The challenges of potency assay development for cell-based medicinal products in Europe

Potency is considered a critical quality attribute (CQA) for any biological product, and the implementation of relevant assays is often at the centre of many challenges and discussions among developers and regulators throughout product development. Potency assessment is indeed important not only for manufacturing, as a tool to assess product quality and consistency, but also for clinical development to help predict product clinical efficacy and create a link to the dose. This link is difficult to establish for biologicals in general, and for cell therapy medicinal products the challenge is even more complicated. This is because these cellular products present a high level of molecular complexity, and their modes of action (MoA) may not always be fully understood and often involve multiple pathways.

The status of knowledge on the MoA supported by literature, together with the collection of development data and the use of a risk-based approach, will constitute important elements to build and defend the potency assessment strategy throughout product development and for registration.

This article provides an overview of the EU regulatory expectations in terms of potency assessment for cell-based medicinal products (CBMPs). The retrospective analysis of the approach followed by both regulators and developers for some of the approved products illustrates how defining an incremental potency strategy early on can help alleviate certain challenges and strengthen overall product development.

Keywords
Potency assessment; Critical quality attribute (CQA); Cell-based medicinal product (CBMP); Advanced therapy medicinal product (ATMP); Mode of action (MoA); Incremental potency strategy; Chemistry, manufacturing and controls (CMC).

Abstract
Among critical quality attributes which need to be monitored during drug development, potency is considered essential for its direct link to product efficacy. Developing the relevant set of potency assays for cell-based medicinal products comes with numerous challenges due to the complex nature of the products. This paper highlights several approaches to consider when designing the potency assay strategy in the framework of current EU regulatory expectations.

Potency: Definition and evolving requirements
Potency is defined in the ICH Q6B guideline as: “The measure of the biological activity using a suitably quantitative biological assay (also called potency assay or bioassay), based on the attribute of the product which is linked to the relevant biological properties.”

Historically, the concept of potency has been applied to all types of drugs (starting with plants and chemicals) at the time when elucidation of structure was limited. Verification of potency was the only way to guarantee that the active substance would fulfill its properties when used in patients. When establishing the first Pharmacopoeia referential, the monographs made reference to “biological activity measurement”. Nowadays, chemically synthesised small molecules usually have a well-defined structure and elucidated MoA, hence the measure of strength (“content”) by a dosage method is often sufficient. This “content” approach (in mass unit) could in fact be considered as the premise of the surrogate approach to potency assessment. The potency measurement requirement has been kept for the quality control of vaccines and other biologicals, which are complex structures and for which their activity is also largely dependent on the integrity of the molecular structure. This holds true for the new class of medicinal products, defined as advanced therapy medicinal products (ATMPs) in the EU legislation.

Biological products, including ATMPs, feature much more complex molecular structures while their pharmacological activities and resulting MoA may not always be totally elucidated, as they sometimes involve multiple pathways. Content (in mass units) is therefore not a sufficient measure of the actual biological activity (later referred to as potency) and specific assays capable of detecting the functional activity of the product are required as part of overall quality assessment and quality control strategy. For most biologics, the main MoA is often at least partly defined and will help guide the design of the potency assay strategy. Challenges tend to be much greater for CBMPs, considering the intrinsic product variability, presence of cell mixtures, and extremely complex biological activities.

In the context of such complex therapies, product knowledge gathered throughout development will help refine and build piece-by-piece the relevant potency assessment strategy that may start with a simple indicator, such as a specific cell surface marker, and evolve towards more relevant and reliable quantitation of biological activity. Product understanding, elucidation of the MoA, analysis of development data and the drawing of a correlation between quality attributes and clinical outcome will all contribute to the design of the final potency measurements. It is therefore expected that potency assessment evolves throughout development before reaching the final adopted tests for any given product. In fact it took nearly 35 years to develop a suitable potency assay for the Pertussis vaccine, and more than ten years to move from animal testing to a more reliable in vitro “content assay” for insulin.

Regardless of these challenges, potency testing is seen as the cornerstone to a solid product development and relevant quality control strategy. Since it is the only quality attribute for which the test should be designed based on anticipated MoA and/or anticipated physiological function(s), the potency assay is critical to many aspects of development.

Authors
Valerie Pimpaneau, Senior Director, Voisin Consulting Life Sciences; François Gianelli, Regulatory Scientist, Voisin Consulting Life Sciences; Jean-Hugues Trouvin, Professor, Paris Descartes University, School of Pharmacy, Paris, France; Anne Dupraz Poiseau, Executive Vice President; Voisin Consulting Life Sciences, St Grégoire, France.

