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Advancing Potency Assay Development for Advanced Therapy Medicinal Products: A Comprehensive Approach and Regulatory Insights

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A Comprehensive Review of Scientific, Technical, and Regulatory Considerations

Presented by Mike Merges

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Potency is central to product quality, release, and regulatory success

How I got here...



Mike Merges, M.S.

35+ years of analytical development & validation experience



Since 2020 at Autolus...

- initially focused on analytical procedure development, validation and product release
- recently led analytical, vector, and cell process development, as well as quality control
- now focused on CMC oversight



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Background and Context

Cell and Gene Therapies (CGT) [FDA] or Advanced Therapy Medicinal Products (ATMPs) [EMA]

- CGTs or ATMPs are medicines based on **gene therapies, tissue-engineered products or cell therapies...or combinations.**
- These therapies **target high unmet medical need** and **rare diseases**
- **Potency is a critical quality attribute** linked to biological activity and intended therapeutic effect
- **Robust potency procedures** are essential for characterization, lot release, comparability, and product lifecycle control

ATMPs are groundbreaking new opportunities for the treatment of disease and injury

Author's Study Objective and Core Question

Core question: how can robust and reliable potency procedures be designed for ATMPs?

- The challenge is to **accommodate product-specific complexity** while **aligning with evolving regulatory standards**
- The author's aim was to translate **scientific evidence** and **regulatory guidance** into a **practical approach**

No single potency procedure fits all ATMPs

Methods

Systematic literature review covering 2018–2024 academic publications

- Review of **industry reports, webinars, and white papers**
- **Comparison of FDA and EMA regulatory frameworks**, including newer potency assurance concepts
- **Database** analysis of potency procedures used in **approved, rejected, and withdrawn CGT/ATMP products**

Provided a framework to address ensure the robust assessment of potency in CGT/ATMP therapies agnostic to product type plus throughout different product development stages

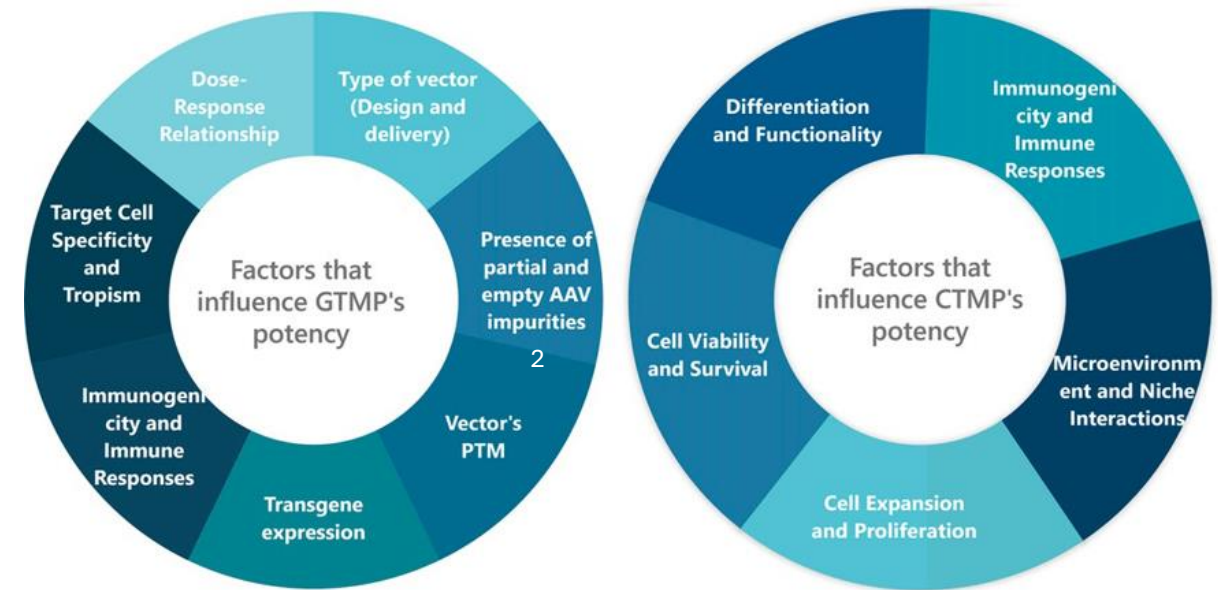
Results

Key Challenges in Potency Procedure Development

- Starting material high variability, especially for autologous and heterogeneous products
- Complex or partially understood mechanisms of action
- Limited batch(s) size(s), short shelf life, and restricted material for testing
- Lack of suitable reference standards and challenge linking in vitro readouts to clinical outcomes

Figure 1. Factors that influence gene and cell therapies product potency.

