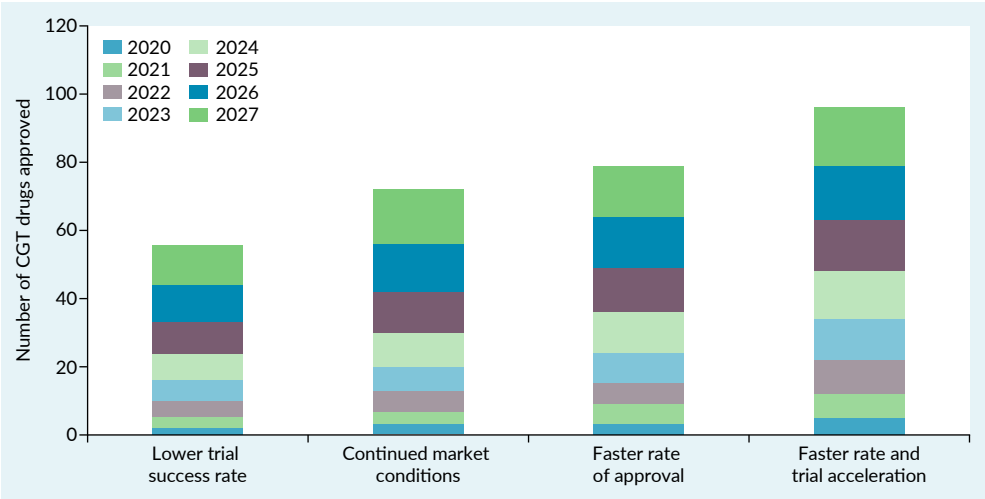
This article aims to evaluate the industry's ongoing efforts to meet and exceed regulatory requirements, particularly concerning the transportation of advanced therapies. The temperature-controlled supply chain must ensure that products remain within specified temperature ranges to preserve their viability and efficacy. Any deviation can result in the degradation of the therapeutic product, rendering it ineffective or unsafe for patient use [2]. This is where ISO 21973 plays an especially crucial role, providing a framework of minimum requirements to ensure the safe transport of these sensitive products.

This evaluation highlights best-in-class risk mitigation strategies that can be implemented to ensure the safe and effective delivery of sensitive materials, such as CGTs, within the temperature-controlled supply chain. In this context, the ISO 21973 standard, introduced in June 2020, is critical in providing a comprehensive framework for transporting cells for therapeutic use [3]. The introduction of ISO 21973 was driven by the need for standardized practices across the industry to mitigate risks associated with temperature fluctuations, contamination, and logistical disruptions during transport.

Coordinated by the Standards Coordinating Body (SCB), this standard represents the culmination of efforts from over 20 experts across government institutions, membership organizations, and

FIGURE 1

CGT drugs approved by scenario.



Comparison of the number of new therapies approved by the FDA by scenario with a breakdown by year of approval. There is a notable acceleration of therapeutic approval by year as well as an accelerating trend in terms of how quickly trials are turning into approvals. Data from Bain analysis.

industry bodies and establishes minimum requirements for IT infrastructure, Chain of Custody systems, centralized logistics management, transportation protocols, shipment tracking, and monitoring [3]. These guidelines are essential for mitigating risks and ensuring the quality and safety of advanced therapies during transit. By adhering to these guidelines, stakeholders can ensure product integrity and patient safety, which are vital in the rapidly growing and evolving field of advanced therapies.

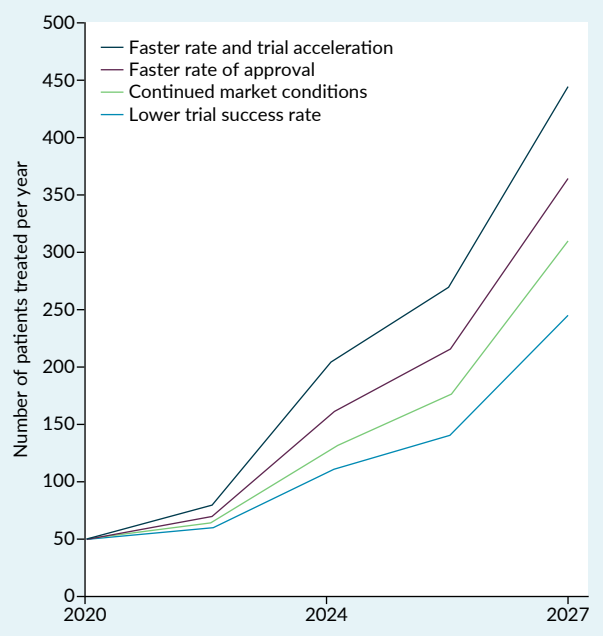
REGULATORY COMPLIANCE

ISO 21973 provides a comprehensive framework for the transportation and storage of cells for therapeutic use, including temperature-controlled and cryopreserved materials. This standard emphasizes the importance of robust IT infrastructure, comprehensive Chain of Custody systems, and centralized logistics management, as illustrated in Figure 3. The CGT industry requires end-to-end precision and traceability, everything from Chain of Custody to Chain of Condition and Chain of Identity. Adhering to these guidelines that incorporate complete traceability of the equipment, processes, and logistics used in managing the environmental control of the CGT while it is in transit ensures that operations meet the highest standards of quality and safety.

By centering operations around ISO 21973 guidelines, stakeholders can be confident in the safety and efficacy of transported therapies. This assurance is critical for product developers, healthcare providers, and patients alike. Moreover, compliance with these standards helps streamline operations, reduce risks, and enhance efficiency, ultimately supporting the successful commercialization of advanced therapies.

FIGURE 2

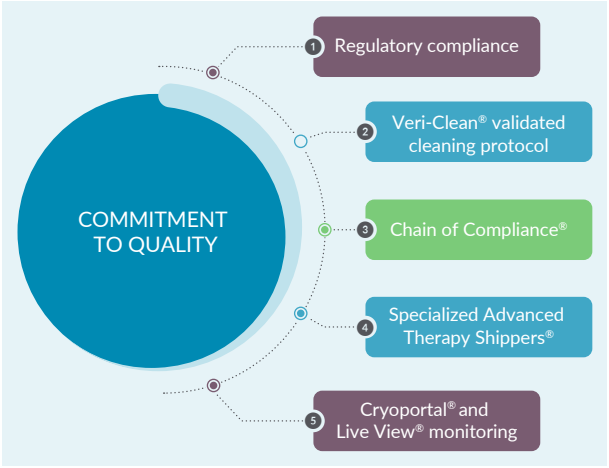
Patients treated per year via CGT clinical trials and/or commercially approved CGT therapies by scenario.



Growing access to advanced therapeutic treatments in coming years. Some therapies, such as those focused on treating hepatitis A and B, will drive higher patient populations. To avoid predicting the success of individual therapies, we have assumed a standard number of treatable patients per therapy based on average incident rates across indications currently in trials. This rate assumes that the total patient population treatable by each therapy does not wholly 'replenish each year' given the rare nature of many diseases that CGT targets. These therapies are highly sensitive to environmental conditions, particularly temperature, necessitating stringent control measures to mitigate risks associated with temperature excursions, contamination, and logistical disruptions [4]. Data from Bain analysis.

FIGURE 3

Multi-pronged, integrated approach to quality and compliance.



Integrated approach to regulatory compliance. An integrated approach to quality and regulatory compliance brings together critical standards and processes to ensure proactive risk mitigation from a collection of starting materials through to patient delivery.

