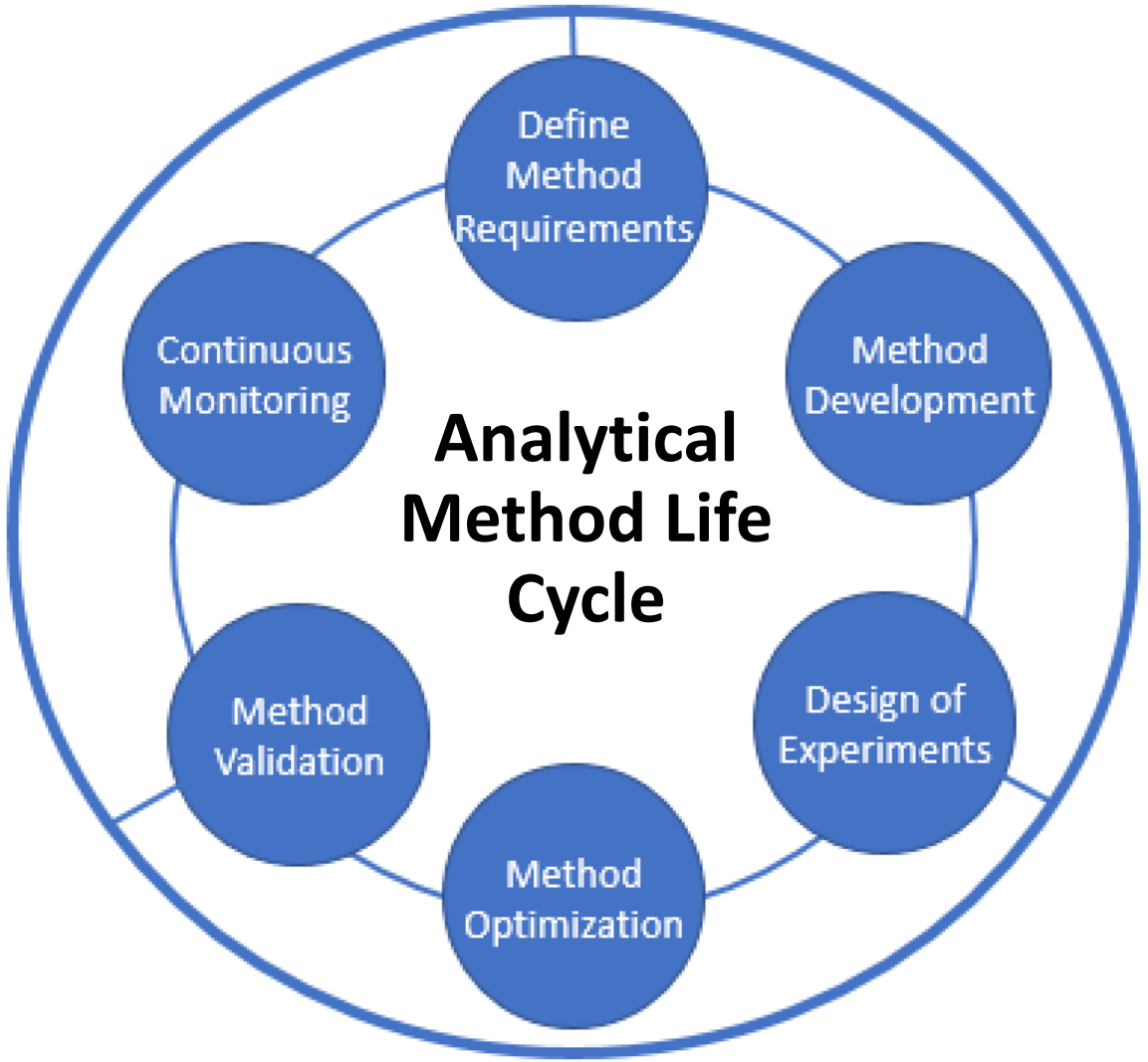
Gene Therapy Potency Methods: Method Lifecycle Management and Optimization

BIOMARIN®

MATCHING PROVEN SCIENCE WITH PROVEN NEEDS

Wandong Zhang, PhD
Associate Director, Quality Analytical Technologies
BioMarin Pharmaceutical Inc.

