COMMENTARY: A guide for potency assay development of cell-based product candidates

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A RELEVANT AND robust potency assay is not only a regulatory requirement, but it is also the basis for producing and delivering a product that works, and is therefore one of the most important and challenging issues when developing and testing cell based therapies.

The potency assay plays a key role in determining the quality of biological products including cellular therapy products (CTPs). As defined in the U.S. Code of Federal Regulations (21 CFR 600.3), the potency is the specific ability or capacity of the product to affect a given result. The potency assay is a quantitative test that confirms the therapeutic product provides a particular response at a certain dose. Since a mechanism of action (MoA) refers to the specific interaction through which the CTP produces its pharmacological effect, it is ideal that the potency assay will represent the product's known or intended MoA.

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Throughout the drug discovery and development process, new data is accumulated on the product candidate's MoA. Thus, a potency assay used in early stages of product development may be deemed non optimal in later stages of the development, as a deeper understanding of the MoA for a specific therapy is gained. For example, in a proposed anti-inflammatory potency assay for a specific product candidate with what is initially believed to be an anti-inflammatory, MoA may require an alternative, non-anti-inflammatory potency assay as additional data is accumulated from *in-vitro* and *in-vivo* studies, suggesting a new MoA product candidate. As a result, an initial potency assay may be replaced. It is also important to note that during early product development stages, a potency assay may be developed to target the most critical biological activity of the product candidate known at this time, while in later stages, additional relevant potency assays may be added.

Stages in potency assay development

The development of a potency assay for cellular therapy products entails many steps of innovations and discoveries, while keeping in mind the end user and the requirements of the regulatory authorities.

The first step in developing a potency assay for a specific cellular therapy product is to understand the biological basis of the disease(s) that the product aims to treat, as well as having a good grasp of the product's MoA. For example, if a cellular therapy product is hypothesized to elicit a pro-angiogenic response which may be mediated through pro-angiogenic cytokines, the specific pro-angiogenic pathways and relevant factors should be identified and based on those scientific results, a potential potency assay could be developed.

The second step in the assay development should be the convenience of the assay to the end user. Since potency assays are performed as routine quality control (QC) tests, the analysis and quantification of the developed assay should be as simple as possible for the person performing the analysis. *In-vivo* tests as a potency assay should be avoided, as these are too complicated, time-consuming and expensive. It is also recommended for cellular therapy product not to use single protein detection for a potency assay, as cells are likely to mediate their therapeutic function via multifactorial MoAs. Therefore, potency assay should on one hand reflect *the in-vivo* process, and on the other hand, should be able to easily and routinely performed by QC personnel.

In the early stages of product development, the potency assay results may not have defined acceptance criteria, and may be collected and documented for information purposes only. However, during clinical development, as part of the final stage in potency assay development, regulatory authorities expect manufacturers to define the potency assay acceptance criteria, particularly prior to the initiation of pivotal Phase III clinical trials. The acceptance criteria for the assay should be based on accumulated data collected from assays performed during all phases of product development and clinical trials, and should be based on a correlation with efficacy as determined in an *in-vivo* test.

To obtain a biologics license, a validated potency assay with defined acceptance criteria must be described and justified in the biologics license application (BLA). Only a validated assay with defined acceptance criteria can be used, which assures manufacturing consistency. Without such parameters, there is no certainty that patients receiving the product candidate will get a consistent potent cellular therapy product.

Potency assay as a tool

In addition to measuring a cellular therapy product's activity, a potency assay is also used as a tool to test the comparability between lots, which were manufactured either following upgrades or as a result of changes to the manufacturing process. Manufacturing process changes may include changes in harvesting procedure, in final product filling or when establishing a new manufacturing line in a new facility. Such upgrades or changes may occur during product development and clinical trials. Therefore, the potency assay has an important role in comparing the products manufactured before and after any upgrades or changes.

A potency assay is also a central tool to test product stability, and therefore should be included in all stability programs. Although viability/recovery of the cells may indicate the stability of the product, viable cells may lose their biological activity during storage. Only cells shown to be potent post-storage can be used.

