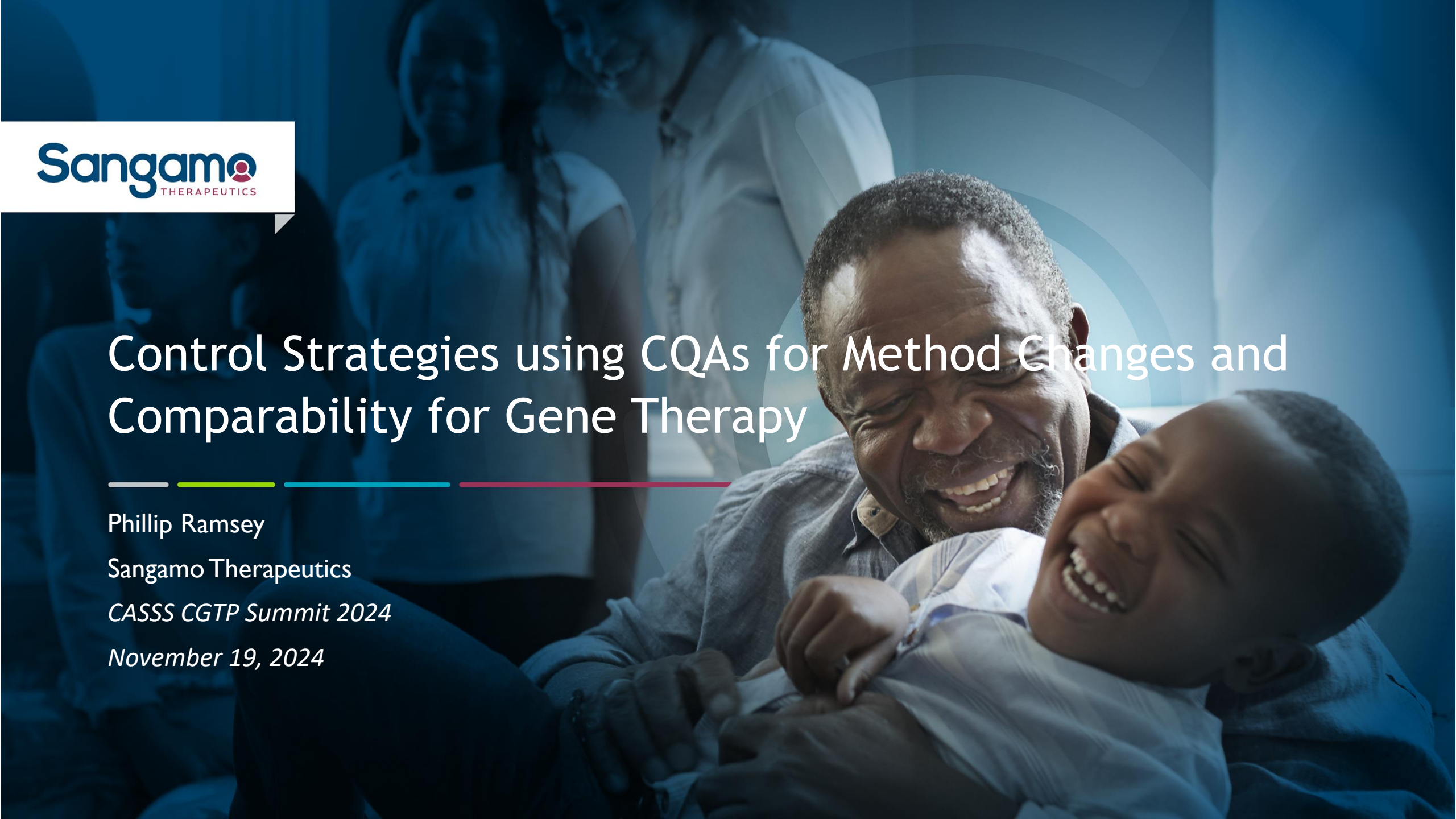


Control Strategies using CQAs for Method Changes and Comparability for Gene Therapy

Phillip Ramsey

Sangamo Therapeutics

CASSS CGTP Summit 2024

November 19, 2024

Outline

- Sangamo Therapeutics Overview
- CQA role in meeting the challenges of Gene Therapies
- Case Study 1: Control Strategy for Analytical Methods Measuring a CQA
- Case Study 2: Control Strategy for Process Change Leveraging Analytical Comparability
- Summary

Sangamo is a differentiated genomic medicine company focused on treating debilitating neurological diseases

Sangamo THERAPEUTICS

Potent zinc finger epigenetic regulation technology, with neurology programs advancing towards the clinic

Industry-leading AAV capsid discovery platform enabling non-invasive intrathecal and intravenous delivery to the brain

Powerful research platform **continually innovates in new modes of genome modulation** to support value creation for both wholly owned programs and potential partners

SHARP STRATEGIC FOCUS IN NEUROLOGY

Successful partnership track record with $50 million in expected near-term payments from Genentech and $220 million in potential milestone payments* from Pfizer. **Fabry partner discussions ongoing, with clear pathway to potential registration.**

