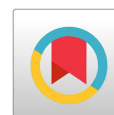


COMMENTARY

Regulatory Perspective on Bioassay Implementation Strategies for Potency Evaluation of Biopharmaceuticals



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Potency is a Critical Quality Attribute (CQA) for biopharmaceutical products and it must be appropriately measured throughout the whole clinical development journey. The development strategy of a biological assay that is able to measure potency accurately and precisely needs to be based on both regulatory and business requirements. Regulatory expectations on potency tests are detailed in international guidelines and constitute a legal requirement for the registration of the drug in the region of submission. However, the development and implementation of biological assays are known to be expensive and time-consuming. This fact opens a debate on how to balance the companies' business needs with the requests for Mode of Action (MoA)-mimicking cell-based assays to determine the potency of increasingly complex biopharmaceuticals. This article overviews the current regulatory requirements for the registration of new biological drugs in USA, Europe and Japan giving a focused perspective on bioassay development strategies.

The foundation of the International Conference of Harmonization (ICH): the first step to cost-effective drug development

During the 1980s, the increasing complexity of new biopharmaceutical products together with the challenging demands from national regulatory authorities led to a significant increase in the cost of the whole drug development process [1]. Many pharmaceutical companies struggled to cope with the much more expensive drug development processes and ended up merging or restructuring. During these times, the European Commission started to work on a single market for pharmaceutical products across the Member States of the European Union, while holding bilateral discussions with the Food and Drug Administration (FDA) in USA and with the Japanese Ministry of Health and Welfare on technical aspects related to guidelines and regulations for pharmaceuticals development [1]. In 1990, the representatives of regulatory agencies and industry associations of Europe, USA and Japan met in Brussels to define a program of harmonization on Quality, Safety and Efficacy aspects to avoid unnecessary and costly repetitions of toxicological and clinical studies. With this meeting, the International Conference of Harmonization (ICH) was officially formed [1,2]. Since then, the ICH has evolved: after twenty-five years dedicated to the development and update of ICH guidelines, its mission is now also focused on the extension of the harmonization benefits to countries that are outside the current ICH regions. To this end, in 2015, the original ICH has been reorganized as a legal entity now called International Council for Harmonization with the aim of

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ensuring that, worldwide, pharmaceuticals meet the highest safety, quality and efficacy targets whilst being produced in a resource-efficient manner [2].

All efforts made so far resulted in a set of harmonized guidelines that lay a common foundation for the design of toxicological, clinical and analytical studies. However, although ICH documents try to embrace all region-specific pharmacopoeias, the registration of biopharmaceuticals in USA, Europe and Japan still has its complexities and challenges. Therefore, when deciding on a strategy for bioassay development, all differences in region-specific guidelines should be considered.

The registration journey of biopharmaceuticals in USA, Europe and Japan

The registration process of pharmaceuticals shares a similar path in USA and Europe. In USA, any sponsor wanting to test in humans a drug that has never been previously authorized for marketing needs to file an Investigational New Drug Application (IND) to the FDA [3]. An IND document should prove that the new pharmaceutical product meets all the safety requirements for being administered to humans and that it holds a pharmacological activity worth its commercialization [4,5]. The amount and depth of chemistry, manufacturing and control (CMC) information on the biopharmaceutical product is very much dependent on the clinical phase but should always assure the adequate identification, quality, purity and strength of the drug [6]. All data collected in the IND documents and produced throughout the clinical studies of the proposed drug will eventually become part of the Biologic Licence Application (BLA) to introduce a biopharmaceutical into interstate commerce.

European guidelines for the registration of medicinal products are in line with the FDA requirements for the most part. The submission of an Investigational Medicinal Product Dossier (IMPD) is required to obtain permission for starting clinical trials in any of the European Union member states [7], [8]. As for the IND, the IMPD contains all the information regarding the quality and safety of the drug product together with up-to-date information on the clinical trials ongoing at the time of submission [7,8].

As the Investigational Medicinal Product (IMP) progresses through clinical phases, the Clinical Trial Authorization (CTA) procedure requires the sponsor to file a new or updated IMPD in each of the Member States where the clinical trial is planned to be carried out. However, following successful clinical development phases, biological drugs can undergo a centralized authorization procedure through the submission of a Market Authorization Application (MAA) to the European Medicines Agency (EMA), resulting in a single marketing authorization in all European countries [9,10].

