# Navigating Challenges in Cell Therapy Potency Assays

## Developing cell-based potency assays for cell therapies requires meticulous coordination.

WEIHONG WANG

Cell-based therapeutics are defined as "articles containing or consisting of human cells or tissues that are intended for implantation, transplantation, infusion, or transfer into a human recipient" as per *Code of Federal Regulations* Title 21, Part 1271.3(d) (1). With their unique mechanism of action, cell therapy products hold the potential to treat many diseases that are refractory to current remedies in therapeutic areas such as regenerative medicine, immunotherapy, and cancer therapy. Technological advancements and the approval and successful commercialization of several chimeric antigen receptor T cell (CAR-T) products since 2017 have promoted exponential expansion of the cell therapy landscape over the past several years. The global market size is estimated to increase from $9.5 billion in 2021 to $23 billion in 2028 (2).

### IMPORTANCE OF BIOASSAYS

As with all biopharmaceuticals, thorough characterization and a robust lot-release testing program are required to ensure product safety and efficacy of novel cell-based therapeutics. The reliable assessment of product potency, a critical quality attribute (CQA), is of particular importance. To this endeavor, bioassays that can measure relevant biological responses and/or characteristics in accordance with a product's expected mechanism of action serve a significant role.

*In-vivo*, animal-based bioassays, when appropriately developed, can be more relevant to clinical response than *in-vitro* bioassays but often suffer from higher inherent variability and lengthy assay time. In comparison, *in-vitro* cell-based assays tend to offer more robust assay performance with less intrinsic variability and faster turnaround of results. In addition to the general effort of adopting the principal of three Rs (replacement, reduction, and refinement), it is also recognized that for most cell-based therapeutics, a relevant *in-vivo* assay for potency measurement is understandably often not possible due to the fact that test samples are live human cells. Therefore, in this article the author focuses on discussing the unique challenges of developing and implementing *in-vitro* cell-based assays for the potency measurement of cell therapy products.

**WEIHONG WANG** is technology development director at Eurofins BioPharma Product Testing.

## THE CELL THERAPY CHALLENGE

Compared to protein therapeutics, cell therapy products come in a much wider range of diversity and complexity. With the large panel of analytical tools available for protein products being non-applicable, potency assessment becomes the focal point and the most challenging aspect of product characterization of cell therapeutics. While there are simpler cell therapy products for which measuring cell viability and specific cell surface markers may be sufficient as potency assessment, the majority of cell therapeutics require significant potency assay development efforts.

Potency assays first and foremost need to reflect a product's mechanism of action. In the case of cell-based therapeutics, there are often multiple modes of action; and in many cases, not all of these modes are completely understood/delineated. Accordingly, it is crucial that potency assay development focuses on carefully selecting the appropriate assay(s) that is/are reflective of key mechanism(s) of action, which naturally leads to a "matrix approach". For example, cytokine release and antigen-specific cell killing are considered the most relevant modes of action for CAR-T products. Correspondingly, functional tests measuring these responses, in conjunction with assays for cell viability, transgene expression copy number, and the phenotypical characterization of cell surface receptors and CAR expression, make up a typical CAR-T potency testing matrix.

It is worth noting that additional assays are often developed and used to more thoroughly characterize the product and to assist process development during early stages of clinical development. Failure to develop an adequate assay matrix to address complex mechanisms of action(s) may delay product licensure, as in the case of lifileucel, an autologous tumor infiltrating lymphocyte (TIL) therapy, for which the sponsor's testing scheme, with a single potency assay, was considered inadequate and rejected by FDA (3). Additional assay development effort was subsequently undertaken by the sponsor and a test matrix was proposed and implemented, including a functional cell co-culture assay, to measure potency with multiple aspects of the product. In some cases, direct functional assays may suffer from difficulties, such as lengthy assay time and poor performance, that prevent them from serving as quality control (QC) methods. In such cases, upon discussions with regulatory agencies and with sufficient justification, surrogate assays may be utilized for release testing; however, a functional assay should be developed as a characterization tool, and correlation between the two methods should be demonstrated.

The complex nature of cell therapy products presents additional practical challenges for potency assay development and implementation. These may include inherent product variability due to starting materials (donor cells), limited material available for testing, the lack of reference standard and an urgency for quick product release. For protein therapeutics, sample potency is measured against a well-characterized reference standard and expressed as relative potency calculated from comparing the median effective concentration (EC50) or half-maximal inhibitory concentration (IC50) of the dose response curves. In the case of cell therapeutics, however, a true reference standard often cannot be generated, especially in individualized therapies.