OPTIMIZING ASSET VALUE

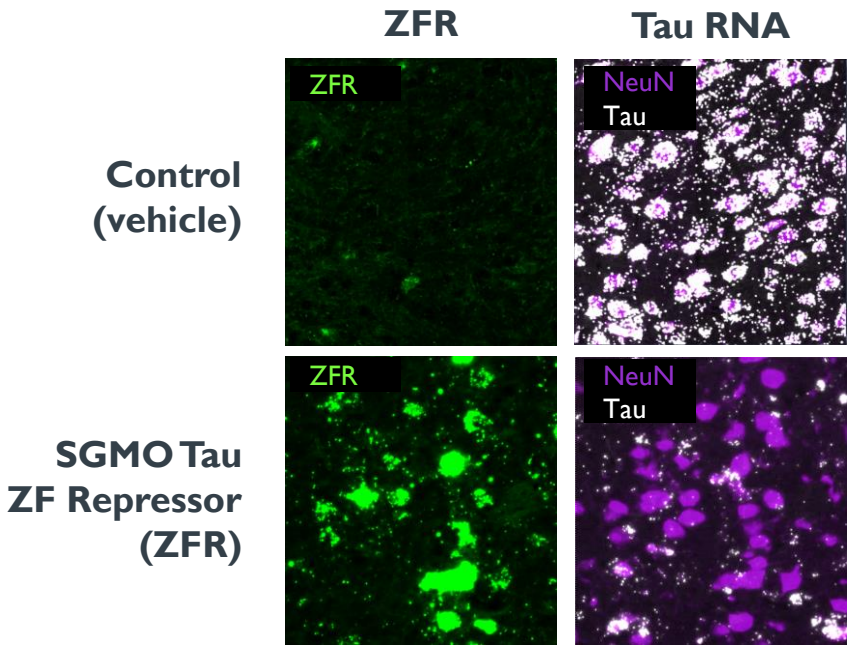
Sangamo pairs the epigenetic regulation *and* capsid delivery capabilities needed to create neurology genomic medicines

Genome-Targeting Cargo

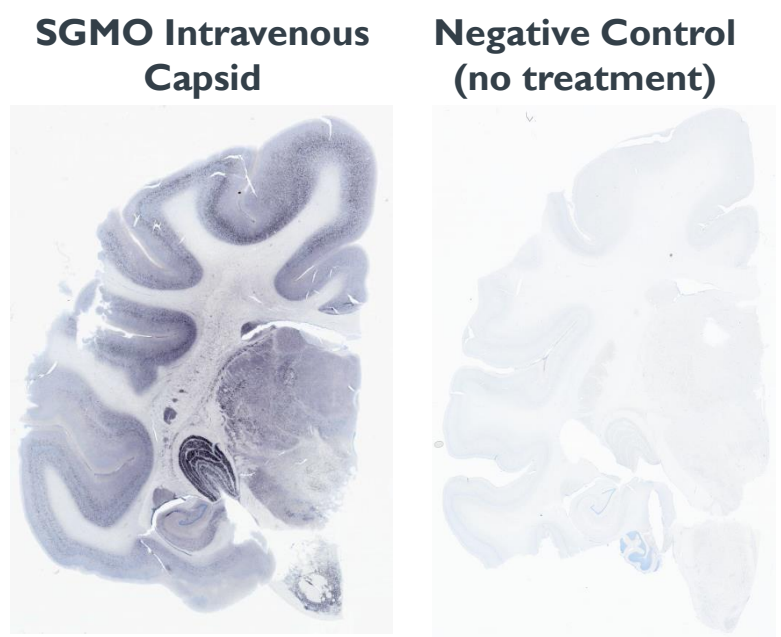
Epigenetic regulation platform

Capsid Delivery Engine

AAV capsid delivery platform via intravenous delivery



Non-human primate brainstem



Non-human primate

Future of Neurology Genomic Medicines

Company pipeline and business development opportunities

CORE NEUROLOGY PIPELINE

Indication	Preclinical	Phase 1/2	Pivotal	Partner	Commentary
Chronic Neuropathic Pain *(Nav1.7)*	*Data presented at ASGCT 23*			-	Nav1.7 IND-enabling activities continue to advance
Prion Disease	*Data presented at ASGCT 24*			-	Prion CTA-enabling activities continue to advance
Tauopathies	*Data presented at ASGCT 24*			Genentech A Member of the Roche Group	**August 2024:** Announced epigenetic regulation and capsid delivery license agreement with Genentech
Undisclosed				Genentech A Member of the Roche Group	
ALS/FTD	*Data presented at ASGCT 24*			Alexion AstraZeneca Rare Disease	
Huntington's Disease				Takeda	