The registration process of a biological drug in Japan requires the submission of a Clinical Trial Notification (CTN) to the Pharmaceutical and Medical Devices Agency (PMDA). This document contains information regarding the pharmaceutical product, pre-clinical data, clinical trial protocols and patients. As in USA, upon completion of the clinical trials, all obtained data are reported in an NDA, which is evaluated for approval or rejection by the Ministry of Health, Labour and Welfare (MHLW) [11]. A peculiar aspect of the drug development process in Japan is the consultation service that PMDA provides to pharmaceutical companies in order to make sure that all key Japanese regulatory requirements are fulfilled throughout the whole development process. This system allows global companies to seek advice from an assigned PMDA review team, which guides the clinical development strategy of the sponsors from the pre-clinical phase until the launch of the product into the market [11].

Despite the efforts made in the past 30 years towards a harmonized regulatory system conceived to aim at a simultaneous global drug development process, Japan still faces a delay in the submission of new drug applications compared to USA and Europe. Indeed, it is a Japanese regulatory requirement to include clinical data on the pharmacokinetics, safety and efficacy of the new drug tested on a large group of Japanese citizens. The

availability of data generated on the Japanese population should prove that the response to the new drug is not affected by ethnic diversity. This requirement initially meant that clinical data produced in other countries were not acceptable for marketing the drug in Japan and most of the time all clinical trials had to be repeated in Japanese subjects. Therefore, Phase I clinical trials would start in Japan when Phase III studies were already completed in Europe and USA [11]. This lag in the availability of new drugs between the rest of the world and Japan, referred to as 'Drug lag', became a critical issue that brought the Japanese Regulatory Authority and ICH to seek possible solutions. These discussions opened the possibility of registering in Japan clinical data obtained in foreign countries, provided that bridging studies were conducted to prove that data on pharmacokinetics, dosage and safety of the drug can be applied to the Japanese population. Today, the inclusion of Japanese individuals in multiregional clinical trials (MRCT), together with the simplification of the consultation system, has already decreased the 'Drug Lag' from 3.5 to approximately 1.5 years [11].

Bioassay development: better early than late?

The method used to determine the potency of a new biopharmaceutical is of crucial importance throughout the clinical development process. Bioassays represent the only way of establishing that the developed drug shows the intended biological activity on an *in vitro* cell-based system that mimics the clinical situation. The information gained from a well-developed potency assay is often decisive in determining lot-to-lot variability and stability of the drug in Quality Control (QC) studies, biosimilarity with another manufacturer's product, or in proving product quality and consistency after changes in the manufacturing process [12-15].

Therefore, a company should ideally design and develop a bioassay that is:

1. Mimicking of the drug MoA *in vivo*,
2. Easy and suitable to be applied in a QC environment for routine testing,
3. Able to determine the stability of the drug under a variety of stress conditions,
4. Accurate and precise enough to support the specification limits

The development of one or more assays with the above-mentioned characteristics is not trivial and often requires a big investment in terms of time and resources, which is the reason why the best bioassay development strategy is still under debate.

There are mainly two approaches adopted by companies for the development of their bioassays:

1. A progressive multi-step approach, which takes into consideration the clinical development phase of the drug, from pre-clinical and early-stage clinical development to late-stage clinical development and market approval.
2. A one-step approach, where a MoA-representative cell-based assay is developed from early clinical phases and, apart from minor improvements, maintained throughout the whole clinical development process up to the marketing phase.

Both approaches have advantages and disadvantages that each company takes into consideration when defining its development strategy, but the two main factors that dictate the choice for one or the other are time and cost.