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It is performed not only as part of product characterisation, but also as part of release testing, to monitor stability and, ideally, to correlate with the expected efficient dose creating a link between quality criteria and efficacy. For CBMPs, processes are prone to progressively and significantly evolve in the course of development from first experimental process up to commercial process with scaling-up and technology transfer for industrialisation. In this context, the potency assay along with other analytical tools will also be key to demonstrate comparability supporting process changes and batch-to-batch consistency (see Figure 1).

The regulatory landscape and expectations regarding potency
Most available guidelines discussing quality aspects of CBMPs refer back to the ICH Q6B guideline, which describes specifications’ expectations for a biological product. However, ICH Q6B came into effect in 1999, well before the ATMP Regulation was issued. The scope was mainly for biologics with a rather complex structure (as compared to small chemical entities) but accessible to characterisation and identification of molecular features contributive to the expected pharmacological activity. The first European guideline covering quality and process changes and batch-to-batch consistency (see Figure 1).

This guideline was superseded by the currently guideline in force1 which acknowledges the challenges in characterising biological function of a CBMP. With regard to potency assessment it indicates the possibility that an in vitro assay could be based on the expression of markers that have been demonstrated to be directly or indirectly (surrogate markers) correlated to the intended biological activity of the CBMP (such as cell surface markers, activation markers, expression pattern of specific genes). It is thus acknowledged that several potency assay formats could be considered. Indeed some assays are more qualitative and describe a given quality attribute (eg, cell marker) whereas others may quantify a functional activity (eg, expression rate of a given protein). It is further specified that if the intended biological function of the product is mainly based on the capacity of the cells to secrete specific molecule(s) to repair a metabolic disorder, to promote growth, or to release a metabolite, then the potency assay needs to be based on the detection of the active molecule(s) produced, which trigger(s) the product’s in vivo biological activity. Therefore it appears that if the MoA involves a metabolic activity, it is very likely that surrogate identification markers alone will not suffice to cover potency assessment. The guideline also states: “The selection of the dose should be based on the findings obtained in the quality and the non-clinical development of the product and it should be linked with the potency of product.” This reveals an ambitious requirement to set potency assay specifications reflecting as much as possible the efficacious dose based on correlations between potency results and (non)clinical results.

Still in the field of ATMPs, the guideline on medicinal products containing genetically modified cells2 is also product-specific and indicates that potency assessment can combine several assays such as the number of genetically modified cells, the gene copy number, the expression level of the transgene and the product activity level, as shown to be efficacious in clinical studies. The presence and expression of the new gene by design in genetically modified cells allows for more targeted directions with regard to the potency assay development than unmodified cells for which potency strategy can rely on a large variety of markers and biomolecules to monitor.

Figure 1: Potency at the core of product development.
Unlike in the US, where guidelines on cell and gene therapies provide technical recommendations, including list of tests and incremental timelines, European guidelines focus more on the conceptual aspects, and aim for the requirements to be met “at the time of marketing authorisation application (MAA)”. In fact, in April 2013, during the public consultation after five years of Regulation (EC) No 1394/2007 on ATMPs, contributors from industry considered that the quality requirements should be further adapted to the specific characteristics of ATMPs and required more specific guidelines taking into consideration the specific constraints of ATMPs. In particular, feedback referred to the challenge of the development and validation of relevant potency assay(s) and required further guidance on potency testing for ATMPs making reference to FDA guidelines. 

That being said, the EMA is in fact giving more latitude to the developers with possibility to apply science and risk-based approaches to product development, allowing defining a pathway relevant to each product rather than guiding within a more rigid framework with predefined requirements. This is clearly creating an interesting opportunity to develop CMC [chemistry manufacturing and control] approaches in a fit-for-use mode rather than adhering to specific requirements, and provides an opportunity to adapt and justify the development strategy based on the specificities of each product, as long as a solid scientific rationale is provided along with supportive data. In fact, several products have received approval using the data.

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Challenges of potency assays development

Pritchett and Little summarised a list of criteria that an “ideal potency assay” would meet based on US requirements. Taking into consideration the criticality of potency assessment and the EU regulatory expectations described above, the desired characteristics of potency assays are summarised in Figure 2. It should, however, be acknowledged that none of the abovementioned guidelines set such requirements for an “ideal potency assay”. Instead, the guidelines leave to the sponsor the responsibility to elaborate the best strategy and justify it.