VALIDATED CLEANING PROTOCOL

CGTs are often single-dose, one-time curative therapeutics, making the stakes of successful transportation incredibly high. Any risk of contamination could result in the loss of a potentially life-saving therapy. Given the unique nature of these treatments, which are often created for individual patients, there is no margin for error. Unfortunately, the majority of the industry does not employ validated or standardized cleaning processes for the systems used to transport these sensitive materials. Many providers make no claims or guarantees related to decontamination, leaving a critical gap in the safeguarding of advanced therapies. ISO 21973 emphasizes meticulous documentation and control of all stages of the transportation process, including equipment performance, cleaning, and equipment-use history.

Eliminating the risk of cross-contamination via a comprehensive decontamination process designed to be effective against bacteria, fungi, and viruses reduces external contaminants to virtually zero. This proactive approach further mitigates additional, avoidable risks to sensitive shipments of advanced therapies.

Veri-Clean® is Cryoport Systems' validated cleaning and disinfection process, establishing a new benchmark in the life sciences logistics industry. As the first and only validated process of its kind, Veri-Clean is designed to eliminate the risk of cross-contamination by decontaminating all shipping systems and stainless-steel accessories after every use. This innovative protocol is crucial in ensuring the safety and integrity of advanced therapies during transport, where it has achieved a >6 log (99.9999%) reduction of tested biological indicators as depicted in Table 1.

The Veri-Clean protocol is fully validated by an independent, accredited laboratory to ensure its efficacy and reliability. Through the Veri-Clean methodology, any contaminants on returned shippers are effectively eradicated to provide a robust safeguard against potential risks. Additionally, residual cleaning agents are virtually eliminated as part of this process, with <10 ppm detected once the Veri-Clean process has been completed. Additionally, it is supported by specially developed requalification protocols that certify each shipper in the active lines can support the necessary physical sustainability, LN2 capacity, and a minimum required hold time threshold. If any of the equipment does not meet the requalification specifications, it is immediately removed from the fleet after a final quality assurance (QA) evaluation. The protocols are universally applied across all Cryoport Systems facilities, ensuring consistent and high-quality cleanliness standards globally. Veri-Clean ensures that every shipping system and stainless-steel accessory undergoes rigorous decontamination and documentation procedures and maintains detailed records of each cleaning and disinfection cycle in full compliance with ISO 21973.

TABLE 1

Reduction in colony-forming units of contaminants following the cleaning and disinfection process via Veri-Clean.

Contaminant name	Reduction in CFUs following Veri-Clean
Escherichia coli	$>10^6$
Klebsiella pneumoniae	$>10^6$
Staphylococcus aureus	$>10^6$
Pseudomonas geruginosa	$>10^6$

Veri-Clean virtually eliminates the risk of cross-contamination by decontaminating all shipping systems and stainless-steel accessories. Through a comprehensive validation process that evaluates the initial bioburden when a shipping system is returned as well as manual cleaning validation and low-level disinfection validation, every shipping system achieves a >6 log (99.9999%) reduction of tested biological indicators at every use. CFU: colony-forming unit. Data from Cryoport Systems.

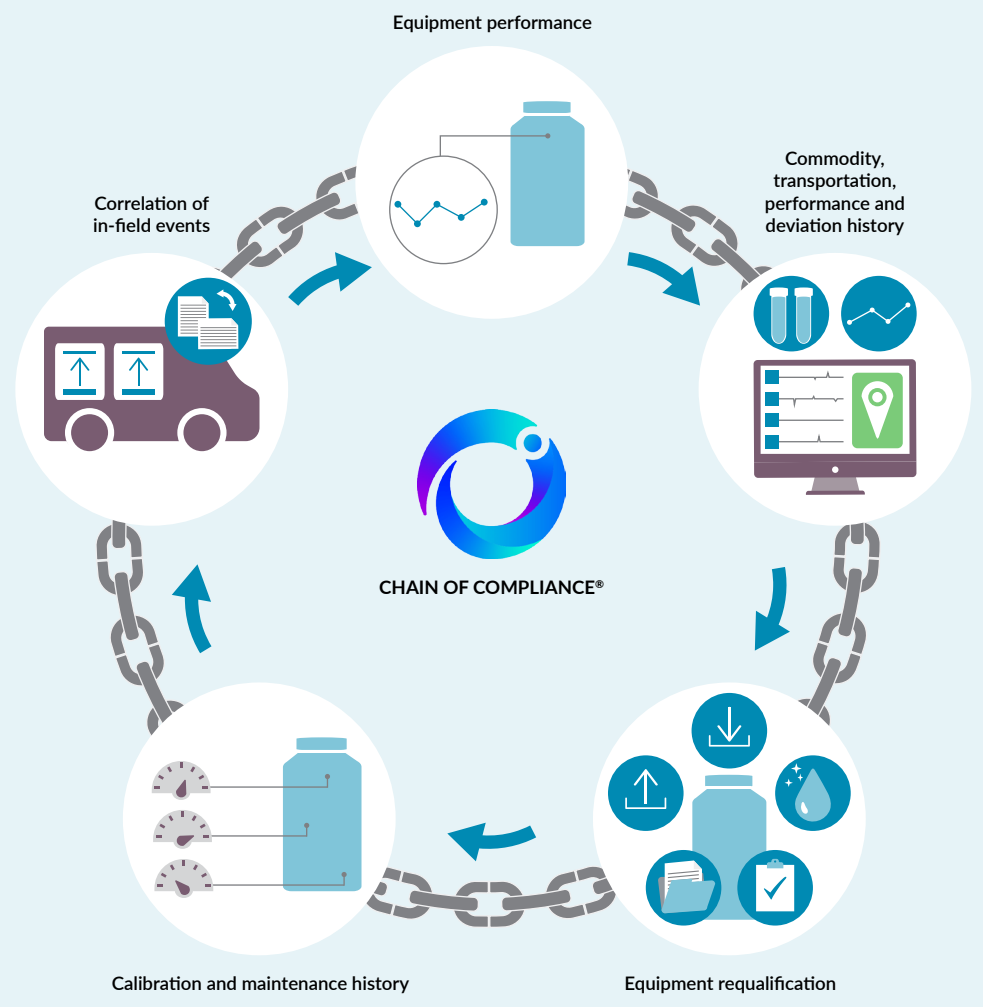
Integral to the Veri-Clean process is the manual confirmation that each shipping system is free from environmental flora to further enhance the decontamination efficacy. By meticulously validating the cleaning process, Cryoport Systems guarantees that all residues are thoroughly removed to achieve the highest level of cleanliness for each shipment. This level of cleanliness sets a new standard in the industry, providing peace of mind that advanced therapies are transported in the safest and cleanest environment possible.

CHAIN OF COMPLIANCE®

Complying with regulations from agencies, such as the US FDA, and adhering to the stringent requirements of the ISO 21973 standard involves complete tracking and traceability across three essential elements: Chain of Custody, Chain of Condition, and Chain of Identity. The Chain of Custody provides a detailed record of who has handled the therapy across every stage. This is achieved through the serialization of the shipper and its components as well as the documentation

FIGURE 4

Support of regulatory compliance with Cryoport Systems' Chain of Compliance.



The Chain of Compliance provides full traceability of the equipment and processes used in managing the environmental control of biomaterials. This assists in mitigating risk throughout the entire supply chain to ensure the safe delivery of invaluable materials.

of comprehensive data on the performance history of the shipper and courier. The Chain of Condition monitors the environmental conditions under which the therapy is stored and transported, utilizing advanced data analytics and near real-time monitoring to maintain optimal conditions. This element also includes the calibration data and history for the data logger used to track environmental conditions. Finally, the Chain of Identity safeguards the identity of each therapy, ensuring it reaches its intended destination without compromise.

