INNOVATOR INSIGHT

Product Characterization: the evolving analytical toolkit

Dr. Baghbaderani is the head of Cell Therapy Development, Emerging Technologies at Lonza. He has over 13 years of experience in stem cells engineering and bioprocessing. Dr. Baghbaderani holds a PhD degree in Biomedical Engineering from the University of Calgary (Calgary, Canada), where he developed bioreactor protocols for large-scale expansion of human neural stem cells for clinical applications. He completed nearly three years postdoctoral program including a two-year postdoctoral fellowship at the National Institutes of Health (NIH) / National Institute of Neurological Disorders and Stroke (NINDS). His postdoctoral research at the NIH focused on generation of human induced pluripotent stem cells, bioprocessing of both human embryonic stem cells and iPSCs and controlled differentiation into neuronal lineage. Since joining Lonza in 2011, he has been working on developing new technologies and manufacturing processes around human pluripotent stem cells. As the head of CT Development, Dr. Baghbaderani is currently leading the process development and bioassay services, focusing on the development of cGMP compliant processes and cell characterization assays for different cell therapy applications.

Q Product characterization is central to developing scalable manufacturing processes – what technologies and approaches are currently available in the cell therapy characterization toolkit?

From a Cell Therapy manufacturing perspective, assay development and product characterization activities need to start concurrently with the process development and optimization activities. In this respect, it is important to take a systematic approach towards product characterization and quality by focusing on identifying the Critical

Quality Attributes (CQA) of the process and understanding their relationship with the critical materials attributes (CMA) and critical process parameters (CPP). After establishing such a relationship, the focus will be ensuring the quality of the final product is based on safety and efficacy. Depending on the phase of application, the scope of bioassay activities and product characterization will depend on whether an analytical method is needed as part of the final product release methodology (or in-process control assay), or a strategy to gather more information about the key steps of the manufacturing process or final product (i.e. For Information Only [FIO]). For the In-Process Control and Product release assays, it would be important to optimize and qualify (or even validate) the assays depending on the phase of application. For FIO assays, having an optimized and reliable assay would be sufficient. Once sufficient information is gathered based on the FIO assays, the specification and release criteria can be defined and such assays can be qualified in later stages of clinical trials.

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One of the major challenges in developing assays for cell and gene therapy products is cell characterization, not only at the genomic level and the protein expression level but also based on the functionality. The mechanism of action of a cell therapy product could be very complex and it may be difficult to establish a direct correlation with the clinical indication. This is mostly due to the fact that usually the functionality is triggered by a combination of cells, proteins, and immune function. Moreover, it is important to develop assays that can quantitatively measure cellular characteristics after setting the specifications. For instance, we proposed to use some of the common assays used in generation of human induced pluripotent stem cells (e.g. evaluation of human iPSC colony morphology, plating efficiency of iPSCs post-thaw using alkaline phosphatase (AP) staining, and embryoid body (EB) formation) as FIO characterization assays due to the challenges with respect to the qualification of these assays [1]. But, perhaps the most challenging task is to develop a process with limited knowledge of the final product. It is very difficult to come up with a final manufacturing process with appropriate controls and tools sensitive enough to monitor the changes and improve the process.

Q You recently published an article recommending additional characterization steps to further define your product. What would be the rationale for a cell and gene therapy company implementing these additional tests?

We started defining the scope of assay development activities and product quality characteristics at the early stages of process development. As mentioned earlier, it is critical to establish a relationship

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between CQA and CPP at the early stages of the process development activities in an attempt to develop a robust and GMP compliant manufacturing process. For the iPSC manufacturing process, it was critical to establish appropriate final product release testing by considering identity, safety, purity, and viability of the final products. In the absence of specific guidelines for characterization of iPSCs, we classified the testing methods as release assays or FIO assays based on the criticality of the assay (i.e. indicating safety, identity, or purity) (Table 1). Considering that iPSCs are mainly input material (or intermediate materials developed from starting tissue such as cord blood $CD34^+$ cells or peripheral blood mononuclear cells) to make fully differentiated cells, we reasoned that no animal tests would be required at the iPSC stage and that the criteria for release of the final product and use for further manufacturing process could be established by *in vitro* differentiation assays and agreed upon quality control (QC) criteria for pluripotency. As with other products that may be used for autologous or allogeneic manufacture, we assumed that the functional characterization and equivalency of the end product with any necessary in vivo or human studies would occur on the final manufactured product. The iPSC lines manufactured by Lonza will be used by a number of individuals or organizations (after gaining access through an agreement with the National Institutes of Health) and utilized to generate a number of different functional products, which will most likely be produced based on different downstream differentiation process and different companies. Therefore, we rationalized the need for additional characterization assays and generation of a database to monitor changes in the cells in culture. In our characterization paper [2], we describe the detailed characterization of two cGMP-compatible iPSC lines using whole genome sequencing (WGS), array-based analysis and aCGH SNP analysis. Our goal was to provide data to the end users to determine which subset of tests will be required for on-going monitoring, how such tests should be used to evaluate the use of subclones for preclinical studies or cell therapy, and how comparability between manufacturing sites needs to be established.