Commercial



Clinical Development

Clinical Development

Potency Assay Development and Validation Strategies

Considerations for Product Specific Reference Material

Lifecycle Management Strategies for Analytical Methods

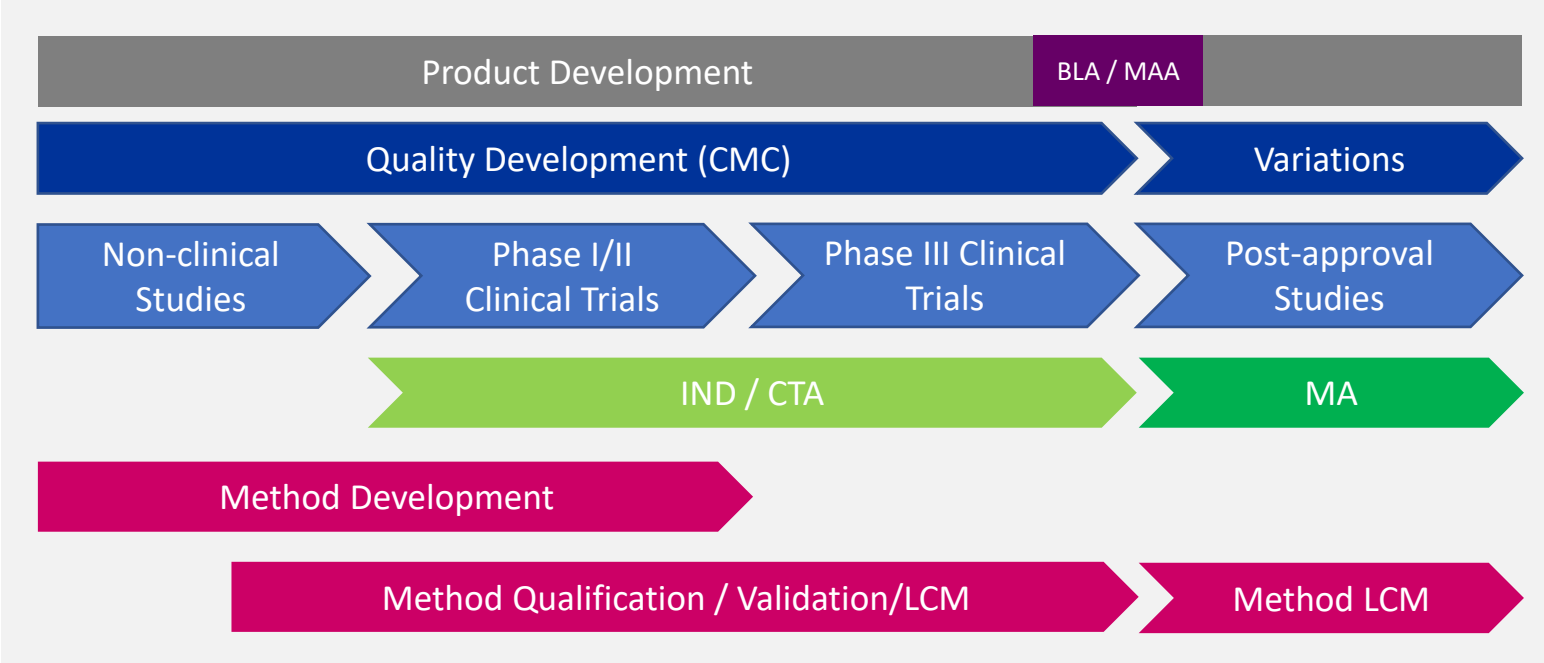
Considerations for Assessing Potency Method Comparability

Introduction

- Potency is a critical quality attribute for gene therapy products that demonstrates **strength**.
  - Mechanisms of action, specific to clinical indication
- Potency is one of the most challenging methods in quality development:
  - Product-specific method
  - Multiple steps of mechanisms of action for viral vector product: transgene sequence, capsid tropism, promoter, vector infectivity, viral uncoating, formation of transgene episomes, and gene and protein expression
    - Requires robust infection of host cells: specific cell type representative of target tissue
    - Measures protein expression and proper folding / modification
    - Measures functional protein
  - Bioassays are typically variable (20% CV or more)

Potency Assay Development Strategies

- Potency methods need to be:
  - In vitro assay strongly preferred
  - Timely release of products
  - Quantifiable
  - Can be validated (Accurate, Precise etc.)
  - Robust, Reliable, Transferrable
- Development strategies aligned with:
  - Clinical timelines
  - Regulatory agency expectations



- A focus on potency method development early on in product development can save time later
  - Knowledge from non-clinical studies
  - Functional assay vs surrogate assay for release vs characterization
  - Incremental, risk-based approach is the key to balance resources, program requirements and timelines

