Cell-Based Potency Assays for QC: Considerations for Development and Analytical Transfer

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Introduction

- Potency is the only critical quality attribute (CQA) that can demonstrate biological function.
- The potency assay must be relevant to the intended mechanism of action (MOA) and relate to clinical response where possible.
- Potency assays are notoriously difficult to develop and transfer and require considerable expertise and understanding to both perform and troubleshoot.
- Advanced Therapeutic Medicinal Products (ATMPS) add another level of complexity over that of large molecule therapeutics due to several potential or even unknown mechanisms of action.

Aim

- To inform key considerations, from over a decade of experience, in developing cell-based potency assays for QC release.
- To highlight challenges associated with the analytical transfer of cell-based potency assays.

Assay Design and Development

The initial phase of any assay design is selection of the relevant cell line(s) to provide a relevant and sufficiently large response; selection of a dose range that provides a good data distribution across the assay biological response is essential (Figure 1).

The assay response data is described using an appropriate mathematical model, typically a 4-parameter logistic curve fit (Figure 2).

Figure 1. Data point distribution and signal intensity encompassing the biological response.

The ‘Goodness-of-Fit’ of the chosen regression model should be evaluated using a suitable approach. One such recommendation for assessing is through measurement of residuals, differences between the actual data and the model prediction (Figure 3).

Figure 3. Evaluation of model fit through measurement of residuals. For this data set the residuals are well-distributed across the assay plate.

The Log-Transformed Response

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Unequal variance of the response across the dose range should be evaluated; failure to address this can lead to inaccuracies in regression (curve-fit) analysis and final potency value reporting. However, this can often be improved by transformation of the response data (Figure 4).

Figure 4. Increasing variance with dose, with logarithmic transformation of the data leading to a more linear relationship.

Positional effects across the assay plate can be assessed using single dose concentrations. Absolute response can be heat-mapped for visual examination (Figure 5) or FCV can be calculated by row and column.

Figure 5. Assessment of plate positional effects, flow line is generated across the full plate at three different therapeutic concentrations representing the full assay response. The data is measured using the full plate assay response and then transformed to assess potential drift across or down the plate. Often large effects associated with the addition of a new cell type or evaporation rate in the outer wells can be seen using this approach.

The Reference Standard

- During early phase product development an international reference standard or, if a biosimilar, an innovator molecule may be available for use as a reference standard.
- From pivotal trials onwards a well-characterised, product-specific primary reference material must be developed that is representative of the clinical material (ICH Q8).
- For cellular and gene therapy products the reference standard choice should be supported by a clear scientific rationale including how and why the specific reference was chosen. Again must be well-characterised and either a clinical lot or directly relevant to the properties of the clinical material. Should, prior to implementation, and in line with regulatory recommendations, be discussed with your regulatory contact prior to proceeding.
- For all molecule types a well-defined strategy for the qualification of new reference standard lots should be in place well ahead of when needed. A two-tiered approach is highly recommended.

Measuring Parallelism

- If a 4-PL model fit of the test material is exactly parallel to the reference material, then the difference is simply the relative shift along the x-axis.
- If the biological activity of the test material is different, then the log dose shift will differ across the dose range and the curves become non-parallel.
- A parallelism test is always performed before calculating a relative potency.

Analytical Transfer

- Do not assume that if the assay performs well in the sponsor lab it will also perform well at the receiving lab.
- Ensure the receiving lab perform a thorough gap-analysis review of your method prior to performing any physical bench work.
- The training phase is possibly the most important aspect of any analytical transfer; analysts must understand each step of the assay and be able to apply good technique associated with the relevant steps of each individual assay.
- Shortcutting the training phase in an attempt to meet timelines nearly always causes problems in validation and risks significant delays at a later point.
- If possible, the training phase should incorporate testing at the extremes of potency (outside specification limits if known) to pre-assess methodologist performance.
- During the transfer phase we recommend that both receiving and donor laboratories analyse identical samples.

ATMP Potency Assay Challenges

Advanced Therapeutic Medicinal Products, e.g. cellular products and gene therapies, present further significant challenges in addition those seen with large molecules. A comparison (Table 1) highlights specific considerations that may impact the development and transfer of such assays.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Large Molecules</th>
<th>ATMPS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Structure</td>
<td>Can be elucidated</td>
<td>Extensively complex</td>
</tr>
<tr>
<td>Structure Function</td>
<td>Defined/known</td>
<td>Multi-faceted</td>
</tr>
<tr>
<td>MIA</td>
<td>Due to cell biology</td>
<td>Multiple or may be unknown</td>
</tr>
<tr>
<td>Assay Conditions</td>
<td>Single potency assay, sufficient</td>
<td>Multiple potency assays may be required</td>
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<tr>
<td>Test Material</td>
<td>Easily available</td>
<td>Limited availability</td>
</tr>
<tr>
<td>Reference Standard</td>
<td>Using control cell lines</td>
<td>Using control cell lines</td>
</tr>
<tr>
<td>Viability</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

In reality, two curve fits are never identical; we must determine if this difference is either due to variation or that the test material is indeed biologically ‘different’ from the reference material.

For cellular and gene therapy products the reference standard choice should be supported by a clear scientific rationale including how and why the specific reference was chosen. Again must be well-characterised and either a clinical lot or directly relevant to the properties of the clinical material. Should, prior to implementation, and in line with regulatory recommendations, be discussed with your regulatory contact prior to proceeding.

Conclusions

- Cell-based potency assays are complex, difficult to develop and require considerable scientific know-how to achieve success.
- Key stages in assay development are presented.
- A thorough training regime in the potency assay is invaluable prior to performing any analytical transfer.
- Cellular and gene therapy products present unique challenges over and above that of large molecule therapeutics.

References and Further Reading

1. Spies, Andrea Nikola; Natalie Neumeier. An overview of ATMPs. PharmaToday; 2015: 30-6