To ensure the integrity of advanced therapies during transport, a robust system is required to track the complete history of each shipment, including its Chain of Custody, Chain of Condition, and Chain of Identity. This system must not only be safe and secure but also offer redundancy and reliable backup to prevent data loss or breaches. Given the critical nature of the materials being transported—many of which are potentially life-saving treatments—it is imperative that the system provides robust tracking and monitoring of critical data points like location, temperature, shipper orientation, humidity, and shock, among others, as well as comprehensive data logging. This level of traceability guarantees that any deviations in temperature, handling, or other environmental conditions can be identified and addressed immediately, ensuring the product's safety from start to finish.

Cryoport Systems' Chain of Compliance is an advanced, integrated framework designed to ensure the highest standards of quality, traceability, and accountability for temperature-controlled supply chains. The Chain of Compliance, as seen in Figure 4, integrates validated requalification procedures. This solution goes beyond basic logistics to provide end-to-end traceability and robust data-driven risk mitigation using advanced tools like the Cryoportal® logistics management system. This system maintains detailed records of every shipment, including commodity history, deviation history, transportation history, and maintenance/refurbishment history of the shipper. Additionally, to further safeguard against equipment failure, every shipper undergoes requalification after each use, ensuring it meets stringent performance standards before being deployed again. Given the irreplaceable nature of these therapies, where even a small error could lead to the loss of a life-saving treatment, this rigorous, data-driven approach is essential to maintaining product integrity throughout the supply chain. This extensive data collection and management capability allows for the anticipation and prevention of potential issues. By leveraging advanced data analytics and near real-time monitoring through the use of Smartpak II® (Smartpak), an advanced condition monitoring system integrated with the Cryoportal logistics management system, in-field events were correlated to equipment performance, thereby continuously improving the processes and mitigating risks. This seamless integration allows for immediate interventions if any anomalies are detected, ensuring that environmental conditions remain within the required thresholds throughout the entire journey.

The ISO 21973 standard was created to address the critical challenges associated with transporting sensitive, temperature-controlled therapies that are derived from human cells. These therapies, many of which are irreplaceable, one-time curative treatments, cannot afford any compromise in their quality during transit. The standard establishes clear guidelines for managing the Chain of Custody, Chain of Condition, and Chain of Identity, all of which are vital to ensuring that therapies remain safe, viable, and effective upon delivery. Cryoport Systems' Chain of Compliance supports regulatory compliance requests from agencies such as the US FDA and adheres to the stringent requirements of the ISO 21973 standard. Ultimately, ISO 21973 ensures that every possible measure is taken to safeguard the integrity of these therapies, from the moment they leave the manufacturing facility to their final delivery to patients in need.

SEGREGATED FLEET OF ADVANCED THERAPY SHIPPERS

Regulatory requirements are evolving to meet the highest standards of safety, integrity, and compliance for the transportation of engineered human CGTs and human-derived cellular and biological materials. A fully segregated fleet for human-derived advanced therapies adds an additional layer of risk mitigation by virtually eliminating the potential for contamination from non-human-based materials. Cryoport Systems developed a proprietary Advanced Therapy Shipper® (ATS) fleet that is segregated from the General Purpose (GP) fleet. Each ATS shipping system is exclusively dedicated to human CGTs. This specificity ensures that these critical materials are transported under the most stringent conditions.

The ATS fleet's design and operational protocols are purpose-built to align with the latest regulatory requirements, including ISO 21973. By adhering to these rigorous standards, Cryoport Systems guarantees that all shipments maintain the highest levels of quality and safety throughout the supply chain. The Certificate of Conformance adds an extra layer of assurance, certifying that each shipping system has only handled human CGT products. This certification is verified, signed, and kept on record for at least 10 years.

In response to growing market demand and evolving regulatory landscapes, the ATS fleet is engineered to ensure the safe and reliable transport of critical patient therapies. By anticipating and addressing future regulatory requirements within the temperature-controlled supply chain, Cryoport Systems ensures that the shipping systems and services remain at the forefront of innovation and compliance. The ATS fleet's rigorous validation protocols and exclusive use for human-derived materials provide certainty in the safety and efficacy of transported therapies, maintaining both good manufacturing practice (GMP) and good distribution practice (GDP) standards.

ADVANCED DATA MONITORING AND LOGISTICS MANAGEMENT

In response to evolving industry developments and the increasing market need for robust temperature-controlled logistics, it is increasingly critical to harness innovative informatics technology to ensure unparalleled safety and efficiency. The advanced monitoring solution offered by Cryoport Systems, comprised of the Smartpak condition monitoring system and the Cryoportal logistics management system, provides comprehensive near real-time tracking and data analytics. This combination facilitates near real-time monitoring and risk management, promoting the integrity and traceability of every shipment through the Chain of Compliance processes. This allows the customer service team, who monitor all shipments 24/7, to immediately respond to any early warning signs of potential issues. By continuously tracking shipment conditions, such as temperature or location, the team can intervene before problems escalate, ensuring that these sensitive therapies remain within safe parameters throughout their journey.

The Smartpak condition monitoring system plays a crucial role in maintaining near real-time oversight of critical shipment parameters, including location, temperature, pressure, anti-tamper status, orientation, humidity, and shock. Parameters like tilt can directly affect the effectiveness of the liquid nitrogen coolant used in cryogenic shipments. Even a slight tilt can affect liquid nitrogen evaporation rates, thereby drastically reducing hold times, potentially jeopardizing the safe transport of therapies, and potentially putting entire CAR T-cell immunotherapy clinical programs at risk [6]. This system not only safeguards the integrity of shipped materials but also delivers meticulous analytics to aid in planning, en-route mitigation, and reporting. When the system alerts the team to a potentially catastrophic issue, like a liquid nitrogen shipper placed on its side in transit, the customer support team can intervene to expedite the delivery, ensuring safe arrival

without temperature excursions that could compromise the integrity of the irreplaceable materials housed within [7]. By capturing and relaying comprehensive environmental data, the Smartpak enables interventions to secure the transport of invaluable biological materials.

The Cryoportal is an integral part of logistics management. It is an innovative, web-based platform that integrates ordering, tracking, paperwork, and communications into a single streamlined portal. Additionally, the Cryoportal platform supports enhanced security and compliance with regulatory standards, such as 21 CFR Part 11, featuring robust audit logging and the latest web security measures. It is also validated to demonstrate compliance with the International Society for Pharmaceutical Engineering, Good Automated Manufacturing Processes (ISPE GAMP). This system ensures thorough logging, capturing a comprehensive record of all activities, and securely storing this data for a minimum of 10 years. It maintains full knowledge and traceability of every piece of equipment, from its performance history to any maintenance or refurbishments. This detailed level of record keeping enables tracking of the entire lifecycle of each shipper, providing confidence that every shipment is managed with the highest standards of care, traceability, and security. Additionally, the Cryoportal ensures complete transparency through the provision of Chain of Condition, Chain of Custody, and Chain of Compliance data, facilitating comprehensive traceability and accountability. This advanced logistics management framework exemplifies Cryoport Systems' commitment to innovation, reliability, and the highest standards of service in the temperature-controlled supply chain industry.

CONCLUSION

As the demand for advanced therapies continues to surge, the temperature-controlled supply chain for CGT faces unprecedented challenges. Cryoport Systems has addressed these challenges through the implementation of next-generation risk mitigation strategies, adherence to ISO 21973 standards, and the development of innovative solutions such as the Veri-Clean validated cleaning process and Chain of Compliance traceability framework. The ATS and state-of-the-art data monitoring systems further enhance the safety and integrity of these sensitive therapies. These approaches are not merely reactive but anticipatory. By staying ahead of regulatory requirements and market needs, Cryoport Systems is committed to providing clients with novel solutions that ensure the safe and efficient transport of products. This proactive stance ensures readiness to support the scaling of advanced therapies from clinical trials to commercial distribution. By providing a comprehensive and integrated approach, Cryoport Systems ensures that the unique requirements of the CGT supply chain are met with the highest level of precision and reliability.