Potency Assay Development Strategies

Method	Development Phase	Pros	Cons	Recommendation / Notes
in vivo potency	Non-clinical / early-stage clinical	• Leveraging tox models	• Colony management • Highly variable • Long TAT for results	• Avoid in vivo models if possible; replace with in vitro method prior to clinical development
TCID50 (surrogate for potency)	Non-clinical / Phase I	• Platform approach • Serotype Specific	• Not product specific • Transgene not assessed • Highly variable	• Leverage method if no alternatives exist in early development
Platform approach	Non-clinical / early-stage clinical through marketing	• Streamlined development • Reduces training requirements	• May not be disease-relevant cells • May not align with pipeline	• Platforming transduction is a big win even if method read-outs are different
Expression assay	Phase I through marketing	• Measures protein expression, protein folding	• Does not assess protein function	• Use for early phase development • Consider replacement with functional assay
Biological Activity / Functional assay	Phase I through marketing, Expected for Phase 3 by many jurisdictions	• Measures protein levels (indirectly), protein folding / function	• Does not directly measure protein expression	• Prioritize functional assay development; may replace all other methods

Potency Assay Development Strategies

- **Early QC interaction with Development Laboratories can lead to:**
  - Guidance on developing robust methods to meet QC needs
  - Identification of issues in previous methods can be avoided for new programs
  - Consult ICH Q14

Variables to assess for cell-based portion		Variables to assess for assay read-out	
Screening of cell lines for transduction efficiency	Determination of cell number for best signal	Determining best technology for assay read-out	Determining conditions for protein extraction
Screening of transduction enhancers	Determining appropriate incubation time	Determining conditions for supernatant storage	Determining best mode of analysis (USP 1034)

Development can be streamlined by leveraging DoE Studies / Biostatistician expertise

Other considerations:

- Plate layout to prevent bias
- Reference standard availability and bridging strategies
- Number of sample replicates / pseudoreplicates
- Replicate testing
- Assay control
- Cell line and critical reagent qualification and acceptance criteria
- Automation
- Throughput requirements
- Licensing of reagents

Product Specific Reference Material in Potency Assay

- Absolute vs relative quantification for potency
- Relative potency assay for AAV-GT is common
  - A valid and stable product specific reference material as calibrator
    - For CGT, compendial or universally recognized reference are generally not available
  - Requires to establish a product specific reference material, usually a well characterized clinical lot from in house production
    - Qualification of reference material can be similar to release of a CGT product lot
    - Reference material has assigned expiry and requires renewal
    - New lot needs to be qualified and bridged to original reference material

Case Study 1: Characterize reference material against a universal protein standard

- Potency method for AAV-GT is very different from that of a protein therapeutic product
  - GT functional assays: measure collectively viral infectivity of host cells, transgene transfer, proper transcription, translation, and post translational modification or localization, functional readout
- Functional readout of the potency assay of the reference material can be calibrated to a universal standard of transgene protein
  - Calibration against the universal protein standard is a characterization assay only
  - Calibration could provide a link back to a well known protein universal standard for the reference material

	Potency (relative potency) method	Link back to a protein universal standard
Reportable of Reference Material	Defined as 100%	Reported as IU/MOI per mL or per well
Precision	Precision meets validation criteria	Characterization assay, more variable

  - The calibration result obtained is specific to transduction method conditions such as host cell, cell numbers, MOIs, volumes, incubation time as well as the functionality assay

Potency Assay Validation Strategies

- Validation of analytical methods should be in accordance with ICH Q2(R2) and internal procedures
- Refer also to USP <1032> , USP <1033>, and USP <1034>
- Results from qualification (pre-validation) studies should be utilized for setting validation acceptance criteria.
- Robustness should be included in validation if not completed during development.
    - Robustness may assess appropriate cell passage numbers, several lots of reagents, storage conditions and times for reagents and / or plates, etc.

Life Cycle Management of Methods

- Methods are rated for 7 categories:
  - Impact to Disposition (number of samples per lot, STAT, or stability requirement)
  - SOP Clarity
  - Robustness
  - Critical Reagent Control
  - Processing Method
  - Equipment continuity
  - Validation Package
- Ratings are subjective but are confirmed with assessment by multiple team members
- Prioritization of method improvement will be dependent on:
  - The severity of the issues with the method
  - The type of method concerns
  - Method importance (i.e. dosing and potency would be prioritized over TCID50 regardless of scoring.)