OTHER PROGRAMS

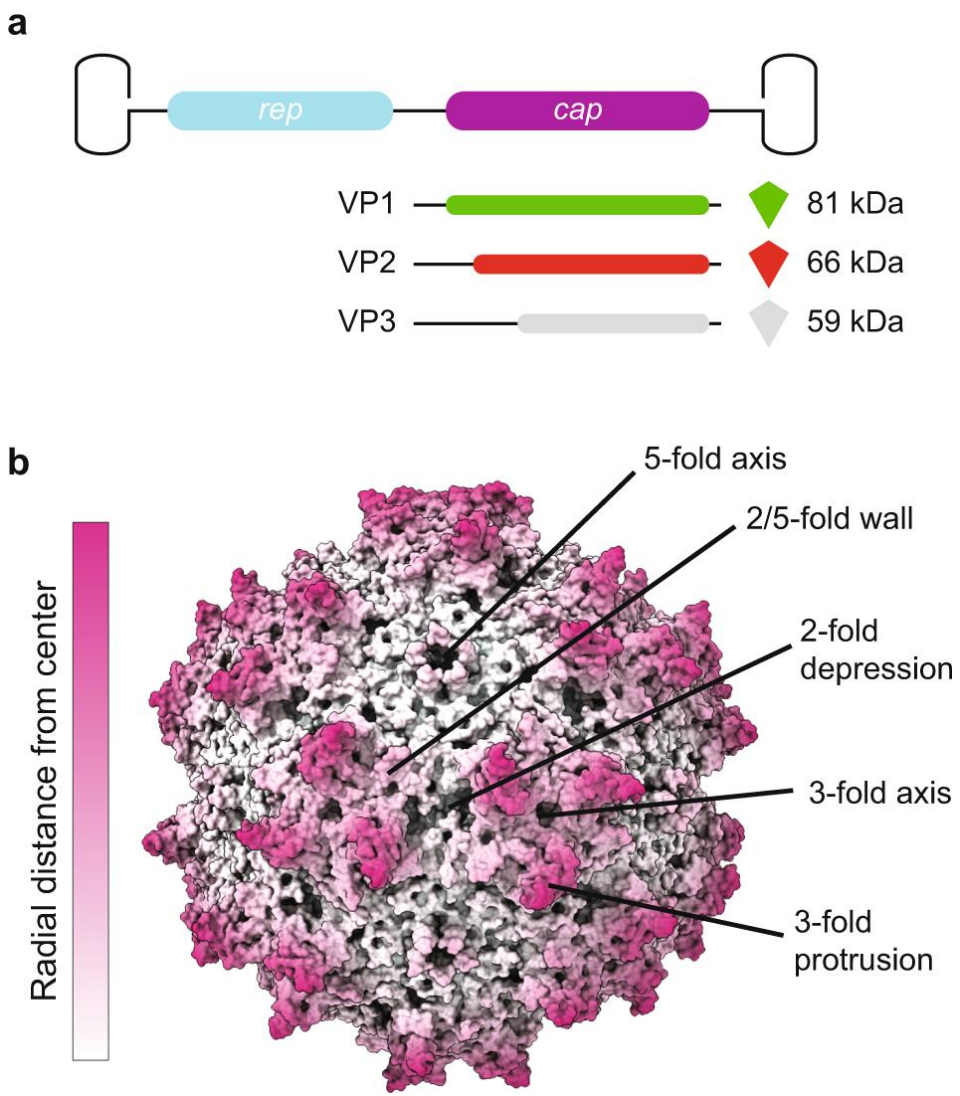
Indication	Preclinical	Phase 1/2	Pivotal	Partner	Commentary
Hemophilia A (Giroctogene fitelparvovec)	*Data presented at ASH 2023*			Pfizer	**July 2024:** Positive topline readout in Phase 3 AFFINE trial. Pfizer plans to discuss data with regulatory authorities in coming months.
Fabry Disease (Isaralgagene civaparvovec)	*Data presented at WORLDSymposium 2024*			-	Continue to amass encouraging clinical data. Potential partnership discussions ongoing.

CQA role in meeting the challenges of Gene Therapies

slide courtesy of Global Genes " Allies in Rare Diseases"

Common Challenges to Control Strategies in CGT products

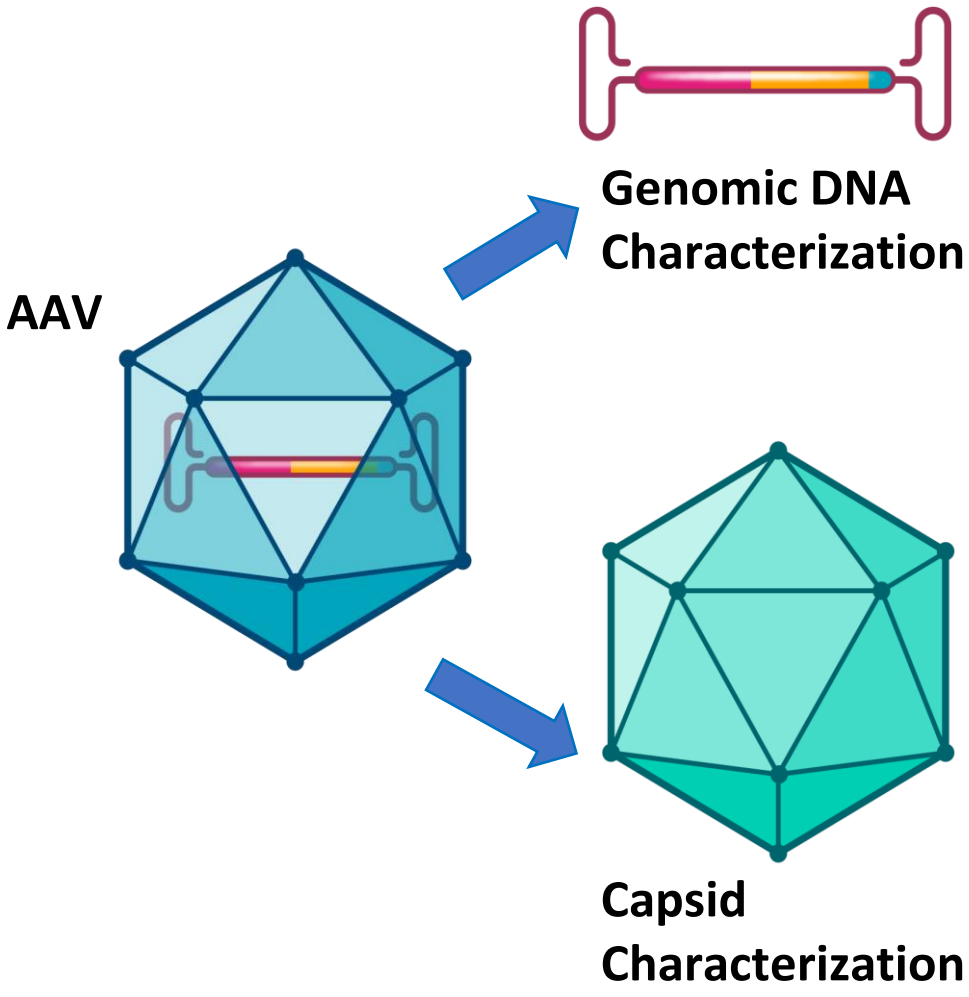
- Limited understanding of CQAs for Novel CGT modalities (AAV, LV)
- Low # of lots (development/clinical) resulting in limited process and product knowledge
- Analytical methods evolve during clinical development and retains are essential to perform analysis
- Lack/shortage of process representative material for critical analytical assays
- Compressed timelines for development



slide courtesy Santosh Khatwani and Michael Molony

Worner *et. al.*, Nature Communications, 2021

Analytical Strategy for AAV Products



1. Precise/Fast Genomic Titer or another rapid dose determining assay
2. Genomic integrity
3. AAV DNA modifications
4. Partial DNA - size and **identity**