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Contributions: The named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.
Acknowledgements: None.
Disclosure and potential conflicts of interest: The authors are Cryoport Systems employees and stock holders.
Funding declaration: The authors received no financial support for the research, authorship and/or publication of this article.

ARTICLE & COPYRIGHT INFORMATION

Article source: Externally peer reviewed.
Submitted for peer review: Sep 19, 2024; **Revised manuscript received:** Nov 11, 2024; **Publication date:** Dec 4, 2024.

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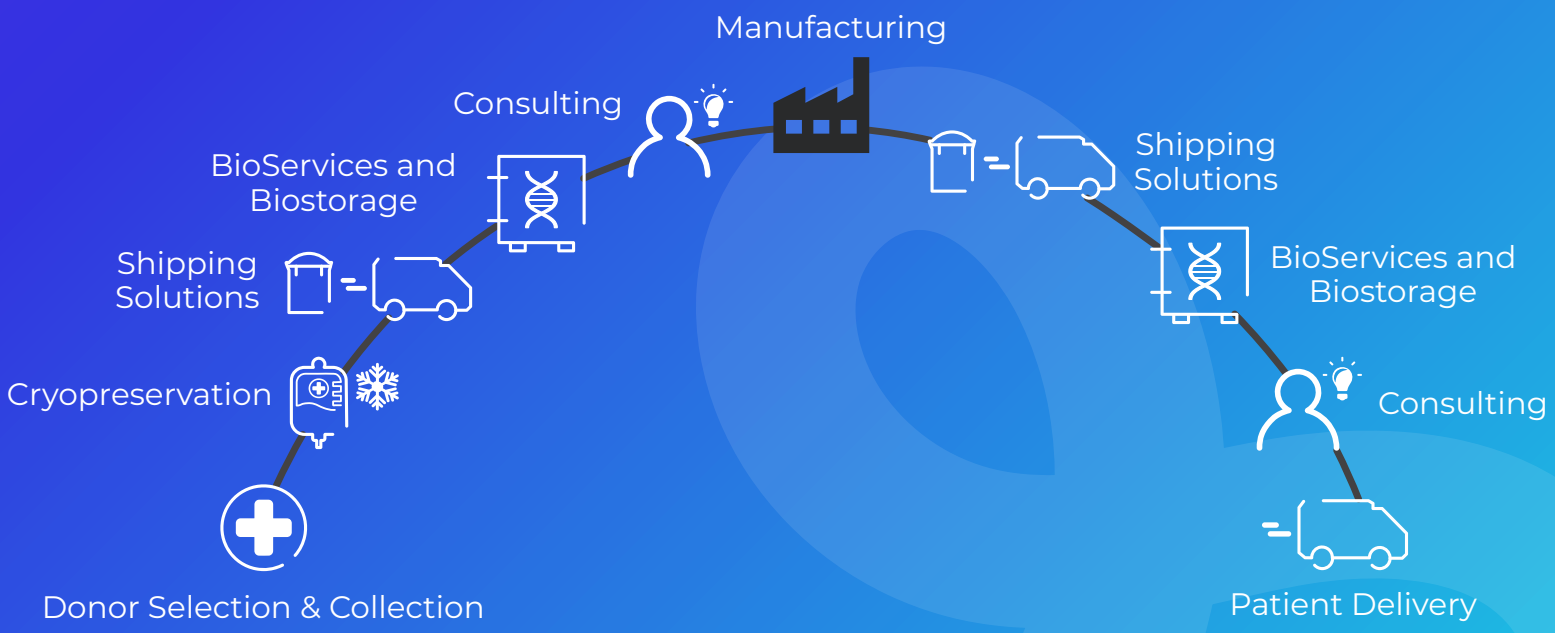

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CELL & GENE THERAPY INSIGHTS

INNOVATOR INSIGHT

Navigating the opportunities and challenges in analytical development of flow cytometry for T cell therapy

Therese Choquette and Minh Ngoc Duong

T cell therapies are derived from autologous or allogeneic starting materials and target various diseases, including cancers, autoimmune diseases, and infectious diseases. This article highlights the rapid advancements in assay validation tools, focusing on flow cytometry as a crucial technique for analyzing T cell products. Understanding the intricacies of both T cell therapies and flow cytometry is essential for successful clinical and regulatory outcomes.

Cell & Gene Therapy Insights 2024; 10(10), 1305–1312

DOI: 10.18609/cgti.2024.149

T CELL THERAPIES AND FLOW CYTOMETRY: AN OVERVIEW

CAR-T cell therapies are generated from autologous or allogeneic starting material, sourced from patient or donor blood. A few autologous CAR-T cell products, such as Kymriah and Yescarta, target leukemia and lymphoma and are based on peripheral blood-derived T cells. Therapies utilizing tumor-infiltrating lymphocytes (TILs), TCR-T cells, and regulatory T cells are also being developed. With rapid advancements, clinical trials are exploring applications for these therapies in solid tumors, autoimmune diseases, and infectious diseases, such as HIV.

A comprehensive understanding of T cell-based starting materials and related validation processes is essential for successful IND submissions, and ultimately, enhanced patient outcomes. The autologous cell therapy process includes validation and quality control assessments at each step, as shown in the examples in Figure 1. Following apheresis, for example, characterizing the starting material involves immunophenotyping various immune cell populations, particularly T cells. Subsequent purity assessments post-T cell

FIGURE 1

Utilization of flow cytometry process using selected tests at various points.

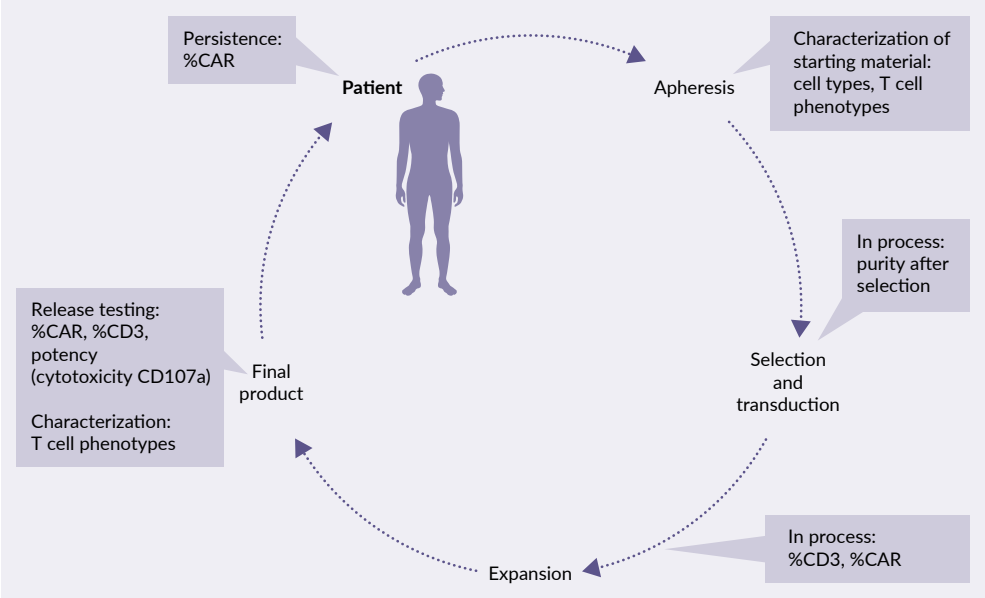


TABLE 1

Parameters requiring determination during the qualification and validation to address flow cytometry challenges.