Potency assay considerations

When developing potency assays, companies have to consider and address several fundamental issues:

- CTP's MoA is a usually multifactorial, was an appropriate assay chosen?
- Is the assay robust and stable?
- For *in-vitro* cell-based assays, does the heterogeneity of the cells used in the assay undermine the stability of the assay?
- What are the proper positive and negative controls?
- What is the proper reference sample?

A cell-based potency assay may have several factors that cause assay variability. Therefore, the sources for assay variability should be considered and limited as much as possible. To this end, critical reagents should be qualified and calibrated, only qualified equipment should be used and only trained analysts should perform the assay. Elaborated standard operating procedures (SOPs) should be written and be used and controls should be included in the assay.

Since the potency assay is critical in assuring the quality and consistency of the product, but must eliminate inherent variability in the test system, a relative potency and not absolute potency should be specified. The relative potency is calculated by comparing the biological activity of the tested sample to a reference standard. The reference standard should be prepared by using the same manufacturing process as the cell-based product.

Several factors should be taken into account while conducting an *in-vitro* cell-based assay: the cells used in the assay should be characterized and banked before being used for routine testing by QC. System suitability consists of prespecified criteria by which the validity of the assay is assessed, and should be integrated in each run of the assay to ensure the quality of the assay results. Outliers, that may be random events, should be predefined and omitted before relative potency analysis.

Most cell-based assays are performed using a cell culture plate. Complete randomization or at least plate layout of samples is the best approach to minimize plate effects.

Although regulatory authorities do not require a fully validated potency assay until the end of pivotal Phase III clinical trials, it is important that the development of an assay be initiated early during the development program to have enough time for the validation parameters. It is expected that accuracy, sensitivity, specificity, precision (repeatability, intermediate precision), linearity and range, system suitability and robustness will be established in order to achieve validated potency assay in the future. ■

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CELEBRATE

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demonstration of stable isotopes for a non-radioactive element. I'd love to get his reaction to the mass resolution achieved today and to using the mass spectrometer for explicating the mysteries of biology. The year 1897 was coincidently the year Thomson established cathode rays as electrons and the chemists at Bayer began exploring acetylated salicylic acid as an improved treatment for pain. It took a while for the drug industry to learn to love mass spectrometry after the petroleum industry showed us the way in the 1950s.

Indiana is blessed with three of the leading academic research centers for analytical chemistry at Indiana University, Notre Dame and Purdue. These three centers supply a significant percentage of the analytical scientists in the country. Purdue has done so for over a century. In fact, our very first chemistry professor, Harvey Wiley, worked with Teddy Roosevelt to establish the U.S. Food and Drug Administration (FDA). The FDA is charged with keeping our food, drugs, medical devices and diagnostics trustworthy—no small task. Our Indiana research universities excel at developing novel instrumentation in chromatography, laser spectroscopies, electrochemical sensors, immunoassays, electrophoresis, microfluidics, ultrasound imaging and nuclear magnetic resonance. These instruments enable the data that leads to an understanding of how things work. Medicine, astronomy, agriculture and food, biology, energy and the forensic and environmental sciences all advance as instrumentation becomes better, faster and more economic. During my engagement with this field, we have reduced both the size of what we can examine and the concentrations of substances therein, each by a millionfold.

The Dreyfus Foundation provided an excuse to celebrate one of the most important tools of physics, chemistry and biology. Mass spectrometry is little more than a century old. It has accelerated as a tool to study complex mixtures over the last 20 years, advancing in no small measure to the contributions of Cooks, his students and his academic colleagues here in Indiana. Purdue University, Notre Dame University and Indiana University have multiple research groups further advancing mass spectrometry fundamentals, instrumentation and accessories.

While we celebrated September as Mass Spectrometry Month in Indiana, our state has long contributed very broadly to the analytical chemistry tools that virtually every reader of *DDNews* depends on for evidence based translational science. I'll drink to that. Red—no bubbles, please. Cheers! ■

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