***CURRENT TECHNOLOGIES:**

qPCR/ddPCR, multiplex PCR, SR-NGS, LR-NGS, CDMS, IP-RP-HPLC, Capillary Electrophoresis

1. Capsid Identity / Concentration
2. Empty, Partial, Full AAV
3. Infectivity/Transduction
4. Viral Capsid Protein Subunit Ratio
5. Capsid Post Translational Modifications
6. Aggregates & SVPs
7. Higher order structure **(viral assembly conformation changes)**

***CURRENT TECHNOLOGIES:**

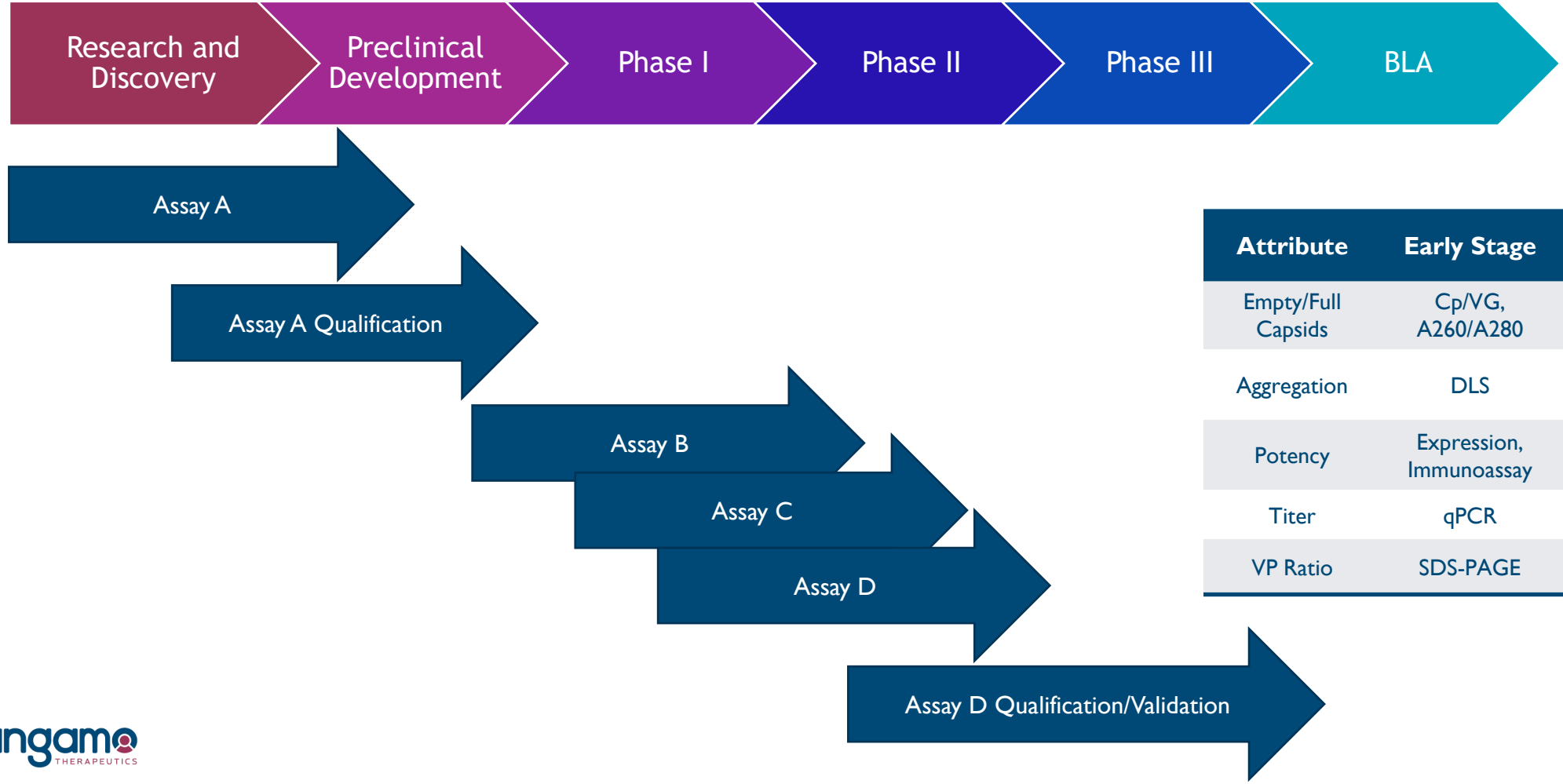
Standard protein analytical technologies—LC/MS, mass photometry, CDMS, AUC, gene expression ELISAs, SEC, A4F-MALS, CE, SDS-PAGE, CrytoTEM, etc

Not so standard for protein analytics: TCID-50 (infectivity) and/or gene expression by PCR