Parameter	Definition	Challenge
Accuracy	Expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found	Lack of reference or standard
Specificity	Is the ability to assess unequivocally the analyte in the presence of component which may be expected to be present	Spike in specific cell populations for detection; heterogenic samples
Linearity and range	Linearity is the ability (within a given range) of an analytical procedure to obtain test results which are directly proportional to the concentration of analyte in the sample; the range is the interval between the upper and lower concentration of analyte which it has been demonstrated a suitable level of precision, accuracy, and linearity	Populations not being 100%, limits the assessment of the range; heterogenic sample with several different cell populations.
LOD, LOQ	LOD is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value LOQ is the lowest amount of analyte in a sample which can be quantitively determined with suitable precision and accuracy	Unspecific background which differ between samples
Precision	Expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogenous sample under prescribed conditions; is considered at three levels: repeatability, intermediate precision, and reproducibility	Cells are heterogenous and may have vial to vial variations in the populations, subjective gating, variability of instruments.

LOD: limit of detection, LOQ: limit of quantitation.

isolation is necessary, and the qualification of %CD3 and %CAR is vital to ensure a quality therapeutic product. Release testing involves additional assessments for cytotoxicity and potency as per regulatory requirements.

Flow cytometry is used throughout the manufacturing process, from initial characterization to monitoring post-administration, as it provides detailed, quantitative, and multiparametric data on individual cells. This makes it an indispensable tool in the development, manufacturing, and quality control of cell-based therapies.

CHALLENGES OF USING FLOW CYTOMETRY IN ASSAY VALIDATION AND QC

Flow cytometry is a powerful analytical technique used to measure physical and chemical characteristics of cells or particles as they flow in a fluid stream through a laser beam. By labeling cells with fluorescent antibodies that bind to specific markers, flow cytometry can identify, quantify, and analyze multiple parameters on individual cells simultaneously, such as size, granularity, and protein expression. This multiparametric data provides a detailed profile of cell populations, allowing researchers to differentiate cell types, assess functions, and track changes in response to treatments. Due to its precision, speed, and capacity for high-throughput analysis, flow cytometry is essential in cell therapy, where understanding cellular behavior at an individual level is crucial for QC.

Despite its critical role, flow cytometry faces several challenges in cell therapy manufacturing, particularly in functional assays like proliferation, cytotoxicity, apoptosis, and cytokine release. One significant challenge is the lack of available reference materials, as assays must be scientifically sound and fit for purpose, especially in the early stages before pivotal trials.

Flow cytometry gating can involve some subjectivity, and precise instrument settings are required. Other challenges include nonspecific antibody binding, cellular autofluorescence, and the tendency of dying cells to interfere with results. These issues complicate the validation, qualification, and analysis of assays.

Determination of linearity is another challenge, as achieving 100% purity in cell populations is rare. Background noise varies between batches, making it difficult to set consistent limits of detection (LOD) and quantification (LOQ). Extensive work is required to evaluate each marker in every cell population within the panel.

Table 1 illustrates the parameters that should be determined during qualification and validation, highlighting specific challenges faced when assessing these parameters via flow cytometry.

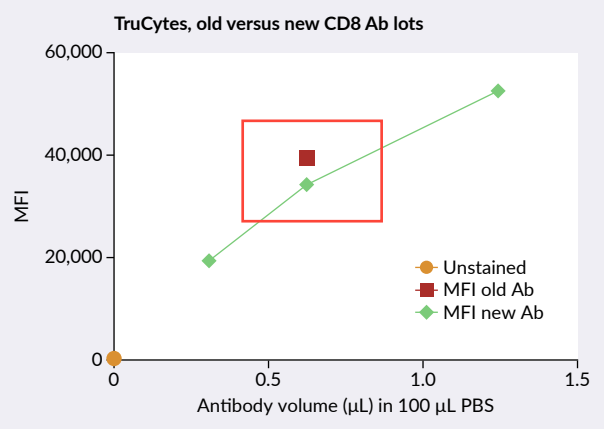
NOVEL TOOLS AND APPROACHES TO SUPPORT ASSAY VALIDATION AND QC VIA FLOW CYTOMETRY

To address these challenges, several innovative tools are available. Lyophilized peripheral blood mononuclear cells (PBMCs), for example, come with a certificate of analysis detailing predetermined percentages of different cell populations, supporting consistency across assay runs, if the same lot is used. Lot-to-lot variability is a significant challenge, as each batch of PBMCs can exhibit differences in cell composition, viability, and marker expression due to donor heterogeneity and variations in collection and processing. This variability impacts the reproducibility and accuracy of assays, requiring additional steps for standardization and rigorous QC to ensure consistent therapeutic performance across different production batches.

Fluorescent particles known as rainbow beads can be used to calibrate instruments and serve as controls. However, the fluorescence spectrum of rainbow beads might not perfectly match the spectrum of the

FIGURE 2

Comparison of TruCyte CD8 cell mimic titration of old and new CD8 antibodies.



Ab: antibody, MFI: mean fluorescence intensity.

fluorophores used in cell samples, leading to inaccuracies in compensation and calibration settings. Rainbow beads are also limited in their ability to mimic biological samples, which can impact light scatter properties. Finally, they may not respond to changes in instrument settings (e.g., voltage adjustments) in the same way as live cells, potentially introducing discrepancies when translating calibration results to real-world cell samples.

CELL MIMICS AS A NEXT-GENERATION SOLUTION

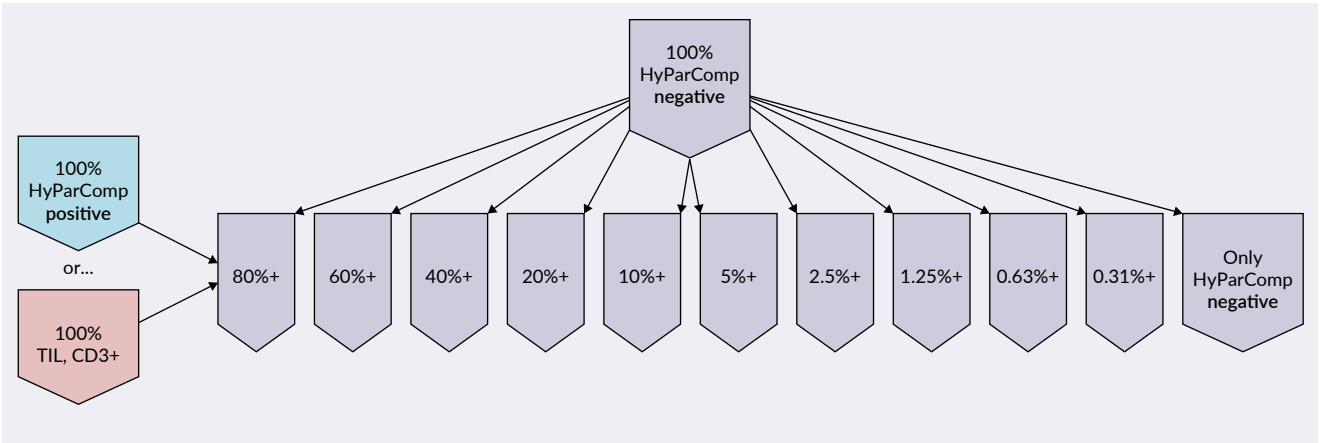
Slingshot Biosciences employs a semiconductor-based manufacturing process to produce polymer-based cell mimics that accurately replicate essential cellular features, such as size, granularity, autofluorescence, and protein expression. These cell mimics are versatile and can be applied across all aspects of flow cytometry analysis, including instrument standardization, compensation, and assay control. With minimal lot-to-lot variability, scalable production, and a shelf life of up to 18 months, these mimics address the limitations of traditional solutions, providing scientists with a reliable and modern approach to overcoming challenges in flow cytometry analysis.

