

Potency assays in gene therapy: from concept to clinic to commercialisation

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1. Introduction

Potency is a cornerstone of quality control for gene therapy products, serving as the most direct analytical link between manufacturing consistency and anticipated clinical activity^{1,2}. Across gene therapy modalities, including viral vectors, non viral delivery systems, and gene editing technologies, potency underpins dose justification, comparability assessments, stability evaluation, and ultimately regulatory approval^{1,3}.

Unlike conventional biologics, gene therapies exert their therapeutic effect through complex, multistep biological processes. Depending on the modality, these may include cellular uptake, intracellular trafficking, nuclear delivery, transcription and translation, genome modification, and sustained expression or editing activity^{4,5}. As a result, potency cannot be reduced to a single molecular interaction but must instead reflect biological functionality within a relevant system^{2,6}.

This complexity makes potency assays particularly challenging to design, qualify, and validate. Assays are often product specific, rely on biologically variable systems, and lack harmonised reference standards^{1,7}. Regulators therefore consistently define potency as a critical quality attribute (CQA) across all gene therapy platforms, while recognising that potency strategies must be mechanism driven and evolve throughout development^{1,2}.

This white paper explores potency assay concepts and strategies across the gene therapy landscape, with a focus on analytical development, CMC integration, and lifecycle management from early research through to clinical and commercial readiness.

2. Defining potency in gene therapy products

2.1 Mechanism of action as the anchor for potency

For all gene therapy products, potency must be defined in the context of the product's mechanism of action (MoA)^{1,2}.

While delivery platforms differ, therapeutic intent generally falls into one or more categories: gene addition or replacement, gene suppression or modulation, or permanent or semi permanent genome modification^{4,6}.

Regulatory authorities in both the EU and the US emphasise that potency assays should be biologically meaningful and plausibly linked to clinical efficacy^{1,2}. In practice, however, expectations diverge in how strictly this principle is applied. EMA guidance and scientific advice interactions tend to place greater weight on demonstrating that the potency assay reflects a functional step aligned with therapeutic intent, rather than an upstream or purely technical measurement^{2,8}. Assays focused solely on delivery metrics are therefore less likely to be accepted as stand alone potency methods for late stage development in the EU, particularly for systemic or long acting products^{2,8}.

2.2 Biological relevance versus surrogate readouts

Ideally, potency assays would directly measure the intended therapeutic effect. In reality, this is often impractical, particularly when the effect is tissue specific, delayed, or observable only in vivo^{4,6}. As a result, many gene therapy programmes rely on surrogate or upstream readouts such as payload delivery, transgene or guide RNA expression, protein production, or editing frequency^{1,3}.

Both FDA and EMA accept surrogate potency assays, particularly in early development^{1,2}. The distinction lies in how long such surrogates remain acceptable. FDA generally allows surrogate assays to support early and mid stage development, provided there is a clear plan to evolve toward greater biological relevance^{1,9}. EMA more frequently expects that, by pivotal clinical stages, potency assays demonstrate functional biological activity or are supported by robust orthogonal evidence that justifies their relevance to clinical performance^{2,8}.

2.3 Regulatory expectations at a high level

Across regions, regulators expect potency assays to be quantitative, reproducible, discriminatory, and aligned with the product's MoA¹⁻³. Importantly, potency assays are expected to evolve into validated, stability indicating methods as programmes progress^{2,3}. While both agencies endorse lifecycle based analytical development principles, EMA typically expects earlier convergence on the final potency strategy, particularly for assays supporting release and stability^{2,8}.

3. Types of potency assays across gene therapy modalities

3.1 Cell based functional assays

Cell based functional assays measure biological activity in a relevant cellular system. Depending on the modality, this may involve measurement of enzymatic activity, phenotypic rescue, pathway modulation, or functional consequence of genome editing⁴⁻⁶.

These assays offer the highest degree of biological relevance and are therefore often favoured for late stage development and commercial release^{2,8}. However, they are inherently variable, sensitive to cell line selection and culture conditions, and resource intensive to develop and validate^{7,10}. Achieving acceptable precision and robustness for GMP use is a recurring challenge^{2,7}.

3.2 Reporter gene assays

Reporter assays measure expression of an engineered reporter following delivery or editing, providing a quantifiable proxy for biological activity^{5,6}. They are generally more sensitive and reproducible than endogenous functional assays and offer broad dynamic range⁷. The principal limitation is their surrogate nature. Reporter expression may not accurately reflect therapeutic transgene behaviour, particularly where expression levels, processing, or functional thresholds differ^{4,6}. As a result, reporter assays are most commonly used in early to mid stage development or as supportive methods alongside a primary potency assay^{1,2}.