A progressive multi-step approach entails the development of a simple binding assay (e.g. ELISA- or Surface Plasmon Resonance (SPR)-based binding assay) in early phases of clinical development. If the product progresses to late development phases, a cell-based assay that is representative of the *in vivo* MoA is developed to comply with regulatory requirements. Although this approach proves to be cost-effective in the early clinical phases, when the fate of the drug is still uncertain, it can certainly have its disadvantages if the molecule progresses to Phase II and Phase III: changing the potency test from a simple binding assay to a more complex cell-based assay along the clinical journey

requires the execution of a bridging study between the two methods to prove that the data obtained with both assays are equivalent. To do that, the two assays must be run in parallel for a certain amount of time (usually at least one year) to gather enough data on different samples and lots that will ensure the statistical significance of the equivalence evaluation. Despite all the effort, in a good number of cases, tests performed in parallel with the binding and cell-based assays show significant differences in the potency results, thereby triggering a series of investigations to understand the root cause of the discrepancies and requiring the sponsor to test all QC samples with both methods for a longer period of time.

In a one-step approach, the early development of a MoA-mimicking cell-based assay allows the sponsor to progress through clinical development stages with the same assay, thereby avoiding investing resources in bridging studies and parallel testing. Also, the increasing complexity of biopharmaceuticals requires the development of complex cell-based assays that need to be appropriately challenged, optimized and proven to be fit-for-purpose before launching the drug into the market. Leaving the development of such assays to late clinical phases might be risky and it could cost the company significant investments in resources for troubleshooting, optimization and revalidation studies.

Finally, having a cell-based assay in place from early phases proves to be of added value also during process development. Potency is a CQA that should be monitored throughout the whole process development and characterization phases to ensure that the production of a biologically active molecule is not significantly affected by changes in any of the manufacturing steps. To this end, the ideal potency test should be able to detect changes in the physicochemical properties of the molecule that are relevant to its MoA, which could occur during process development and in lot manufacturing. Being reflective of the MoA, cell-based assays are more likely to identify these changes compared to cell-free binding assays.

Based on these considerations, the earlier the development of a cell-based assay, the better. However, one should always consider that the set-up and implementation of such assays are time-consuming and expensive. Therefore, while this approach is resource-efficient in the long-term, it could prove to be inconvenient in the short term if the molecule does not progress to later stages of clinical development.

In any case, when deciding what approach to take for the development of a potency test, all regulatory requirements that are reported in the international guidelines on bioassays should be taken into consideration.

The requirements of international guidelines on potency assays

Although it is strongly recommended to start the development of a suitable, MoA-reflecting bioassay as soon as possible in the drug development process, the regulatory guidelines allow a certain degree of flexibility both in the choice and in the acceptance criteria of the potency assay during early phases of clinical development. However, as the proposed biopharmaceutical proceeds to Phase II and Phase III, it becomes mandatory to have in place a bioassay that can ensure consistent monitoring of biological activity between manufactured lots. Also, to the same purpose, the specification limits and acceptance criteria of the method should be tightened. The depth of information on the potency assay that is required to file the regulatory documents to each of the region-specific health authorities mostly follows this stage-dependent approach.

For example, when submitting in the US, an IND for Phase I clinical studies should include a brief description of the bioassay method for determining the biological activity of the investigational drug together with its proposed acceptable limits [3]. If the drug proceeds to Phase II, the sponsor should prove that the developed potency method is stability-indicating, whereas in Phase III the analysis of samples undergoing stress studies should demonstrate the capability and suitability of the proposed analytical procedures [6].

In Europe, a stage-specific approach is also applicable: according to the EMA/CHMP/

BWP/534898/2008 guideline [8], before the initiation of Phase I studies, the sponsor should be able to determine the biological activity of the investigational drug with an appropriate, reliable and qualified method. During Phase II and Phase III, the developed potency assay should be used to test samples undergoing accelerated and stress studies to better understand the IMP degradation profile and support any shelf-life extension. As a result, the method developed to determine the biological activity of the proposed investigational drug should be stability-indicating [8].

In addition, the EMA issued a document that is specific for monoclonal antibodies and related products [16]. This document explains in greater detail the information that should be provided to the authority to ensure that these products comply with the highest quality standards. A section specific for potency assays underlines that the developed method should reflect the biological activity of the biopharmaceutical in the clinical situation. To this end, the development of a binding assay is acceptable whenever the biological activity of the antibody is proven to be solely dependent on its binding/neutralising properties. Should the antibody have effector functions affecting its clinical behaviour, specific potency assays should be developed to allow a combined study of specific and effector functions-related activities. Whenever a cell-based assay that measures both activities is not available, two methods should be developed and used in combination to provide precise information on the biological activity of the drug [16].