Q With many diverse cell types being utilized within the industry does this mean there is very little opportunity for 'universal' assays to be developed?

The main cell characterization challenges for cell and gene therapy applications are demonstrating safety and efficacy of the final products. With respect to the efficacy, different cell therapy products (including CAR-T, iPSC derived functional cells, or tissue specific stem cells) carry unique functional features and need to be tested with specific potency assays. Also, these products predominately grow in custom media, which results in unique matrices for each cell therapy product. Other assays such as flow cytometry are used for different applications (for instance, identity and purity) using different number and type of markers (intracellular versus extracellular markers). Therefore, it is necessary to develop specific characterization, potency, purity, identity and residual

TABLE 1

Assays used to characterize iPSC lines.

Assay release				
Assay	**Objective**	**Evaluation Criteria**	**Category**	**Tested iPSC Line**
Pluripotency Markers	Identity & Purity	SSEA-4 >70%, Tra-1-60 >70%, Tra-1-81 >70%, Oct3/4 >70%; Purity: CD34 <5%	Release assay	All lines
Karyotype Analysis	Safety	46, XX or 46, XY	Release assay	All Lines
Mycoplasma Testing	Safety	Negative	Release assay	All Lines
Sterility Testing	Safety	Negative	Release assay	All Lines
Endotoxin Testing	Safety	Standard QC release (<0.5 EU/ml)	Release assay	All lines
Vector Clearance	Safety	No trace of episomal plasmid DNA detected	Release assay	All lines
STR Genotyping	Purity & Identity	STR Profile of starting population and iPSC line are identical	Release assay	Al Lines
Cell Count & Viability	Viability	% viability >50; minimum cell number/vial	Release Assay	All Lines
Viral Panel Testing	Safety	Standard MCB Release Panel	Release Assay	LiPSC-GR1.1
Characterization assays				
EB Formation	Identity & Potency	Detection of at least one marker per germ layer	FIO*	All lines
Gene Array Analysis	Identity	Clustering with established hPSCs	FIO*	All Lines
Colony morphology	Identity & Purity	Characteristic morphology of culture/colonies; lack of spontaneously differentiated cells	FIO*	All lines
Post-thaw Plating	Thawing Efficiency & Viability	20+ colonies / vial (after 7 days or 50% confluency)	FIO*	All Lines
HLA Typing	Identity	HLA-A, B, C, DRB1 and DQB1 Type	FIO*	All lines
CGH+SNP microarray	Identity	Amplifications and/ or deletions of specific genes	FIO*	LiPSC-GR1.1 and ER2.2
Whole Genome Sequencing	Identity	HiSeq X Human Whole Genome Sequence	FIO*	LiPSC-GR1.1 and ER2.2

Table 1 summarizes the tests that were performed on the three engineering run lines and the two cGMP lines (all). Note that the three engineering lines were generated at different times from the same donor sample (Female), while the two cGMP lines were generated from a different donor (Male). Adapted from [2]. * FIO: For information only. ER stands for engineering run and GR stands for GMP run.

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safety assays for each product. This makes it difficult to develop a universal approach regarding assay development for cell and gene therapy products. However, some aspects of assay development activities can be shared between different cell therapy products, in particular standard safety assays such as sterility, mycoplasma testing, karyotype, and viability. Moreover, standardization of assay development activities would be crucial to increase the efficiency of assay development activities. By applying standard procedures, the assay optimization and qualification activities can be designed based on specific targets after carefully implementing appropriate controls, reference standards, and performing a set of activities designed to reach the goal in an efficient way.