While some of these elements are fully applicable to biologics, it is often challenging for CBMPs to develop a potency assay meeting all of those characteristics. We can distinguish two classes of challenges linked to CBMPs when developing a potency assay:

- **Intrinsic challenges.** The first challenge is based on the difficulty of characterising the MoA, often not fully understood or linked to multiple factors that are not necessarily clearly defined (such as cell stage of differentiation, expression of cellular markers, excretion of signalling proteins, etc). In addition, each of those factors can sometimes be linked to several effector functions that may occur at different stages of the in vivo response. In theory, developing potency assays would require identification of all of the contributive elements of a CBMP, but developing assays that would reflect the complete MoA and qualify every step of the organism response is nearly impossible for those complex products.

In addition, CBMPs are often composed of multiple active cell types with potential biological activity for each of them, interferences or synergy depending on the composition of the product. As an example, during the assessment of Dendreon’s Provenge product, the up-regulation of CD54+ cell population was presented as a potency assay. However, the EMA requested further characterisation of the product, including identification of all contributive cell types, prior to accepting the proposed potency approach. Furthermore, when dealing with stem cell-based products, additional challenges exist in terms of final cell population qualification and measurement of cell activity once differentiated. Indeed, in such cases, the fate of the cells and the resulting biological activity may in fact be inferred only after cells have reached their final destination, hence complicating the choice of relevant potency assay. The reflection paper on stem cell-based medicinal products sets the expectation rather high in terms of potency by providing...
Operational challenges. It is however noted that such assays may not always be suitable for release testing, where the time for testing may be limited. Thus, a combination of different types of assays may be needed to confirm the potency of a stem cell product. For Holoclar, the first stem cell-based medicinal product approved in Europe (in February 2015), the combination of clonogenic assay with specific cell population markers for both active and supportive cells was used as a baseline to design the potency strategy. Cytological characterisation allowed selecting quantitation of a limbal stem cell marker as the product release potency assay, as it was shown to be significantly related to clinical success.

Overall, finding the right functional assay that could be extrapolated to humans remains a challenge if we add to this point the fact that sensitivity of each cell lineage to environmental conditions, post-administration, may lead to functional modifications, including loss of viability, release of cell signalling elements potentially inducing alternative differentiation or degradation of other cell subpopulations. It can be nearly impossible to reproduce, either in vitro or in vivo (with animal or cellular models), the expected overall clinical response.

Finally, in cases of autologous or allogeneic single donors (primary cells), the variability of the starting material should also be considered as it will have an impact on the cell composition heterogeneity, the cell type relative proportions from one sampling to another.

Developing potency assays ensuring that a potent product is released, regardless of the characteristics of the starting material and inter-donor variability, is imposing a tough challenge in terms of analytical development. The assay will have to balance between tolerance with regard to the intrinsic heterogeneity of the product under test and yet the ability to capture relevant potency measurements to drive the release. In certain cases, a comparison of results against a specification set on historical data may not be sufficient, and the design of the assay itself must then anticipate and integrate the technical tools to be able to interpret the results. This could be in the form of a comparison between the product and the starting material (ie, the CD54 up-regulation used for Provenge), or the use of relevant reference standards, further discussed below.

Operational challenges. Developers also have to consider the fact that CBMPs are often living products with a limited shelf life before administration to the patient. In order to be used for release testing, ideally providing results before the product is administered to the patient, “suitable” potency assays have to be fast for their readout. Furthermore, for some products the batch size is often limited due to the small quantity of starting material available (in particular for autologous products or cord blood derived CBMPs, for example). In such cases, any sample taken for quality control purposes reduces the quantity of product available to the patient; this ethical issue may be needed to confirm the potency of a stem cell product.

In light of all these challenges it is not surprising that a relevant potency strategy may take years to develop. Yet developers are encouraged to propose potency assessment strategy from Phase 1 onwards. Table 1 provides examples of questions raised by regulators on filing or during scientific advice, and gives insight on the level of expectations in terms of potency which can be taken into consideration as the potency strategy is designed. Some of the challenges may be appropriately addressed by considering an incremental approach, starting with a first simple potency test to be introduced as early as possible and adding relevant orthogonal methods while gaining increased knowledge on the product and the process throughout development. The design of the overall potency strategy and its acceptability will in fact vary depending on the etiology, the knowledge of the disease, literature available, final product composition and result of characterisation and MoA studies available. All of these factors will collectively influence the strategy for potency assay development, which should in most cases be built as a stepwise approach as follows:

Selection of a first potency test. The first test(s) proposed for implementation early on should in fact come from a thorough review of literature on the disease, the existing treatments (if any), the known elements of the MoA, published data on specific markers or groups of markers of interest for the indication or for desired cell population, and the quality target product profile. This exercise should, as much as possible, take into consideration the list of questions in Table 2. This thorough analysis will help design and justify the first potency assay(s). In certain cases, selected markers to identify the desired cell population would be acceptable as a first assay. This is often the case when tissue replacement is intended or when, for a known targeted indication, there is literature evidence of the biological activity of a certain cell type essentially identified through specific cell markers. In other cases, functional tests may be required quite early on (in particular when MoA involves a metabolic activity).