- GTMP, Gene Therapy Medicinal products
- CTMP, Cell Therapy Medicinal Products.



¹Adeno-Associated Virus (AAV)

² Post-Translational Modification (PTM)

Innovative and tailored development approaches required

Results

Table 1. Key Challenges in Potency Procedure Development...continued...Possible Solutions

Solutions

- Orthogonal Procedures
- Measure gene expression or protein present
- Measure empty/full capsid ratios
- Peptide mapping
- In-vitro models
- Reporter gene procedures

Table 1. *Potency assay challenges for cell and gene therapies and their possible solutions*

<i>CGTs/ATMPs Type</i>	<i>Potency Assay Challenges</i>	<i>Possible Solutions</i>
GTMP	The mechanism of action and the relationship between in vitro activity (or even in vivo in animals) and therapeutic efficacy in patients may be complex or poorly understood	Use of orthogonal assays: Orthogonal assays are assays that measure different aspects of the product's properties or function. By using multiple orthogonal assays, it is possible to get a more complete picture of the product's potency and reduce the risk of missing false negatives.
	Direct measurement of protein activity is impossible	Measuring the expression of the gene that encodes the protein of interest. Measuring the amount of protein present in a cell or tissue. Measuring the activity of enzymes that are downstream of the protein of interest. Measuring the effects of the protein on a cell or tissue.
	Managing partial and empty AAV impurities	AUC and Anion exchange chromatography are widely recognized as the gold standard method for determining the empty/full AAV capsid ratio. Extraction of polyethyleneimine (PEI) from the sample matrix and analysis by UHPLC with CAD to monitor the synthesis/purification, and characterization of linear polyethylenimines
	PTMs	icIEF and LC-HRMS and peptide mapping to precisely determine PTMs
CTMP	Infectivity is cell-type specific and not easily modeled in vitro	Develop new in vitro models that more closely mimic the in vivo environment (3D cultures)
	Killing assays have limitations in serving as quality-control	Reporter-gene assays have emerged as promising alternatives for QC purposes

AAV, adeno-associated virus; ATMP, Advanced Therapy Medicinal Product; AUC, Analytical ultracentrifugation; CAD, charged aerosol detection; CGT, Cellular and Gene Therapy; icIEF, Imaged capillary isoelectric focusing; LC-HRMS, liquid chromatography-high resolution MS; PTM, Post-Translational Modification; UHPLC, ultrahigh-performance liquid chromatography.

Results

Current Potency Procedure Landscape (Table 2 not shown)

- Approved ATMPs use a **wide range of potency approaches**, reflecting product diversity
- Procedure types include **in vitro functional, ex vivo, in vivo models, biomarker-based, and surrogate readouts**
- **Examples** include cytokine secretion, proliferation, vector transduction, transgene expression, and functional protein activity
- The landscape supports a **matrix approach** rather than reliance on a single procedure

All potency assays listed in Table 2 were retrieved from the European Medicines Agency (EMA) website, specifically from the European Public Assessment Reports (EPAR) for each product (<https://www.ema.europa.eu/en/glossary-terms/european-public-assessment-report>).