ANTIBODY TITRATIONS USING TRUCYTES™ CD8 CELL MIMICS

One of the key challenges in flow cytometry is determining the optimal antibody concentration, as titrations are performed with the target cell type, which can vary across batches, especially with primary cells or autologous samples. TruCytes, from

FIGURE 3

Experimental design of assessing linearity and LOQ of flow cytometry using HyParComp beads.



LOQ: limit of quantitation, TIL: tumor-infiltrating lymphocyte.

Slingshot Biosciences, are polymer-based particles with specific protein markers embedded on their surface, that can be tailored to mimic specific attributes of most cell types.

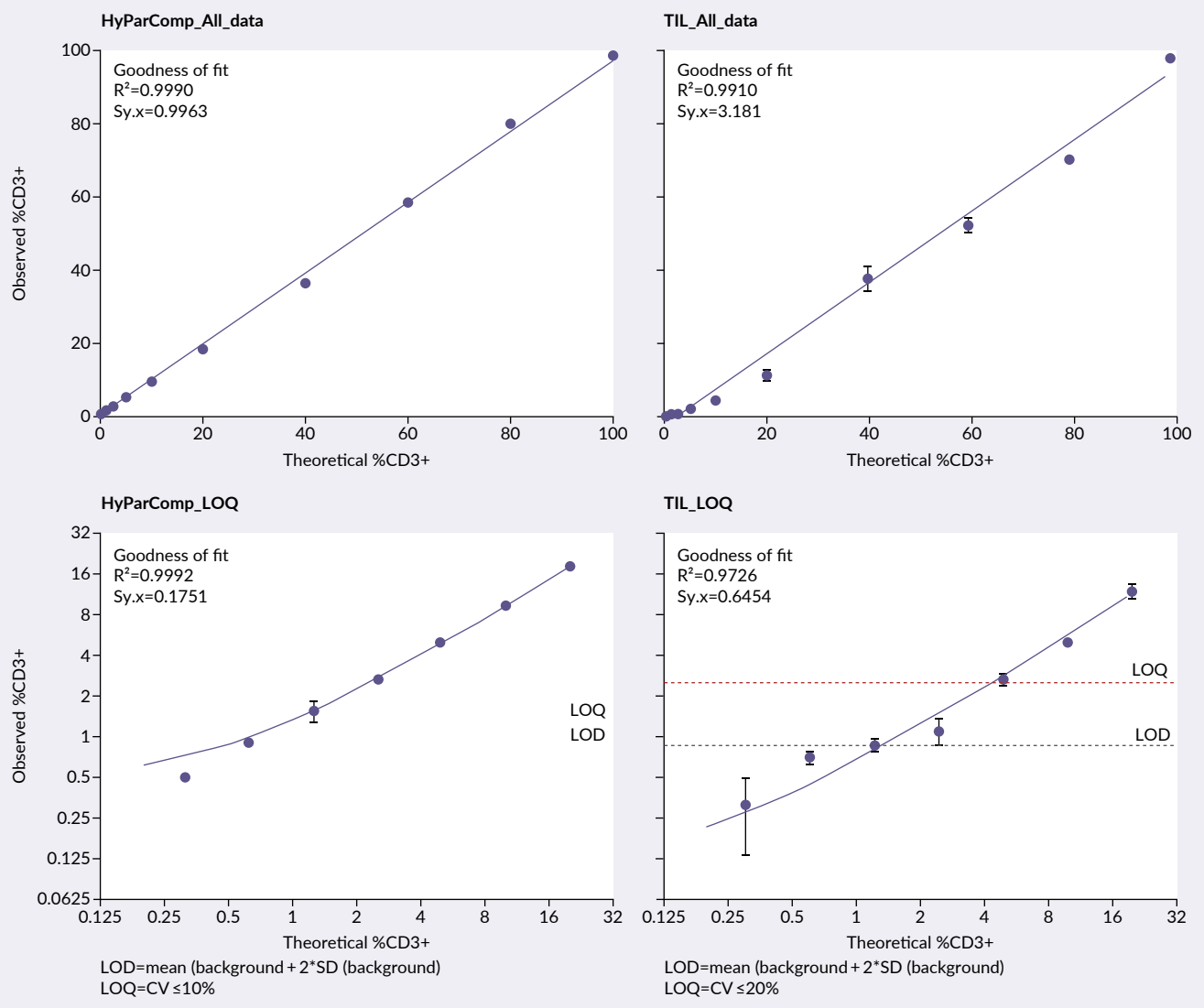
Figure 2 compares old and new CD8 antibodies, titrated using TruCytes CD8 cell mimics. The red square and green diamonds represent the MFI (mean fluorescence intensity) of old CD8 antibodies and the MFI of new CD8 antibodies respectively. This was used to determine the old/new antibody MFI ratio (87%), indicating that the optimal dilution of the new antibody lot was 0.72/100. Of note, the first titration should be done on the same type of cells as the test samples; the following titrations of new lots can be done with the cell mimics for a standardized method of antibody titration.

LINEARITY AND LIMIT OF FLOW CYTOMETRY QUANTIFICATION OF HYPARCOMP™ BEADS

Understanding the LOQ is another crucial component of flow cytometry assay

FIGURE 4

Linearity and limit of flow cytometry quantification of HyParComp beads and TIL cells.



CV: cell viability, LOD: limit of detection, LOQ: limit of quantitation, TIL: tumor-infiltrating lymphocyte.

validation, which represents the lowest reliable quantitative measurement. Linearity is typically assessed by fully diluting a sample to 0%. Finding an appropriate cell type for these dilutions can be challenging; and while cell lines are commonly used, they may not fully replicate the properties of lymphocytes.

HyParComp is a cell mimic designed for compensation and is provided in two vials: one with 100% positive beads and the other with 100% negative beads. HyParComp compensation mimics were used to measure assay linearity from 0–100% and determine the linear range.

The experiment used two approaches, as seen in Figure 3. In the first, TILs at 100% were diluted with negative HyParComp beads, or 'naked beads', from 80% down to 0%. In the second approach, 100% HyParComp positive beads, which bind all antibodies, were diluted similarly with the naked HyParComp beads.

This allowed for the assessment of linearity and LOQ, where the results are shown in Figure 4. The upper graphs represent the linearity assessment across all concentrations, while the lower graphs focus on the 20 to 0% range for the LOQ assessment. The HyParComp beads demonstrated excellent linearity with an R-squared value of 0.999. TIL data showed some deviation with an R-squared value of 0.991.

HyParComp cell mimics showed consistent and clean results, with minimal variation across replicates a LOQ of just over 1%. In contrast, TILs displayed higher variability due to the non-uniform distribution of cells, resulting in higher cell viability (CV) percentage. For the TILs, a %CV of 20% was accepted, setting the LOQ around 3%.

SUMMARY

Flow cytometry, a vial method in T cell therapy, faces many challenges including the need for precise assay validation and standardization, non-specific antibody binding, and subjectivity in gating. Novel approaches such as lyophilized PBMCs, cell mimics from Slingshot Biosciences, and automated instruments help address these challenges.

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AUTHORSHIP & CONFLICT OF INTEREST

Contributions: The named author takes responsibility for the integrity of the work as a whole, and has given their approval for this version to be published.

Acknowledgements: None.

