Bioassay Challenges

Bioassays are used to determine the critical quality attribute (CQA) bioactivity/potency of biopharmaceutical products. They shall reflect the mechanism of action (MoA), therefore bioassays are individual for each product but still need to fulfill all regulatory requirements.

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General Considerations for Bioactivity Testing

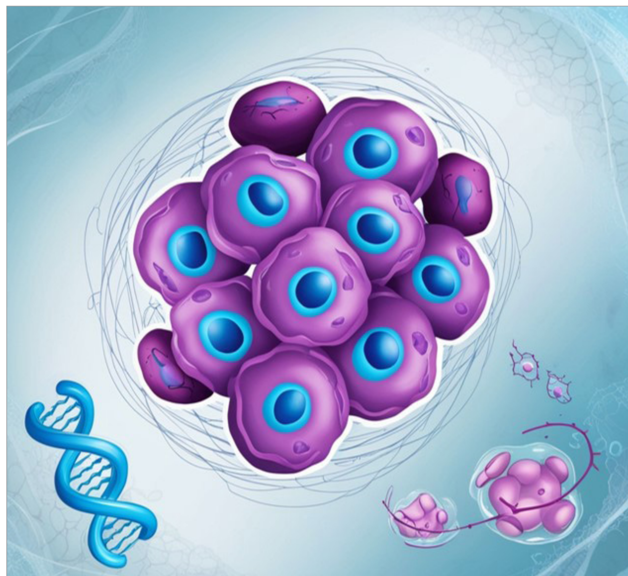
On the one hand, bioassays are challenging and often not easy to establish; on the other hand, they are exciting from a scientific point of view because new products with new mechanisms of action and requirements for assay development are constantly emerging. Most recently, the product group of ATMPs has been added, which in some cases differ significantly from biotherapeutics such as monoclonal antibodies due to their properties, targets, and mechanisms of action.

Due to their nature, cell-based bioassays are usually more variable than classical analytical and biophysical methods because they work with cells, tissues or even living organisms. Nevertheless, they must have sufficient robustness, precision and specificity for the purpose of lot release and stability testing.

Regulatory authorities typically agree with a phase-appropriate approach to bioassay development over the life cycle of a product. Less complex and generally easier to set up methods for activity determination, such as cell-free binding assays with different readouts like ELISA or SPR, are acceptable for the early phase. This allows developers to gain time for the development of more complex bioassays (usually cell-based). Nevertheless, it is strongly recommended not to start too late with the development of the relevant cell-based assay, as the development often takes more time than initially thought and the assay also provides valuable information for understanding the impacts of the product and of processes on the assay. In addition, the correlation between the data from the cell-free assay from the early phase and the cell-based MoA-reflecting assay can be considered at an earlier point in time. Cell-based bioassays are typically qualified and monitored over the time span of clinical development to improve understanding of the critical steps and components of the assay.

What's the best strategy for therapeutics that do have more than one MoA which is relevant to the patient, e.g. in case of monoclonals? Most of the products are capable to induce parts or all classical immunological pathways of the innate immune response in the patient, that are known from the first generation of therapeutic antibodies, namely ADCC, ADCP and CDC whereas the newer ones in addition target specific signaling pathways, e.g. the immune checkpoints from the adaptive immune response. Another example are bispecific antibodies which address more than one cellular target, which often rises the need for development of individual assays targeting the effects of each of the targets. In addition, where multiple MoAs exist, a combination assay that covers all MoAs in a single assay will be requested. If such a method fulfills the requirements of guideline-compliant method validation and stability indicating properties most likely it will be the method of choice for QC purposes. The minimum requirement for switching from one assay format to another is a bridging study, that confirms accuracy, precision and stability indicating properties, e.g., by using degraded samples.

A frequently asked question is whether a MoA-reflecting, cell-based assay is required for approval in every case for biopharmaceuticals and ATMPs. The question cannot be answered with a simple yes or no. In cases where the mechanism of action is binding to the target, a direct or competitive binding assay will most likely be sufficient for the authorities. Even in cases where a cell-based MoA-reflecting bioassay would be difficult to validate because the variability is too high, it may be possible, for example, to get approval with a binding assay to test the CQA bioactivity. The cell-based assay then migrates to the characterization panel.



Risk assessment tools and life cycle approaches are becoming increasingly important for developing and maintaining phase-appropriate biological activity tests. Statistics are also gaining importance beyond the actual evaluation of relative activity, e.g. in the form of design of experiments (DOE) and trending. The aim is to have a validated method that is robust, feasible and provides trustworthy results.

To meet expectations, the optimal control of critical reagents and the cells used in the assay, the use of automation and the inclusion of quality by design (QbD) and, if appropriate, artificial intelligence are essential. Automation improves reproducibility and reduces the risk of human error, while the use of assay ready cells, for example, significantly reduces the variability in the process caused by continuous cell culture. Another advantage of assay-ready cells is improved flexibility to start testing as needed. Besides the ICH Q2 (R2) / ICH Q14 strong recommendation to use QbD for assay development and robustness studies needed for validation it is understood as a great tool to optimize assay conditions starting from early method development, e.g. to optimize incubation times, cell density, tissue culture times, optimize buffers and so on. The biggest advantage of QbD over one factor at a time assay (ofat) optimizations is that interactions of factors can be detected at an early stage and taken into account in the assay design.

Nevertheless, a comprehensive understanding of the underlying biology is essential and still gains importance for development, as the mechanistic principles and therefore requirements for classic protein therapeutics, cell and gene therapy products and vaccines can be very different.

The regulatory requirements also pose a challenge, as both the strict specifications of general guidelines, such as those for the development and validation of analytical procedures and the statistics for the evaluation, must be met, as well as specific local guidelines, such as those for cell and gene therapy products. At the same time, due to the very individual nature of the mechanisms of action, there are very few monographs and, if at all, only very limited international reference and control materials.