Procedure developers deployed novel to well-established procedures

Potency Procedure Strategies

General Best Practices and Regulatory Direction

- Use a **lifecycle-based, risk-based** potency **strategy** that **evolves with development stage**
- **Link** potency assays to **quality target product profile** and potency-related **critical quality attributes**
- Apply **orthogonal** or **matrix-based** procedures where mechanism of action (MoA) is **multifactorial**

Align potency procedure development with increasing regulatory expectations
from early clinical phases to licensure

Gene Therapy

Main potency procedure challenge is complex Mechanism of Action (MoA)

- Potency should address **both gene transfer and expression of the therapeutic product**
- Recommended readouts include **infectivity, transgene expression, protein expression, and biological activity**
- Assays should be **quantitative, dose responsive, stability indicating, and well controlled**
- **Emerging tools** such as ddPCR and optimized bioassays can improve sensitivity and precision
- Consider a **matrix approach**

Give a think to the need for a matrix: procedure length, intense material and resource drain

Gene Therapy...continued

Potency procedure recommendations

For gene therapy products, the following potency assays are recommended

Recommended

- Infectivity
TCID50
- Transgene
Expression
- Protein
Expression
- Biological
Activity

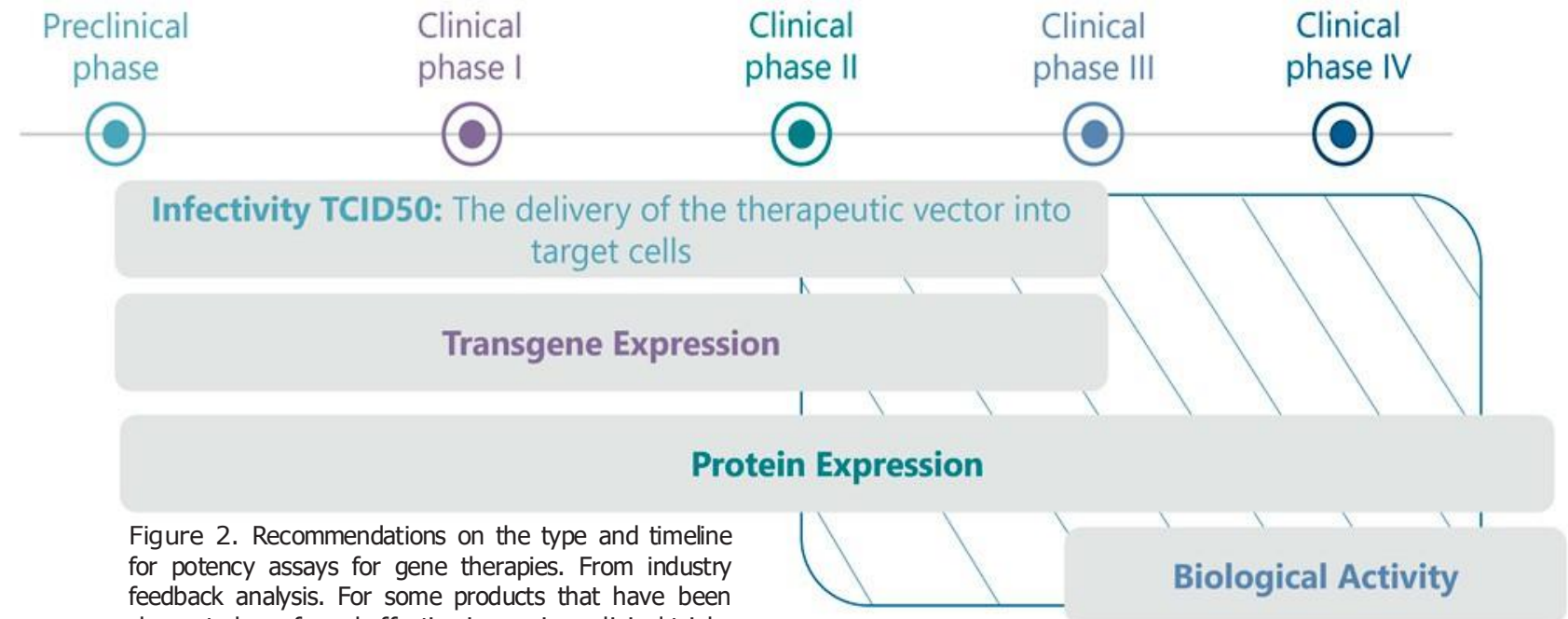


Figure 2. Recommendations on the type and timeline for potency assays for gene therapies. From industry feedback analysis. For some products that have been shown to be safe and effective in previous clinical trials, and that have a well-understood Mechanism of Action (MoA), there is no need for phase 3 and phase 4 trials.