As described in the EMA/CHMP/BWP/532517/2008 guideline, the development of complementary assays and, hence, the implementation of an assay matrix approach, is highly recommended by regulatory authorities, especially in cases where the drug has complex or multiple biological activities (e.g. receptor-blocking activity and Antibody-Dependent Cellular Cytotoxicity (ADCC)) or when the existing surrogate assay (e.g. ligand binding assay) is not fully representative of the drug's MoA [16]. Although testing samples with more than one assay can be demanding for QC labs, this approach can ultimately avoid incurring in registration problems or hard-to-fulfil post-market approval commitments.

Unlike US and EU, the requirements and expectations of PMDA related to the development of bioassays for potency testing are mainly based on conference presentations held by representatives from PMDA and Japan Pharmaceuticals Manufacturers Association or on published book chapters [11]. According to these references, PMDA requires the development of a potency assay that reflects the clinical mechanism of action in the indication for use, regardless of the clinical phase. To this end, the development of cell-based assays for potency testing is recommended as it ensures the monitoring of the biological activity of the product through a method that mimics the MoA of the drug *in vivo*. There are instances where PMDA might accept the approach of using a binding assay as potency test. In these cases, however, factors like the involvement of effector functions in the biological activity of the molecule, the availability of extensive characterization studies and broad manufacturing experience, significantly contribute to the final PMDA evaluation and decision [11].

It is worth mentioning that, unlike USA and Europe, Japan has not yet issued a guideline on the development and validation of bioassays. Only recently, a new research group funded by AMED (Japan Agency for Medical Research and Development) and formed by representatives of pharmaceutical industries, academia and regulatory agencies has been constituted to enable the efficient development of biopharmaceuticals by combining scientific and regulatory aspects [17]. In the field of bioassays, the aim of this team will be the definition of standard procedures on the calculation of relative potency, development and validation of cell-based assays and definition of system suitability criteria, in line with the corresponding EU and US pharmacopoeia [17].

The preference for a MoA-representative bioassay right from the early phases of clinical development could represent an obstacle for those companies that decide to reg-

ister their biopharmaceutical product in Japan whilst holding a progressive multi-step approach for bioassay development. Currently, most of the times this approach does not represent a problem for pharmaceutical companies because Japanese citizens are often not included in the first Phase I and Phase II clinical trials: when a sponsor decides to file a drug for Phase I clinical studies in Japan, the same biopharmaceutical is likely to be already at the end of Phase II or beginning of Phase III in EU and US. Therefore, by that time, a cell-based assay is likely to be already in place. However, as mentioned before, this 'Drug Lag' resulting in a delayed registration of new drugs in Japan compared to USA and EU has already started decreasing, mainly thanks to the inclusion of Japanese individuals in MRCTs [11]. Hence, the definition of a development approach for potency assays should take into consideration the company's registration strategy of a new drug to avoid rejections, delays, or last-minute commitments with regulatory agencies.

Conclusion and future perspectives

What is the best strategy for bioassay development? This question is at the center of a long-standing debate that sees, on one hand, the growing need for developing MoA-mimicking cell-based assays for increasingly complex molecules, and on the other hand, the high costs associated with this approach from early clinical development phases. When trying to answer this question, one should also keep in mind the characteristics of the ideal cell-based assay and the importance to have it in place as soon as possible in the clinical development journey. Therefore, how can we develop the best and most appropriate method(s) to determine the biological activity of a drug while decreasing the costs associated with its implementation? The future strategy for bioassay development should include investments both in the research for the best MoA-reflecting assay and in the identification of ways that would allow its cost-efficient implementation from early phases of clinical development. Innovative technologies and ideas, such as end-to-end automation or the introduction of Ready-to-Use cells [18], are already contributing to shaping a new concept of bioassay: from highly variable to highly reproducible, from long and complex to fast and standardized. Only by investing resources in the development of cost-efficient bioassays, companies will be able to reconcile the need for a MoA-reflecting cell-based assay from early clinical development phase with their business requirements.

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