Disclosure and potential conflicts of interest: Slingshot Bio provided the Slingshot Bio material needed for the study free of charge (cell mimics and comp beads). All material besides this was purchased or provided by Tigen Pharma. Therese Choquette and Minh Ngoc Duong are employees of Tigen Pharma SA.

Funding declaration: Support was given for presenting at the Cell Therapy Analytical Development Summit, Boston, Dec 3–5, 2024.

ARTICLE & COPYRIGHT INFORMATION

Article source: This article is based on a webinar held by Slingshot Biosciences.

Revised manuscript received: Oct 14, 2024; **Publication date:** Nov 25, 2024.

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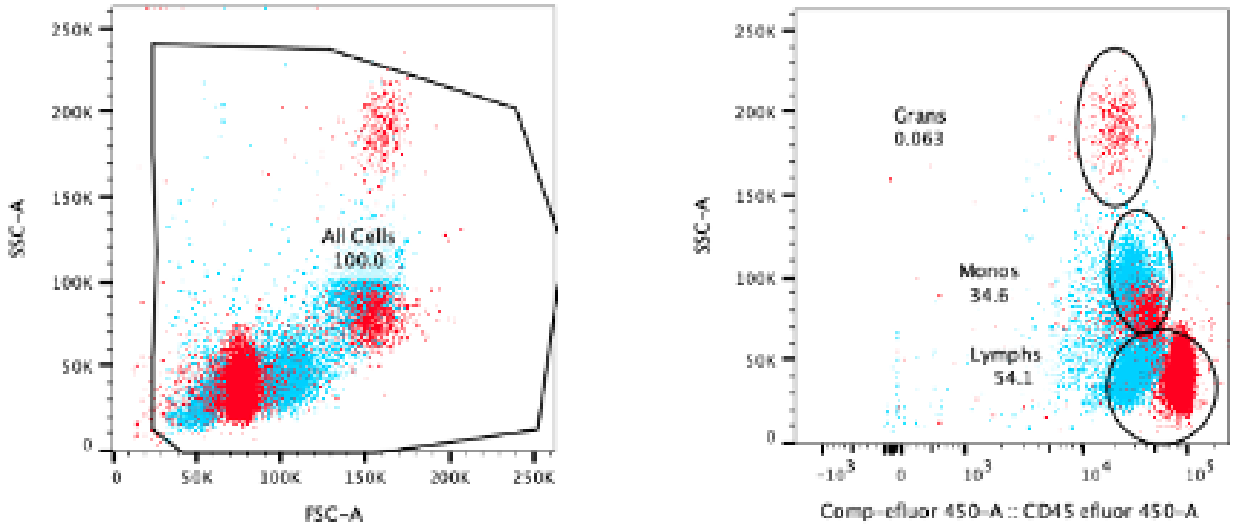

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FASTFACTS

Redefining the economics of upstream mAb and viral vector bioprocessing: how process intensification boosts productivity

Rachel Legmann, Senior Director of Technology, Gene Therapy, Repligen Corp

The higher complexity of viral vectors compared to monoclonal antibodies creates additional production and purification challenges. This poster presents case studies on how integrated viral vector bioprocessing solutions with perfusion production, continuous clarification, harvest, purification, and process analytical tools simplify and speed up the process leading to a significant intensification of potent lentivirus and adeno-associated virus titer yield.

MANAGING COMPLEX VIRAL VECTOR MANUFACTURING PROCESS

Manufacturing challenges across the advanced therapy workflow include meeting high titer demands without compromising on quality, safety, cost, or speed. Repligen offers an agnostic end-to-end manufacturing solution for viral vector intensification, using various cell lines and vectors, from seed train to production and purification. To address the challenges posed by the complexities of viral vector-based modalities, it is critical to move from a batch or fed-batch process to perfusion from the early development phase to ensure high productivity and low cost.

UPSTREAM PERFUSION TECHNOLOGY

KrosFlo TFDF® perfusion-based intensified cell culture offers the combined benefits of tangential flow and depth filtration, enabling high cell density with high product transmission. This technology contains a 2–5 µm pore size tubular depth filter operated in TFF mode, delivered with single use closed γ-irradiated flow paths. The KrosFlo TFDF® technology enables scalable perfusion production processes for lentivirus from a 2–2,000 L bioreactor scale. This advanced viral vector bioprocess solution enables the generation of more doses per batch, offering 3–10× adeno-associated virus (AAV) and >20–50× functional lentivirus (LV) productivity.

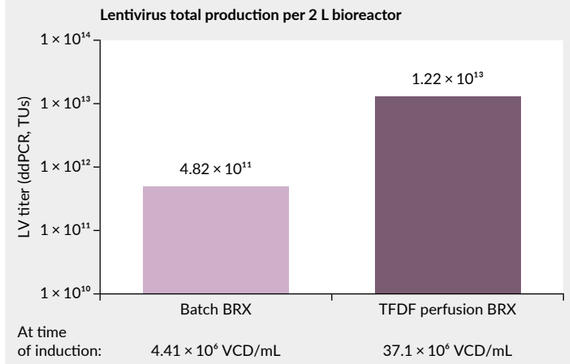
Figure 1 shows data from a customer proof-of-concept case study illustrating how upstream process intensification through growth and clarification using mammalian stable cell lines can be achieved using KrosFlo TFDF®, enabling 25× more potent LV doses per 2 L bioreactor. Additional optimization can be employed to increase yield further to provide sufficient LV doses for large patient populations at affordable cost.

Table 1. LV manufacturing process scenarios data.

	1. Batch–depth filtration (one harvest)	2. Batch–TFDF® (one harvest)	3. Batch–TFDF® (two harvests)	4. Perfusion–TFDF® (continuous harvest)
Cell culture mode	Batch	Batch	Batch	Perfusion (with TFDF®)
Bioreactor seed train (L)	200/500	200/500	200/500	20/50
Production bioreactor volume (L)	2000	2000	2000	200
Viable cell density (cells/ml)	2×10^6	2×10^6	2×10^6	2×10^7
Virus production phase (days)	2	2	3.5	3.5
Filtration technology	Depth filtration	TFDF®	TFDF®	TFDF®
Harvest/retention yield (%)	70	90	90	90

LV: lentivirus.

Figure 1. Process intensification of LV production through growth and clarification: control batch versus TFDF perfusion process.



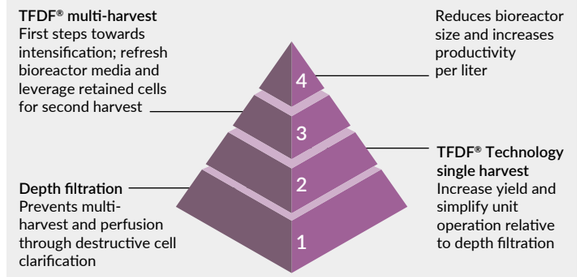
LV: lentivirus, TFDF: tangential flow depth filtration, TU: transducing unit, VCD: viable cell density.

DOWNSTREAM BIOPROCESS SOLUTIONS FOR HIGH VIRAL VECTOR DEMAND

Process robustness and reproducibility are key for high productivity and cost-effective viral vector manufacturing at scale. To intensify downstream production, process performance and consistency can be achieved by using the KRM™ Chromatography System platform. This comprehensive chromatography solution is designed to improve vector recovery yield, separation, and operational simplicity, whilst also reducing cost and risk.

Theoretical LV manufacturing process scenarios (outlined in Figure 2) were explored for cost analysis, with resulting process performance data shown in Table 1. This data is based on LV production for an autologous CD34+ transduced hematopoietic stem cell indication (inherited immunodeficiencies) at 1×10^{10} TU/dose (1×10^8 cells transduced, multiplicity of infection 100). By level 4 of the intensification pyramid (tangential flow depth filtration [TFDF] continuous perfusion), dose productivity is significantly increased, and consumable cost per dose relative to batch is significantly reduced.