Prioritization

Per Category	Composite Rating	Suggested Next Steps
1	7-8	Method Maintenance and Monitoring
2	9-11	Method Maintenance and Monitoring
3	12-14	Identify appropriate mitigation and prioritize as resources are available
4	15-19	Apply appropriate mitigation(s) and prioritize as resources are available
5	≥ 20	Prioritize for method trouble-shooting and optimization as appropriate

METHOD	RATING	METHOD	RATING	METHOD	RATING
Method 1		Method 12		Method 23	
Method 2		Method 13		Method 24	
Method 3		Method 14		Method 25	
Method 4		Method 15		Method 26	
Method 5		Method 16		Method 27	
Method 6		Method 17		Method 28	
Method 7		Method 18		Method 29	
Method 8		Method 19		Method 30	
Method 9		Method 20		New Method 1	TBD
Method 10		Method 21		New Method 2	TBD
Method 11		Method 22		New Method 3	TBD

Considerations for Analytical Comparability

- Methods will be changed / improved throughout clinical development
  - Better precision and / or accuracy through further development
  - Robust Change Control process
  - Revalidation may be required
- Specifications will be re-assessed following method changes and comparability studies
  - Quantitative specification ranges are required for release and stability results in late-phase programs
  - Refer to ICH Q6B
- Method Comparability Protocols should include acceptance criteria
  - Head-to-head comparisons or side-by-side testing
  - Testing historical lots: It is critical to set aside material for future comparability studies

Case Study 2 – Replacement with an improved method

- Potency version 1 is a release and stability assay and exhibits fairly high variability and robustness issues
  - Version 1 is multi-component bioassay that has two read-outs
  - The method is not able to measure accurately across the proposed specification range
- Potency version 2 is an improved method that has been used for characterization and process development studies
  - Version 2 is a multi-component bioassay that has two read-outs
  - There is no long-term stability data for Version 2; however, Version 2 has been utilized in forced degradation studies for the product
- The goals of the comparability assessment were to:
  - Demonstrate comparability of Versions 1 and 2, while acknowledging there are differences due to method improvements
  - Replace Version 1 with Version 2 in the product control system
  - Set meaningful specifications for Version 2

Case Study 2 – Replacement with an improved method

- Differences of version 1 and version 2

Method Attribute	Version 1	Version 2
Cell line	Higher passage number bank	Lower passage number bank
Media	Proprietary media	Readily available media
Assay Length	Longer assay length	Shorter assay length
Method Read-Outs	Longer hands-on time	Shorter hands-on time
Method Format	24-well format	96-well format
Method Throughput	3 samples across multiple plates	5 samples per plate
System Suitability	Appropriate Criteria	Improved system and sample criteria, implementation of an internal assay control
Method Accuracy	Less accurate at low and high input values	Greatly improved accuracy across analytical range
Method Robustness	Frequent system suitability failures	Reduced system suitability failures

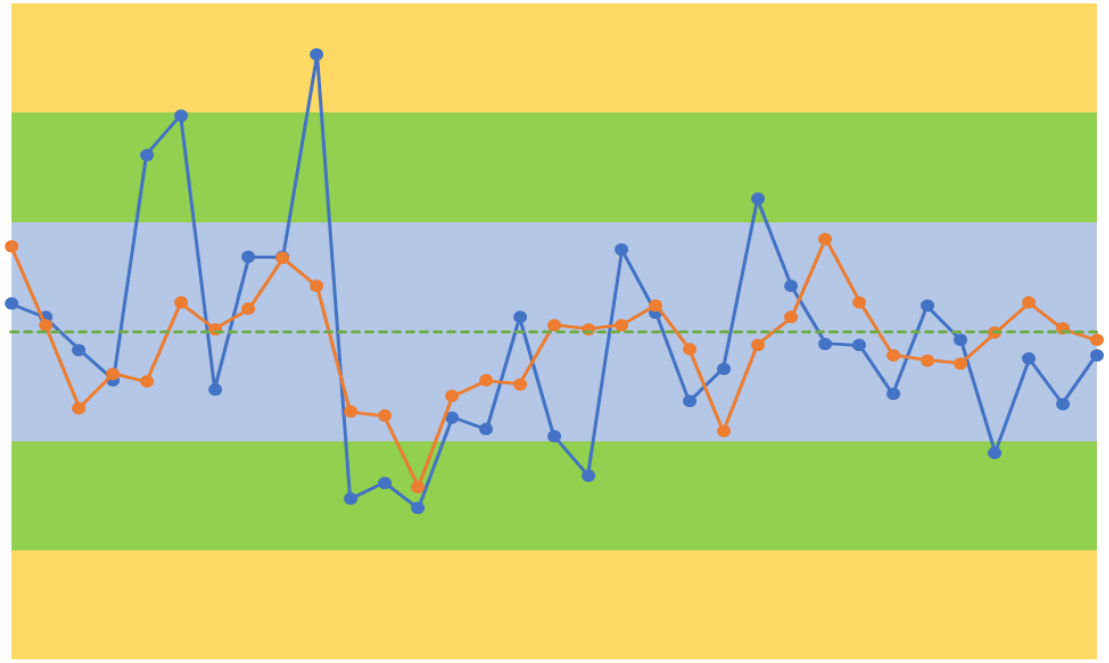
Case Study 2 – Replacement with an improved method

