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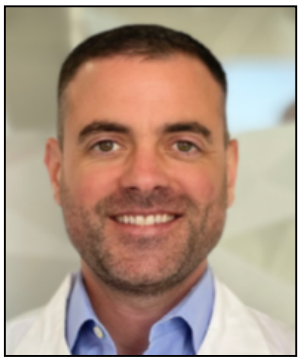
SPOTLIGHT

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.

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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

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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

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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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