Sangamo THERAPEUTICS

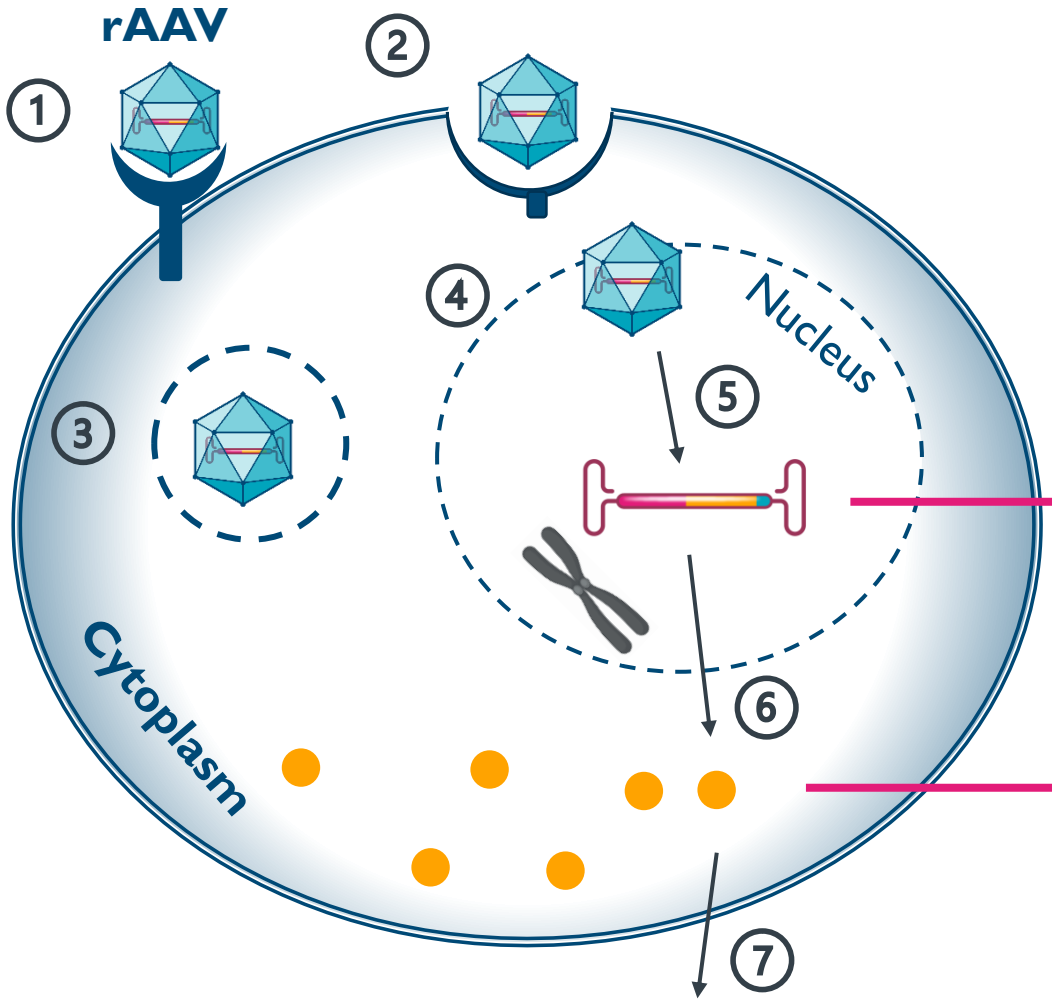
Evolution of Methods

- **Analytical methods evolve during clinical development**
- **New/improved methods are implemented**



Attribute	Early Stage	Late Stage
Empty/Full Capsids	Cp/VG, A260/A280	TEM, AUC, IEX, MS
Aggregation	DLS	SEC-MALS, FFF-MALS
Potency	Expression, Immunoassay	Biological Function Assay
Titer	qPCR	ddPCR
VP Ratio	SDS-PAGE	CE, Labchip

Potency Assay Matrix Approach



Infectivity Assay

Expression Assay

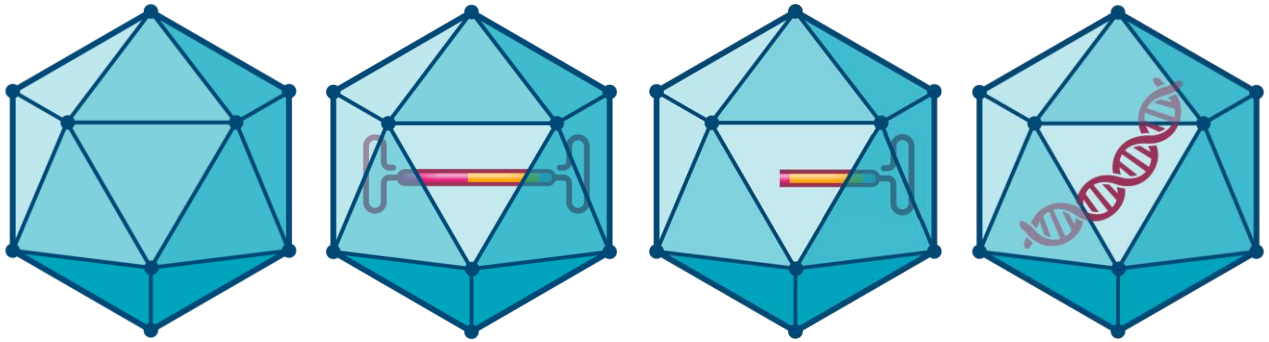
Biological activity → Functional Potency Assay