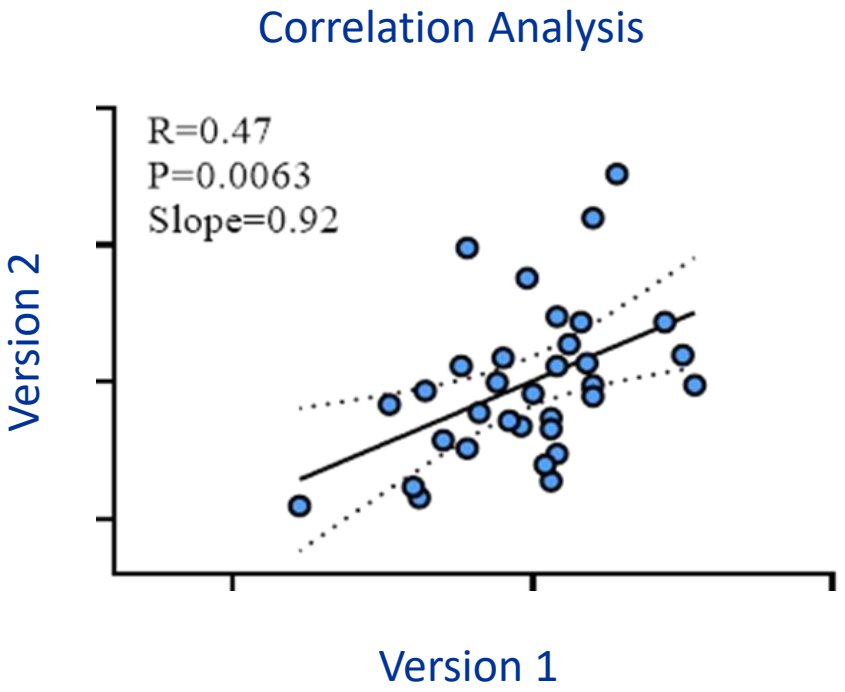
	Version 1	Version 2
Accuracy	Relative Bias: 0.5 – 61%	Relative Bias: 1 – 16%
Repeatability	4%	8%
Intermediate Precision	8%	13%
Linearity	$R^2 = 0.96$	$R^2 = 0.99$

- Stability-indicating potential for each version was also assessed.
- Results shown for one read-out method.

Case Study 2 – Replacement with an improved method

- Batch analysis comparison across lots with version 1 (orange) and version 2 (blue).
- Results generally trend together and version 2 showed higher and lower values, as expected, due to increased accuracy across the analytical range.





- Version 2 was determined to be superior over version 1 due to increased accuracy across the linear range of the method and increased throughput.

Case Study 3 – Remediation of a validated in-use method

- A potency method that had been validated and implemented at multiple sites demonstrated robustness issues:
  - Inconsistent valid results across the analytical range for the same material, leading to OOS results
  - Increased system suitability failures
  - Similar issues at all testing sites
- Some improvements evaluated included:
  - Cell number
  - Plate layout to reduce bias
  - Volumes
  - Dilutions of reagents
  - Additional intermediate MOI concentrations
  - Incubation times
  - Implementation of replicates
- Proposed improvements to the method were evaluated against the validated state of the method.

Case Study 3 – Remediation of a validated in-use method

- After initial evaluation of potential improvements, multiple sites performed a supplemental study to support the validation with the optimized method.
    - The precision and accuracy of the method were improved across the analytical range.
- Multiple improvements were implemented into the method.
- Replicate testing was implemented.
- The validation report was updated with the supplemental study results and the procedures were updated.
- The variability of the method has been reduced from ~20% CV to < 8% CV (n=76), confirming the results from the supplemental validation study.
- The system suitability failure rate has decreased significantly.

Acknowledgements

Quality (Gene Therapy) Analytical Technologies

QC Analytical

Analytical Sciences

Process Sciences


Questions?