Biosimilarity and Interchangeability

In the context of protein therapeutics, the assessment of biosimilarity still involves several challenges, especially for bioassays. On the one hand, similarity must be established with often more than one MoA-reflecting bioassay, and on the other hand, regulatory guidelines for assessing biosimilarity are limited. The biological activity of the biosimilar needs to match the biological activity of the reference product. The concept of interchangeability goes beyond biosimilarity, as not only analytical data are required. Interchangeability studies are intended to demonstrate that the biosimilar can replace the reference product without any additional risk in terms of safety or efficacy compared to the use of the reference product alone. Therefore, for interchangeability usually additional clinical work is required to assess the impact of switching between the biosimilar and the reference product on safety and efficacy outcomes. Clinical studies for interchangeability are specifically designed to assess the impact of switching between the biosimilar and the reference product on safety and efficacy outcomes.

Bioactivity Testing for ATMPs

Particularly for cell and gene therapy products, some of which are even produced autologously, a matrix approach over the life cycle with selection of suitable test procedures to ensure maximum patient safety, but without unnecessarily complicating the release of individual batches and meeting all regulatory requirements, is gaining acceptance.

The matrix approach suggests an incremental development of potency tests. For example, starting with *in vivo* models in the discovery and preclinical phase to show proof of concept, evolving to continuously optimized *in vitro* testing from the early phases of clinical development for a more robust assay for validation. Typically, the transcriptional, translational and (if applicable) functional level are addressed. Often the transcriptional level is sufficient for release in early phases, whereas for later phase functional readouts are required.

The inclusion of orthogonal methods to guarantee the requirements for reproducibility, accuracy and robustness

is also often useful. The acceptance criteria should be selected in such a way that a minimum level of efficacy and a maximum level of safety are achieved.

Potency assays are crucial for the evaluation of the biological activity of cell and gene therapeutics. In the best case, bioactivity is associated with efficacy in patients. However, the development and validation of potency assays for cell and gene therapeutics is associated with challenges.

Cell and gene therapeutics are often very complex and heterogeneous. Cell therapies consist of living cells that can have a variety of functions, while gene therapies introduce genetic materials that can trigger different effects in the patient which shall be reflected in the target cells of the MoA reflective bioassay. This complexity often makes it difficult to develop a single potency assay that covers all relevant aspects of product function. Particularly in the early phase, the mechanism or mechanisms (MoAs) of action are not yet fully understood, which makes the selection and development of suitable potency assays difficult.

Furthermore the biological variability in ATMPs is in most cases significantly higher than in classical protein therapeutics, e.g.: between cell batches. This variability complicates the establishment of potency assays, as on the one hand they should provide reliable results within system suitability criteria (SSC) and acceptance criteria, but on the other hand they must also tolerate the variability of the product as long as this does not negatively affect safety and efficacy for the patient.

Due to their often complex mechanisms of action, ATMPs require a deep understanding of the relevant signalling pathways and biological processes relevant to the product. It is therefore important to acquire this knowledge at an early stage and, if necessary, to iteratively develop the strategy for potency determination.

While in vitro tests are often more practical and cost-effective, they do not always reflect the complex interactions that occur in a living organism. It is therefore a challenge to develop in vitro potency assays that accurately reflect in vivo conditions and effects. However, both FDA and EMA now require compliance with 3Rs for release assays, so there is no alternative to implementation.

Regulatory requirements for potency assays for cell and gene therapeutics are stringent and sometimes vary by country. The assays must not only be scientifically sound and reproducible, but also meet the requirements of the regulatory authorities, which means additional complexity and effort. ATMPs do not fall under mutual recognition, so care must also be taken to ensure that they are approved in the right country.

The development of potency assays often requires specialised techniques and equipment. Readouts that never were relevant for QC of classical protein biotherapeutics are now becoming relevant, since e.g. enzyme activity after reconstitution of a gene by a gene therapy product or impedance as a readout that allows measurement of activation of receptor types including G protein coupled receptors, tyrosine kinase receptors, and some nuclear receptors now need to be taken into consideration. Establishing and validating these methods can be time-consuming and costly. In addition, the assays must be robust enough to be validated according to ICH Q2(R2) and to be routinely used in a production environment.

Both Quality by Design (QbD), as required by ICH Q14, and the use of artificial intelligence (AI) help to set up new procedures so that they are suitable for use under GMP. Furthermore, implementation of automation is an important pillar in minimising variability.

Cell and gene therapeutics are often sensitive to environmental conditions such as temperature and light. Storage and transport must be selected and validated to ensure product stability, and the functional potency assay must have sufficient “stability indicating properties” to reliably detect damage to the product. This is also part of the validation according to ICH Q2 (R2).

In summary, potency assays for cell and gene therapeutics require an integrative approach that combines scientific expertise, technical know-how and a deep understanding of regulatory requirements. Addressing these challenges is critical to ensure the safety and efficacy of these advanced therapies.

To address the increasing need for scientific and regulatory exchange, the ECA Academy, in collaboration with Concept Heidelberg and experts in the field of bioassays and potency assays, has decided to launch a European conference bringing together authorities, industry and laboratories. **The Bioassay/Potency Assay Conference at the PharmaLab Congress on 26/27 November 2024 in Neuss** offers a two-day lecture programme with corresponding opportunities for exchange and a platform to discuss current challenges and requirements with colleagues, speakers and representatives of the authorities. The current regulatory landscape will be presented as well as current case studies from industry and CRO covering the development and GMP implementation of potency assays, automation and digitalization.

For all information, please visit
www.pharmalab-congress.com/bioassays-2024