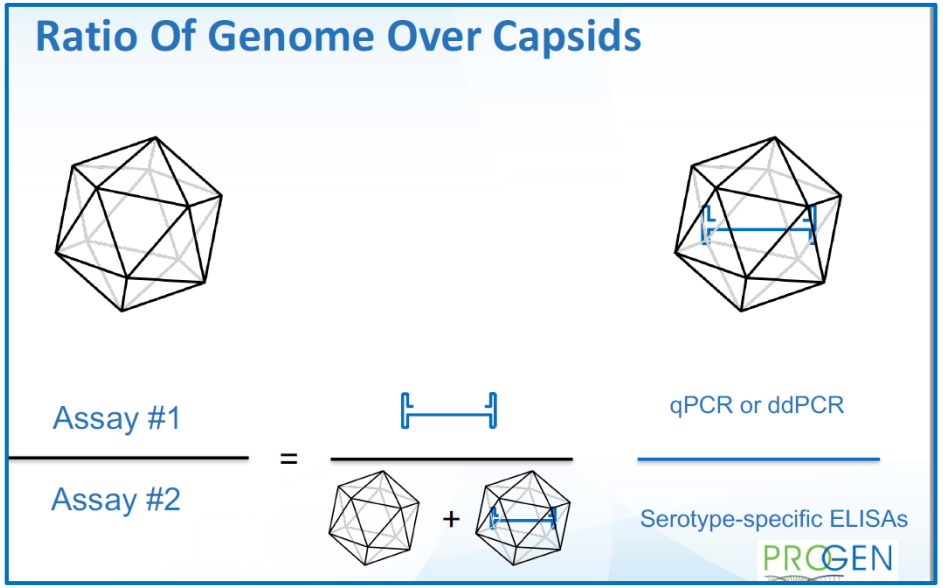
Case Study 1: Control Strategy for Analytical Methods Measuring a CQA

Example applies to the following Control Elements:

- Release Testing
- Stability Testing
- In-process Testing
- Product Characterization

Empty Capsids as CQA





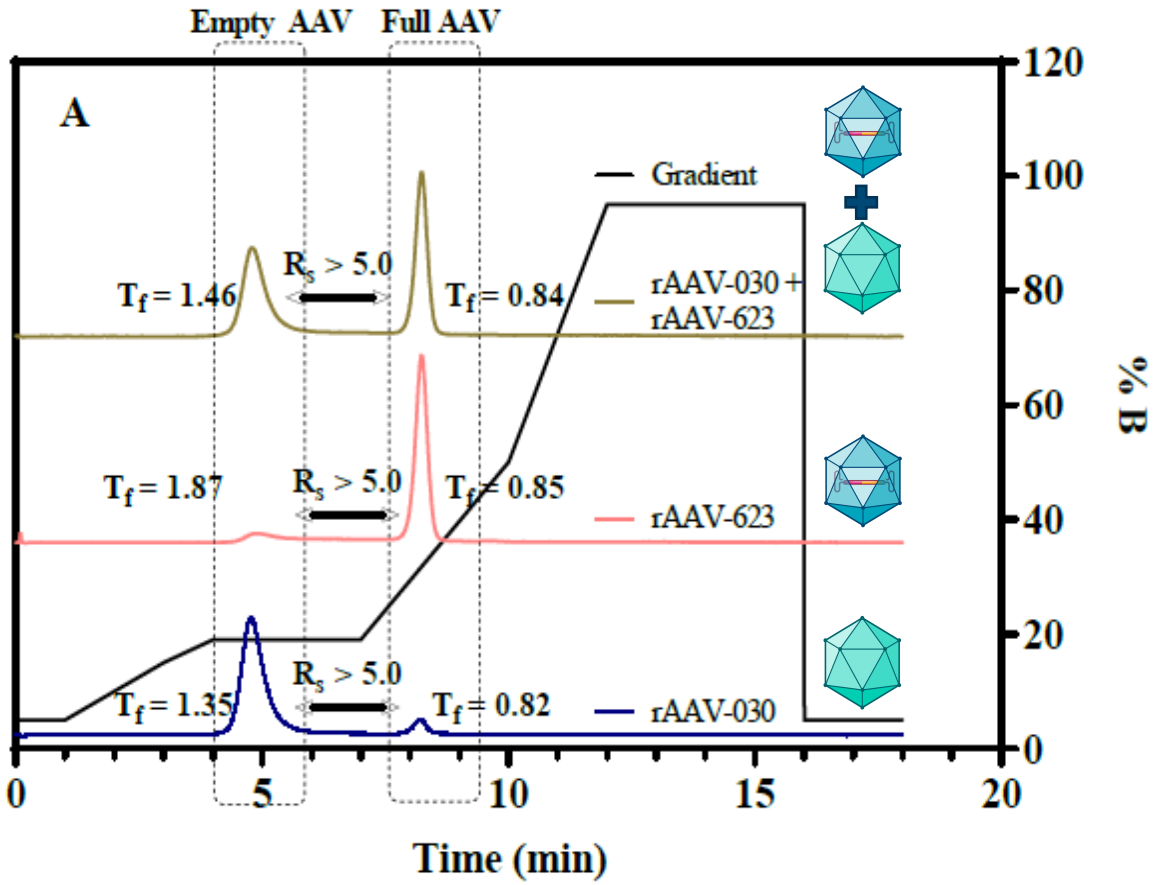
- ***AAV Empty Particles – Can be one of the major product-related impurities***
- ***May act as decoys against neutralizing antibodies***
- ***Has potential immunological consequences***
- ***Higher production cost if significant amount of empty capsids are present***
- ***Important to characterize the AAV preparations***

Cp/vg = 1 (Ideal)

Empty Capsids as CQA

Technology	Assay	Procedure	Advantages	Challenges
Calculation	Capsid Titer and VG Titer	Ratio	Fast TAT	Theoretical number; Highly variable as values are obtained from two different assays
OD	OD260 and OD280	Ratio	Fast TAT; QC-friendly	Highly pure product needed; Assay is susceptible to contaminants
CryoTEM	Staining of capsids	Visual test, software calculates full/empty	Slow TAT	Partial capsids are difficult to measure; Low resolution
AUC	Sedimentation velocities	Ultracentrifugation	Slow TAT	Sedimentation coefficients have to be significantly different for full and partial
IEX-HPLC	Ion exchange separation	HPLC	Fast TAT, QC-friendly, HTP	Partials are difficult to separate
Mass Spectrometry	CDMS	HPLC/MS	Medium TAT	Expensive equipment