Ideal potency procedure would simultaneously measure transfer of genetic material and changes in target gene expression...ex. One-step reverse transcriptase Droplet Digital PCR (RT-ddPCR)

Cell Therapy

Main potency procedure challenge measuring biological activity accurately

- **Early-phase** procedures should focus on **viability, proliferation, differentiation**, and core functional activity
- **Later-stage** development may incorporate **immunophenotyping** and **cytokine or growth factor secretion** procedures
- Traditional **killing** procedures may be **biologically relevant** but are often difficult for ~~routine~~ predictable QC use...more on this later
- **Reporter-gene** procedures offer more **reproducible** and **practical** alternatives **if they reflect mechanism of action**

Biological activity measurement must ensure patient safety and efficacy during manufacturing

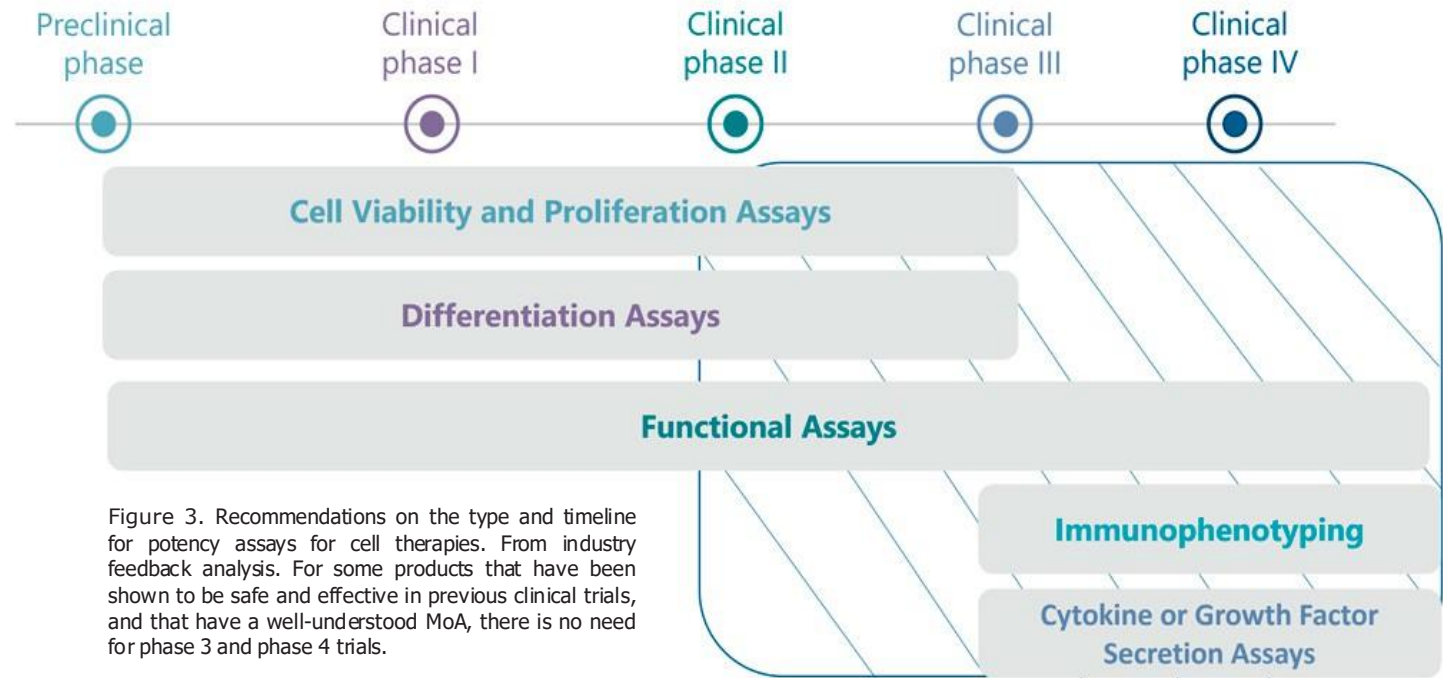
Cell Therapy

Potency procedure recommendations

Recommended

- Cell Viability and Proliferation
- Cell Differentiation
- Cell Function
- Immunophenotyping
- Cytokine or Growth Factor Secretion