Continue to investigate MoA and product characterisation. While early clinical phases are initiated, characterisation of the desired product should continue in parallel and help elucidate other elements of the MoA. Questions should include: is biological activity linked to specific differentiation? Is there secretion of cytokines? Binding to specific cells or receptors? Cell death?

Potency assay development strategy: What, when and how?
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- **Propose orthogonal methods as knowledge is gained.** As pieces of the puzzle are being assembled around the MoA, development of additional orthogonal methods may be possible and addition of several orthogonal methods will enrich the potency assessment strategy. Functional assays should be developed when possible to propose a thorough assessment of the biological activity.

- **Build correlations between tests and select the most relevant ones.** As development moves forward, more batches are produced and tested using the different potency assays available. This will help to initiate correlation between the various tests and with previous animal proof-of-concept_functional studies. Degraded samples or impure samples integrated in this analysis will also help establish assay sensitivity and the ability to detect degraded or subpotent lots. These data will be critical to refine the selection of the most relevant potency assays and specifications.

- **Build correlation with biological activity and clinical outcome.** Analytical results can be analysed in light of the clinical data collected over time to ideally start drawing correlation between potency results and product clinical efficacy. This correlation can be extremely valuable to verify whether the potency assay is capable of detecting meaningful clinical differences between batches and hence allow identification of a link to the dose and strengthen the dosing schedule and its justification.

- **Final strategy using surrogates.** Ideally, if development data can demonstrate that strong correlations are established during the different phases of clinical development, the option of proposing a surrogate assay as part of routine testing might be considered acceptable. The alternative of using a surrogate assay is particularly important to consider where functional assays available have proven to be either highly variable and/or their readout too lengthy to be available prior to product administration. The alternative surrogate assay may provide the advantage of better reproducibility, shorter timeline to release, etc.

  The retrospective analysis of the potency strategy implemented for Provenge, where many constraints were combined (autologous product, limited shelf life, limited product availability), provides an interesting case. The active substance was reported to be autologous PBMCs (CD54+ including APCs), activated ex vivo with the recombinant fusion protein PA2024 (PAP-GM-CSF). However, the active substance was in fact a mixture of cells containing other mononuclear cell types: T cells, B cells, Natural Killer (NK) cells, and APCs (including monocytes and dendritic cells (DCs). PA2024 is uptaken by APC and activates T cells targeting prostate cells. The MoA was based on the induction of an immune response to the target antigen, PAP expressed on tumour cells. Potency measurement proposed was based on the combination of CD54+ cells viability and CD54 up-regulation with no measurement of functional activity justified mainly by the constraints of limited shelf life prior to administration.\(^\text{12}\) The EMA challenged the potency assay strategy and considered that the chosen surrogate assays failed to demonstrate potency due to a lack of specificity to the APC. Therefore, despite the operational advantages of the surrogate assay for release purposes (particularly in terms of being more quantitative, faster with good reproducibility and robustness compared to the functional assay), the heterogeneity of the drug substance composition combined with the lack of specificity of the selected marker challenged its suitability to measure potency and distinguish, on its own, subpotent batches.

  The applicant had to perform extensive complementary characterisation studies, investigating the potential influence of other cell types in the final product on the activity and building correlations between the number of CD54+ cells, the up-regulation of CD54 population and the clinical endpoint (survival). Based on these studies, the MA was granted, with a commitment to present complementary data as soon as available.\(^\text{12}\) In this case, it is clear that the surrogate approach