Sangamo's Proprietary AEX-HPLC Approach for E/F Analysis



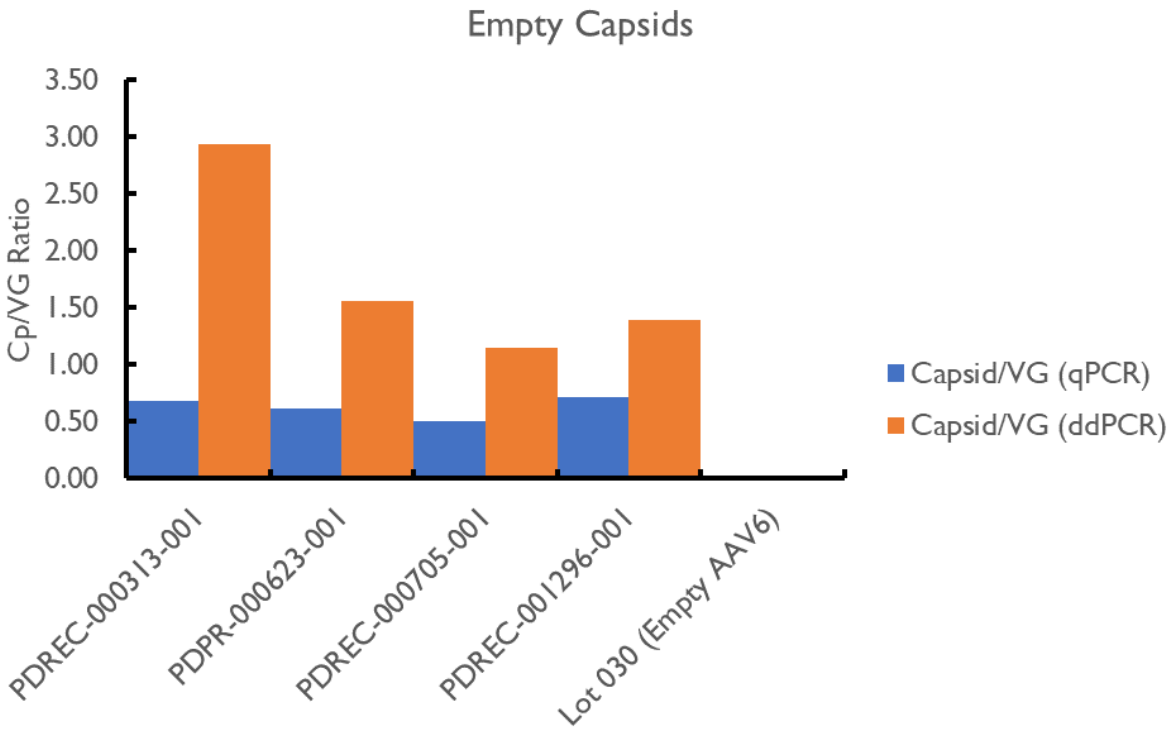
1. Modular/Adaptable Discontinuous Gradient Approach – Isocratic Hold and Two linear gradients
2. High resolution for empty from full capsids (>>> 2.0)
3. Low Peak Tailing for both empty capsids (< 2.0)
4. Retention times < 2% CV

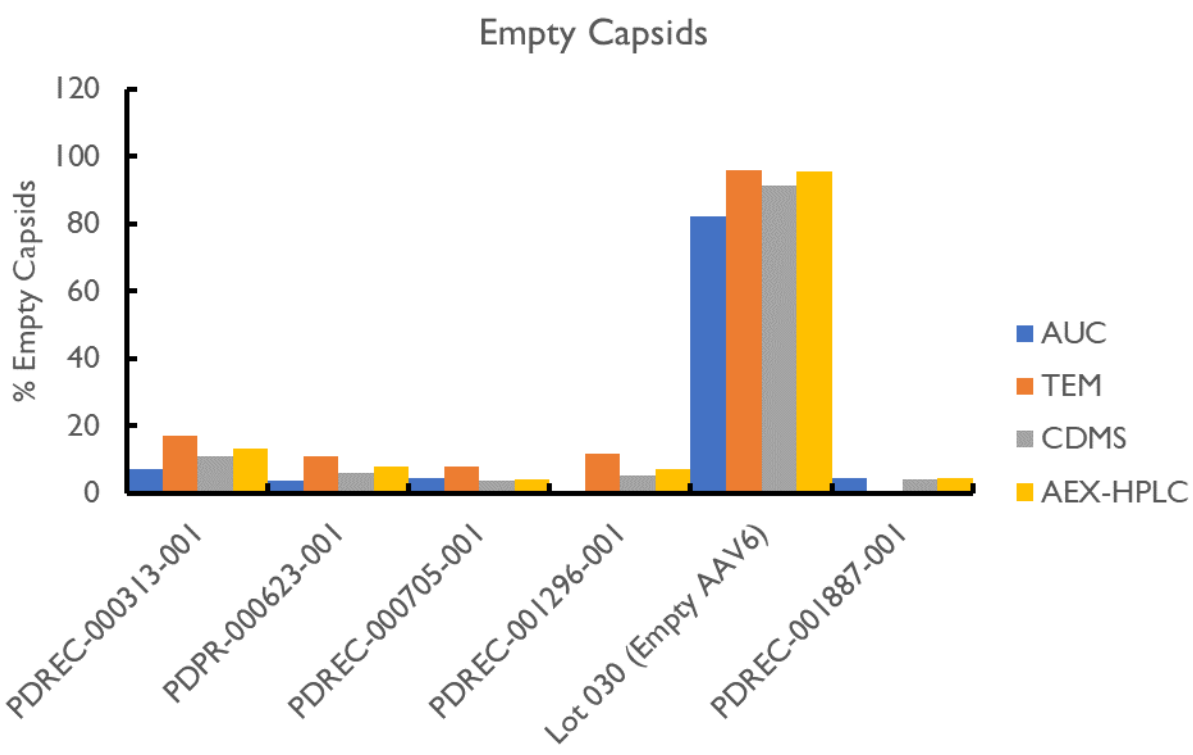
Rs = USP Resolution
Tf = Tailing Factor

Khatwani *et. al.*, Patent (US20210009964)
Khatwani *et. al.*, Mol. Ther. Methods & Clin. Dev., 21, 548-558, 2021