For cell therapy products, the following potency assays are commonly employed



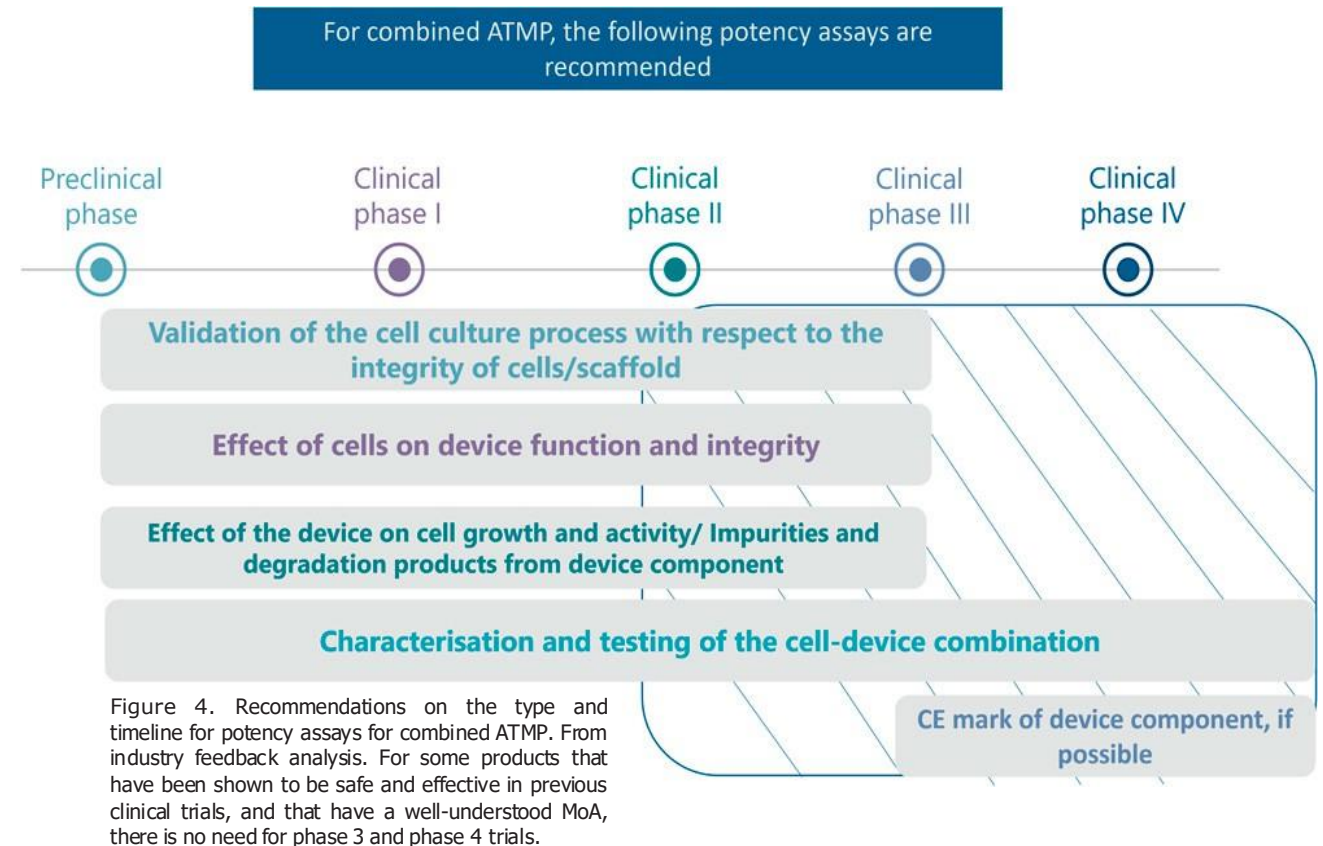
Validate that the procedures accurately reflect the Mechanism of Action