Q You work with a diverse range of clients in the sector – do you get a sense that cell and gene companies understand what assays are required for their products?

The level of regulatory knowledge and experience varies amongst different companies or institutions engaged in cell and gene therapy applications. As cell and gene therapy is gaining more and more traction in the field of biomedical science, the awareness around cell characterization and assay requirements for different CG products are being constantly updated and emphasized. This experience and knowledge largely depends on the field of application. For instance, the use of iPSCs for cell therapy applications is still in a nascent stage and regulatory and quality requirements are still evolving. Therefore, the clients involved in using iPSC products are facing the challenges associated with this young field. Some clients are taking a more conservative approach by considering a wide range of assays to ensure the quality of the final products. The key steps to achieve product characterization goals for cell and gene therapy clients are to (1) engage in early communication with the regulatory agencies to gain proper regulatory guidance, and (2) work with experienced custom manufacturing organizations that can guide them through careful selection of assays (to address safety and efficacy characteristics of the products) and apply relevant specifications and criteria to assure the quality of their products. We encourage our clients to establish early and frequent communication with the regulatory agency while applying our extensive custom manufacturing knowledge to completely understand the process and assay needs and start an assessment process that involves development of a process map detailing the critical process parameters, critical quality attributes, process controls, decision points, mile stones with deliverables and timelines. This enables us to prioritize the assay development activities and develop assay development road maps, which are married to the process development plan that fits into the overall client timeline from IND submission process, start and completion of specific phase of clinical trial, or commercialization.

Q The pace of developments in the field is quite staggering – is assay development mirroring that pace?

While cell and gene therapy is quickly growing, the field still needs to develop appropriate tools to fully understand the function of the cell therapy products. The characterization tools primarily being used are standard safety tests, flow cytometry to study surface and intercellular proteins, molecular biology methods such as PCR and genomic studies to study gene expression levels and to ascertain the safety, ELISA to estimate whether the cells secrets the appropriate cytokines and chemokine to induce autocrine signalling, paracrine signalling and endocrine signalling as immunomodulating agents. It's quite a challenging task to understand the clinically relevant cell population, confirm such cell population exists, or detect the level and significance of impurities included in the final product. Therefore, the main bottlenecks in the assay development field are having access to reliable tools to demonstrate the functionality of the true clinically relevant population of cells and having sensitive tools to exhibit the safety of the final drug products. Moreover, there is a need to harmonize the pace of the growing field of cell and gene therapy with regulatory guidance to apply the best assay development practices. This is particularly true for the new cell therapy applications such as iPSC derived products with limited clinical trials while investigators are still discovering the full clinical potential of this exciting cell type.

Q With the utilization of new biological assays, such as whole genome sequencing, how might they impact the field in the next few years?

As discussed earlier, it is crucial to develop relevant test methods to meet regulatory requirements as part of a product release strategy and develop FIO tests to gather more information around the final product or to understand the significance of process changes. However, it is important to expend the list of FIO assay characterization and incorporate some subset of assays (such as whole genome sequencing) into a routine testing process, particularly for the pluripotent stem cells that are mainly used as starting material for further manufacturing of a variety of cell therapy products. As demonstrated in our recent publication [2], we believe these types of tests can serve important practical purposes. The cells are intrinsically variable and can change during the manufacturing process and after implantation as they respond to the environment. To address the comparability or equivalency of the products developed from these starting materials, it is critical to understand the factors underlying biological variability. New biological assays such as WGS can provide data in an unbiased way that if collected in a database over time allow us to infer the critical parameters that need to be monitored. Whole genome sequencing can be

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used to verify if long term cell culturing and passaging has altered the genetic integrity of the cell lines generated and to verify any contamination that may arise due to certain laboratory practices. In our publication, we have also suggested that a combination of WGS and SNP-CHIP analysis can be used to provide data on the state of the starting material and serve as a reference to any changes that occur in this population over time. The WGS provides a comprehensive source of information with immediate utility in providing a high-resolution map of key genes and variation between individuals that may have predictive value. Moreover, we have shown that WGS can provide significant additional value when used to infer minor blood group antigens and high-resolution HLA typing. New assays such as WGS can overcome the current limitations with the sensitivity of analytical methods and can serve as an unlimited source of information to determine the biological utility of the final cell and gene therapy products.

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