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<th>Table 1: Examples of questions raised by regulators.</th>
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<td><strong>Topic</strong></td>
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| Clarification MoA | • Confirm the types of cells expected to provide an effect  
| | • Confirm other cells are not contributing to the MoA → If other cells contribute to the overall activity, full quantification and qualification to be provided  
| | • Ineffective cell lines identified as product-related impurities, therefore if not removed, there will likely be a need for monitoring and setting specifications (residual limits)  
| | • Ideally, cells that do not contribute to MoA should be removed unless strong rationale supported by data is provided  
| | • Use data from proof-of-concept studies to justify the chosen potency assay  
| Correlation and surrogates | • Correlation between surface binding and cell activation required  
| | • Correlation between cell marker expression and actual biological activity to be established  
| | • Purity or viability not considered acceptable surrogates of potency  
| | • Potency assay not sufficiently correlated to biological activity of product and specification limits not appropriately justified  
| | • Need to correlate potency acceptance criteria with clinical data  
| Specifications | • Specification for potency (target and range) was not justified in terms of batch data  
| | • Company has not studied the level of expression needed to ensure proper efficacy of the product  
| | • Potency values are within specification but the specification is not justified  
| | • Data presented to substantiate relation between potency results and clinical results in pivotal clinical trial premature as the results were generated with non-validated assay  
| | • Acceptance criterion does not ensure only sufficiently potent batches will be released (risk of releasing subpotent product).  

Conclusions

Potency assay strategy should be addressed early on in product development, not only to fulfill its critical role as part of quality assessment but also to initiate the detailed characterization and correlations studies that will justify the overall quality control and release strategy. Several CBMPs obtained EU approval after struggling to fill gaps that could have been at least partially addressed by implementing relevant studies earlier in development. In addition, potency assays were the source of major concerns for other applications which were withdrawn during the evaluation process. The risk-based approach allows designing a strategy based on the specificity of each product, and regular interaction with agencies is strongly recommended to discuss the acceptability of the proposed potency assay strategy. Scientific advice provides the opportunity to discuss the characterization of the active substance, the expectations when mixtures of cells constitute the drug substance or when a complex product or MoA is anticipated and the impact on potency assay development. Correlations between tests and clinical outcome can be drawn incrementally and will help adjust the selection of tests and relevant specifications. Moreover, having additional data generated and building knowledge by using orthogonal methods as early as possible during development will lay the strong basis needed to propose the use of simpler surrogate tests for release. All in all, while this article is based on the EU experience, the same considerations and critical points have been identified by both the EMA and the FDA, allowing developers to consider a global approach of the potency assay development.

References

1. ICH Q6B Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products.
2. Points to consider on the manufacture and quality control of human cell therapy medicinal products (CPMP/BWP/44540/98).
5. Guideline on quality, non-clinical and clinical aspects of medicinal products containing genetically modified cells (CAT/GTP/671639/2008).
15. EPAR, ChondroCelect, EMEA/724428/2009.

Table 2: Points to consider prior to potency assay development.

<table>
<thead>
<tr>
<th>Questions</th>
<th>Possible answers and points to consider</th>
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<td>1. What constitutes the active substance?</td>
<td>A mixed-population of cells, a very specific and pure cell line, autologous vs allogenic etc.</td>
</tr>
<tr>
<td>2. In cases where the product is a mixture of cells, do they all contribute to the MoA?</td>
<td>Identification of the various cell types, relevance of the markers, qualification of the biological activity or contribution to the MoA of each cell type.</td>
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<td>4. What is the cascade of events leading to physiological response?</td>
<td>Based on the etiology of the targeted indication or confirmed with supportive data. Literature can be supportive to some extent.</td>
</tr>
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<td>5. What can be measured?</td>
<td>Qualitative and/or quantitative expression of cell markers, second messenger cascade, expression of a given protein, genomic markers (transcriptome, etc.).</td>
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<td>6. What happens to the product after administration?</td>
<td>The cells are the final form of the product, or further differentiation steps are expected ... Improve knowledge regarding the administration specificities and the events following administration (including fate of the cells, ie differentiation and migration).</td>
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was only accepted once significant characterisation data, correlations to functional assays and clinical data were established.

Similarly, ChondroCelect, a suspension of cartilage-forming cells for use in autologous chondrocyte implantation (ACI) experienced issues related to potency assay during evaluation of the MAA dossier. While the developer defined a genetic profile (positive and negative surface markers) correlated with cells capable of cartilage formation in an ectopic animal model and proposed it as a surrogate potency assay, the EMA challenged the fact that no direct correlation of the surrogate with the cartilage repair in patients could be demonstrated, and requested development of an additional functional assay to allow correlation.15

While developing functional assays, anticipating the pros and cons of all assays and the way they fill gaps from each other is key. Similarly, when building a matrix approach for potency, the choice of assays should consider complementarity regarding MoA coverage and correlation. An assay that cannot be correlated with the others is not useful to the matrix.