Combined ATMPs

Main potency procedure challenge is accounting for the component interactions

Recommended

- Combined ATMPs require potency strategies that account for interactions between biologic and device components
- Validation should include the device where it is integral to performance



Overall therapeutic activity must be measured

RNA-Based ATMPs

Main potency procedure challenge is ATMPs cellular composition and gene expression heterogeneity

- Gene therapy **product classification** remains a **complex** and **unsettled** issue
 - The **FDA's** definition of gene therapy focuses on the **modification of genetic material within cells**, a process that RNA-based therapies, such as mRNA drugs, do not typically involve
 - In the European Union, **mRNA-based therapies** may fall under the broader category of **gene therapy medicinal products** as defined by the **EMA**, especially when the mRNA acts as a recombinant nucleic acid with therapeutic effects.
- RNA-based therapies introduce **distinct challenges** related to transient expression, delivery, translation, and immunogenicity
- Current **regulatory frameworks** can inform development; more tailored guidance is still evolving

Establish consistent and reproducible measurements of
the relevant functional attributes of the combined ATMPs

From Cytokine or Growth Factor Release to Cell Killing

Traditional killing assays are biologically relevant but are often difficult for predictable Quality Control use



Development of a New High Throughput
Quantitative Potency Assay for a CD19
Chimeric Antigen Receptor T Cell Therapy

Louise Webb, Senior Scientist II, Autolus Therapeutics
2024 US Bioassay Conference

Private & confidential

Autolus.com

Challenging transfer and Quality Control validation is overcome by partnership, training and planning

Successful transfer and validation in Quality Control

Label free real time cell growth / death monitoring as opposed to flow cytometry or cell metabolite end-points

Development of a New High Throughput Quantitative Potency Assay for a CD19 Chimeric Antigen Receptor T Cell Therapy

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Conclusion

- New high throughput cellular impedance-based potency analytical procedure developed to replace previous flow cytometry-based procedure.
- Cellular impedance-based procedure shows ease of execution.
- Raji cells are in a thaw-&-use format – continuous culturing not required.
- Cellular impedance-based procedure shows ease of analysis:
 - xCELLigence RTCA software performs majority of analysis.
 - No requirement for subjective FACS gating.
- Method highly robust.
 - Target cell growth monitored ahead of Effector cell addition.
 - Wells masked and/or assay aborted in case of unusual Target cell patient DP not seeded under suboptimal conditions.



Leveraged cross-functional design and communication
to overcome challenges to predictable Quality Control use

Discussion and Future Directions

There is a gap between emerging science and 'routine' approved potency strategies

- **Standardized reference materials** and stronger **links** between in vitro **potency and clinical outcomes** are needed
- **Future** directions include **advanced in vitro models, improved analytics**, and potential **AI-enabled design approaches**

Regulators are moving toward more flexible, science-driven, product-specific guidances

Conclusions and Take-Home Messages

Potency procedure design remains one of the central Chemistry, Manufacturing, and Control (CMC) challenges for ATMP advancement

- **Successful development strategies** must be **product-specific**, **mechanism of action-linked**, and **phase-appropriate**
- **Risk-based, lifecycle-oriented** potency procedure **planning** is increasingly **expected by regulators**

Author's Final Message:

Robust potency strategy is not just an analytical requirement, but a product development enabler

Questions to Consider?

What would a fit-for-purpose potency strategy look like for our own development programs?

- Which potency attributes are most predictive of clinical performance for each ATMP class?
- When is a single assay sufficient, and when is a matrix or orthogonal strategy necessary?
- How early should potency strategy be locked for regulatory confidence without overcommitting too soon?
- Where are the biggest current gaps: reference standards, assay robustness, or correlation to clinical outcomes?
- What emerging technologies are most likely to improve potency assessment in the next few years?

thank you!

BEBPA's next Journal Club is...

October 8th, 2026
9am PT