3.3 Molecular and nucleic acid based assays

Molecular assays quantify nucleic acid species such as vector genomes, mRNA transcripts, or editing frequency^{1,3}. These methods are analytically robust, highly sensitive, and well suited to routine testing^{3,7}. Their limitation, however, is that they do not directly measure biological function.

Regulators therefore view these assays as supportive rather than definitive indicators of potency, particularly for late stage development^{1,2,8}.

3.4 Hybrid potency assays: bridging biology and analytics

Hybrid potency assays integrate multiple mechanistic steps of gene therapy activity within a single analytical framework or within tightly linked readouts^{2,6}. Rather than measuring delivery, expression, or function in isolation, these approaches aim to capture cause and effect relationships that better reflect therapeutic activity^{6,10}.

Examples include assays that combine transduction and endogenous protein expression with expression and downstream functional activity, or editing efficiency coupled with functional rescue⁴⁻⁶. Multiplexed and high content cell based assays that simultaneously assess uptake, expression, localisation, and functional response are also emerging¹⁰.

Hybrid assays offer a pragmatic balance between biological relevance and analytical feasibility². From an EU regulatory perspective, they are particularly attractive because they demonstrate that potency is supported by converging lines of biological evidence rather than a single surrogate readout^{2,8}. Their complexity, however, presents challenges for validation and routine GMP implementation, necessitating careful assay simplification and control strategy design^{3,7}.

3.5 Comparative overview of potency assay types

Functional cell based assays offer high biological relevance but lower reproducibility and higher development burden^{2,7}. Reporter assays provide moderate relevance with improved precision⁶. Molecular assays offer excellent reproducibility but limited biological insight¹. Hybrid assays occupy an intermediate space, offering enhanced relevance at the cost of greater complexity^{2,10}.

4. Key challenges in potency assay development

Potency assay development across gene therapy modalities is constrained by biological variability, dependence on model systems that may not fully reflect in vivo biology, limited reference standards, and assay evolution over the product lifecycle^{1,7,10}. A particularly important regulatory consideration is the expectation—more pronounced in the EU—that potency assays serve as stability indicating methods capable of detecting clinically relevant degradation^{2,8}. Failure to anticipate these expectations can lead to late stage assay redevelopment, delayed submissions, or challenging regulatory interactions^{2,8}.

5. Phase appropriate potency strategy

In early development, exploratory and surrogate assays are generally acceptable to support candidate selection and early clinical studies^{1,9}. In mid stage development, assays should be refined, controls strengthened, and biological relevance increasingly emphasised^{2,3}. Divergence between FDA and EMA expectations becomes most apparent in late stage development. EMA often expects fully validated, biologically relevant, and stability indicating potency assays to be in place by the start of pivotal trials^{2,8}. FDA may allow some continued evolution, provided the endpoint strategy is clearly defined^{1,9}. This difference strongly supports early investment in robust or hybrid potency assays, even if they are not immediately deployed as the primary release method^{2,8}.

6. Orthogonal and multi assay approaches

Given the complexity of gene therapy mechanisms, reliance on a single potency assay is rarely sufficient^{2,6}. Orthogonal strategies that combine functional or hybrid assays with precise molecular or expression based methods allow developers to contextualise variability, support comparability following manufacturing changes, and address regulatory concerns around robustness and interpretability^{2,3,7}.

Independent analytical laboratories with experience across modalities and regulatory regions are particularly well positioned to help design and execute such multi assay strategies consistently across the product lifecycle^{2,8}.

7. Practical considerations for developers

Potency strategy should be integrated early into CMC planning^{1,3}. Common pitfalls include over reliance on convenient surrogates, underestimating validation effort, and deferring robustness considerations until late development^{2,7}. Early engagement with experienced analytical partners can significantly reduce these risks by providing modality spanning expertise, orthogonal method capability, and lifecycle support from development through commercialisation^{2,8}.

8. Conclusion

Potency remains one of the most scientifically demanding aspects of gene therapy development^{4,6}. Across viral vectors, non viral delivery systems, and gene editing technologies, robust potency strategies are essential to demonstrate product consistency, support regulatory approval, and ultimately protect patients^{1,2}.

By grounding potency assays in mechanism of action, adopting phase appropriate and orthogonal strategies, and anticipating regional regulatory expectations, particularly the stricter stance of EMA on validation timing and biological relevance, developers can navigate this complexity and progress confidently from concept to clinic^{2,8}.

9. References

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