In the absence of a reference standard, appropriate assay control(s) should be identified and implemented. Ideally, a significant amount of material generated within the same manufacturing process that behaves similarly to the test sample should be implemented as an assay control, along with other controls that help assure acceptable assay performance. These assay controls should be thoroughly characterized and are of great value in long-term assay maintenance and dissecting assay variability from product variability, which, in combination with other considerations, can provide critical information to help and justify setting product specifications. The reported potency results, even if not possible to be directly compared to a reference standard and expressed as relative potency, should still be quantitative wherever possible.

Another practical challenge for cell therapy products is often the need to release the product quickly. The logistics to ensure a quick turnaround time to generate test results requires efficient coordination between multiple parties involved. It becomes a particularly important consideration when the sponsor works with contract manufacturing and testing organizations. A streamlined, expedited process from sample shipment/receipt, laboratory testing, to final release of certificate of analysis should be well established and tested for potential roadblocks. In rare cases, the short shelf life of a product may warrant an abbreviated release test panel, which may be granted by regulatory agencies on a case-by-case basis. For example, products may be released after assessing key phenotypical biomarkers while awaiting results from lengthy functional bioassays. In this case, the justification for a simplified conditional release test procedure should be clearly described and explained. More importantly, the positive correlation between phenotypical assessment and functional assays should be established well in advance during product development and process validation. Notably, accessibility to this pathway highlights the importance of adopting the "matrix approach" for potency assessment, as discussed in the previous paragraph.

## THE NEED FOR STRATEGY

In general, development and implementation of potency bioassays is an ever-evolving process, and it is particularly true for cell therapy products. Development of relevant, quantitative, and robust QC potency methods has proven to be challenging and time consuming. Typically, sponsors are

expected to "initiate potency assay development by way of product characterization during preclinical and early clinical investigations to obtain as much product information as possible" as recommended in FDA's guidance for industry on *Potency Tests for Cellular and Gene Therapy Products* (4). Over the course of product development, potency testing strategy is refined, leading to a partition between methods intended for lot release versus characterization based on both scientific and practical considerations. Lot-release potency assay(s) should be fully validated, and assay and sample acceptance criteria tightened in preparation of the biologics license application. Once validated and deployed for routine testing, continuous assay monitoring and maintenance is necessary, especially given the assay complexity and the implementation of multiple controls.

The bridging between different lots of controls and other critical reagents needs to be planned well in advance and guided with a clearly defined testing scheme and criteria. In the case where lot release testing is performed in a laboratory different from where it was originally developed, method transfer may post significant challenges. Expertise in some of the highly specialized assays may be difficult to source.

Some assays, such as flow cytometry methods, can present unusual difficulties due to highly customizable instruments and manual input for data analysis. Because the intensity of the fluorescent signal is influenced by the assay-specific instrument settings, data analysis and even acceptance criteria may need to be adjusted, which in turn can lead to the need for a more substantial comparability study. Therefore, a gap analysis should be performed to guide the selection of a compatible partner lab and the implementation of a sufficient training program.

Ideally, the same instruments and filter configurations are utilized and cross standardized between the originating and receiving laboratories. Advanced planning that allows co-validation may allow better resource utilization and an expedited timeline. In the case where substantial modifications are introduced to the methods due to instrument differences, a full validation by the receiving laboratory coupled with a comparability study may be necessary.

## ADVANCING CLINICAL DEVELOPMENT

The excitement around the potential of cell therapeutics has fueled the race for developing the next breakthrough therapy. These complex biological products can present unique technical and regulatory obstacles with respect to potency bioassays as discussed. Initiating potency assay development early on allows sponsors to gain useful information on multiple aspects of their products' function so that a sufficient and practical lot release and stability testing program can be developed that are both scientifically sound and regulatory compliant.

Continuous monitoring and sufficient control of potency bioassay performance is of paramount importance to ensure accurate potency measurement and meaningful interpretation of clinical results. Challenges such as the lack of relevant reference standards, need for rapid product release, method transferability, and a requirement for specialized assay expertise can be mitigated through careful planning and close communications between all stakeholders.

Given the still-emerging regulatory guidance in this new field, discussions with regulatory authorities should be initiated early on to seek necessary advice whenever difficulties in potency testing are observed to prevent delay in the advancement of clinical development.

## REFERENCES


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