Figure 2. Intensification pyramid. Intensifying LV process scenarios from 1) batch depth filtration, to 2) TFDF batch single harvest, to 3) TFDF batch multi-harvest, to 4) TFDF in continuous perfusion mode.



LV: lentivirus, TFDF: tangential flow depth filtration.

Cell & Gene Therapy Insights 2024; 10(10), 1367; DOI: 10.18609/cgti.2024.155

Accelerating gene therapy downstream process development through DoE and scalable chromatography

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

In the competitive landscape of gene therapy development, achieving efficient purification workflows is crucial for success. This Executive Summary delves into how the design of experiments (DoE) methodology can streamline downstream process development (PD), specifically for AAV therapies, and how pre-packed chromatography columns play a key role in this process.

OVERVIEW OF PRE-PACKED COLUMN PROCESS DEVELOPMENT

As biopharmaceutical companies focus on developing new therapeutic modalities like viral vectors and nucleic acids, the demand to streamline downstream processing has significantly increased. Traditional, sequential approaches to purification process development can be time-consuming, and the cost of materials, such as resins, remains high. Therefore, improving yield can result in a decreased CoG. This is where DoE and automation come into play to significantly shorten development timelines to accelerate speed to market.

Repligen's OPUS pre-packed chromatography columns are designed to streamline purification processes at various stages of downstream PD (DPD). The DPD portfolio of these columns is divided into three primary products: where each is tailored to different applications, as seen in **Figure 1**. RoboColumns are designed for resin screening, MiniChrom columns for bench scale process development and optimization, and ValiChrom columns for process validation including viral clearance studies.

The columns offer several key value propositions, one of which is the ability to enable rapid development of purification protocols. Additionally, the columns provide scalable solutions, ensuring that the same platform can be used across different stages of DPD, from small-scale development through to large-scale production.

Figure 1. Three primary OPUS pre-packed chromatography columns (RoboColumn, MiniChrom, and ValiChrom) and their uses at various stages of downstream process development.

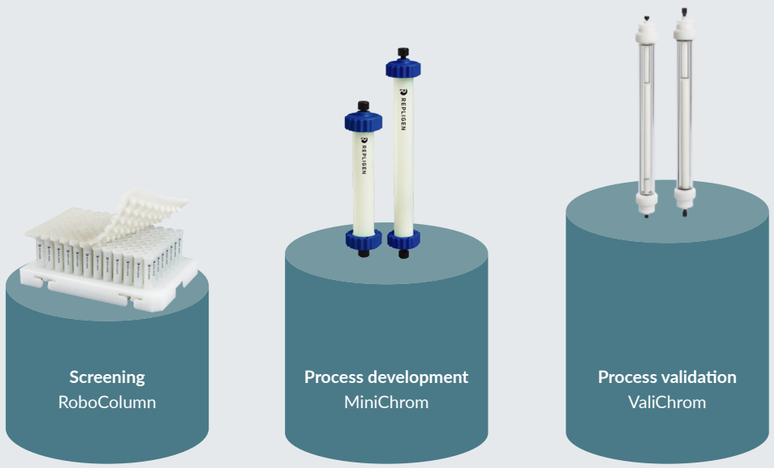
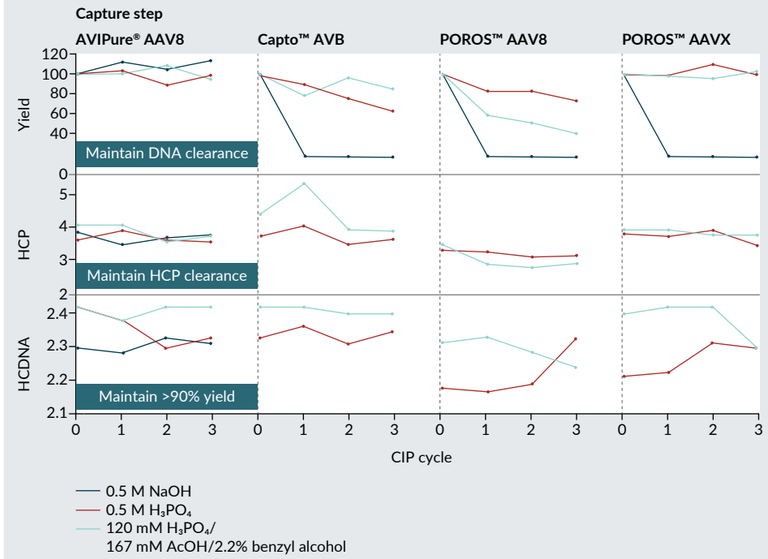


Figure 2. Design of experiment for four AAV capture resins across three cleaning solutions to measure yield, host cell protein, and host cell DNA.

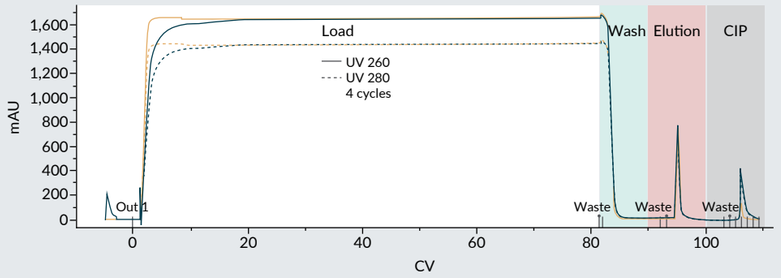


THE ROLE OF PRE-PACKED COLUMNS IN AAV PURIFICATION WITH DoE

RoboColumns, a miniaturized format of chromatography columns, are instrumental in applying DoE to purification workflows. These columns enable high-throughput, parallel chromatography, making it possible to run multiple experiments simultaneously. Pre-packed with over 300 different chromatography resins, RoboColumns offer the flexibility needed for the rapid screening of resins and separation conditions. This approach is particularly useful for new modalities like AAV-based gene therapies.

In this study, four AAV capture resins were tested using three different cleaning solutions, including acetic acid and caustic conditions, shown in **Figure 2**. The experiment measured yield, host cell protein, and host cell DNA clearance across four cycles. In total, 48 chromatographic runs were performed in less than five hours. This process would have taken several weeks using a traditional chromatography approach.

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



The results demonstrated that the AVIPure-AAV8 resin maintained a consistent performance across all cleaning solutions, including caustic conditions. This resin proved to be the only caustic-stable option currently available for AAV capture. The primary takeaway from this case study was the significant time savings achieved by transitioning from a sequential operation to a parallel operation using the RoboColumns.

The data generated with the RoboColums for the AAV capture step was used to translate into the MiniChrom. **Figure 3** illustrates the superimposition of four chromatography runs executed on the ÄKTA system using a 1mL MiniChrom column pre-packed with AVIPure-AAV8 and operated under optimized conditions. This superimposition demonstrated an almost perfect match to the RoboColum, confirming high reproducibility. Additionally, this showed consistent yields in host cell DNA and host cell protein removal between both scales.

SUMMARY

The use of DoE, particularly with RoboColumns, is advancing DPD for gene therapies. By enabling parallel experimentation, researchers can rapidly screen resins, optimize conditions, and scale up processes, all while saving significant time and resources. As the demand for new therapies such as AAV-based treatments continues to grow, the need for efficient and scalable purification strategies will become even more critical. Repligen's OPUS pre-packed chromatography columns offer a solution to address these challenges.

Watch the video and view the poster here

Cell & Gene Therapy Insights 2024; 10(10), 1369 • DOI: 10.18609/cgti.2024.156