Empty Capsids as CQA

- *5 development lots for same product along with an empty AAV lot used with multiple methods*
- *Each lot manufactured with a process change*



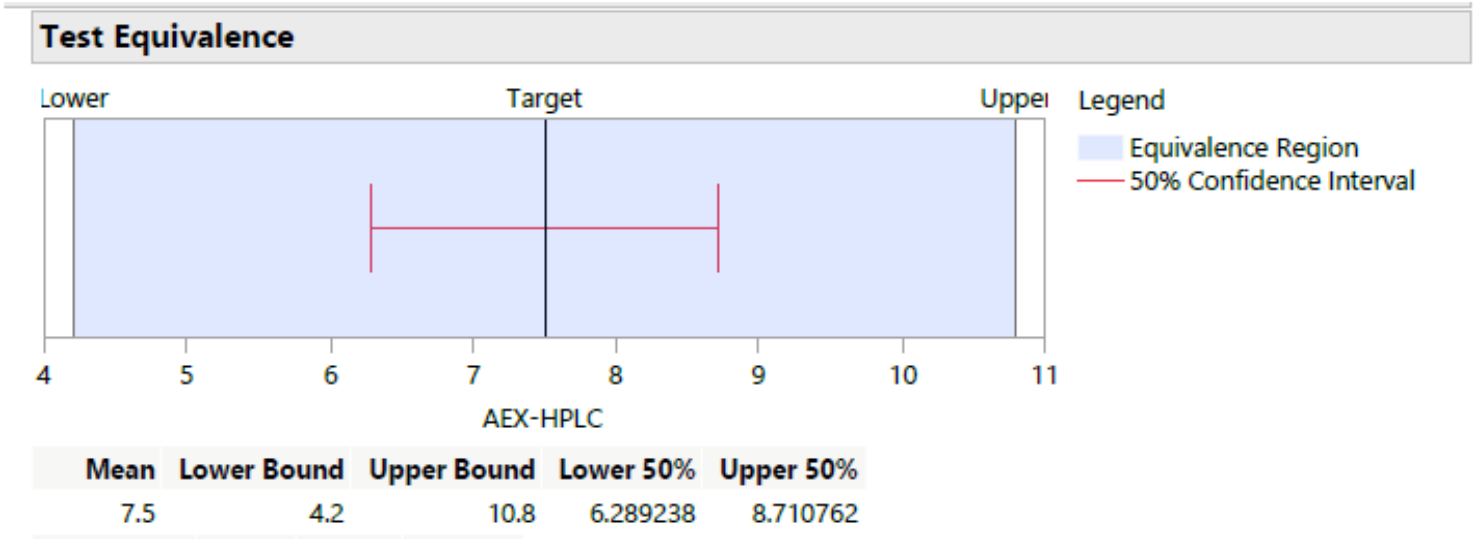


Are methods and lots comparable enough to monitor the CQA?

- ***Example Criteria:***

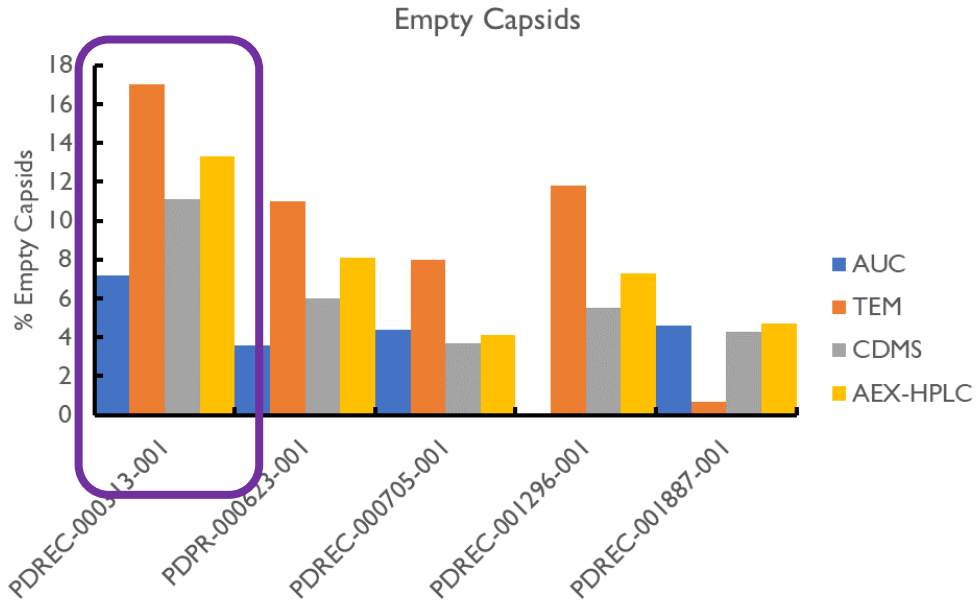
Difference that is practically zero = 50% near LOQ (Based on SME input)

α = 0.25; Confidence level = 1 - α = 0.75

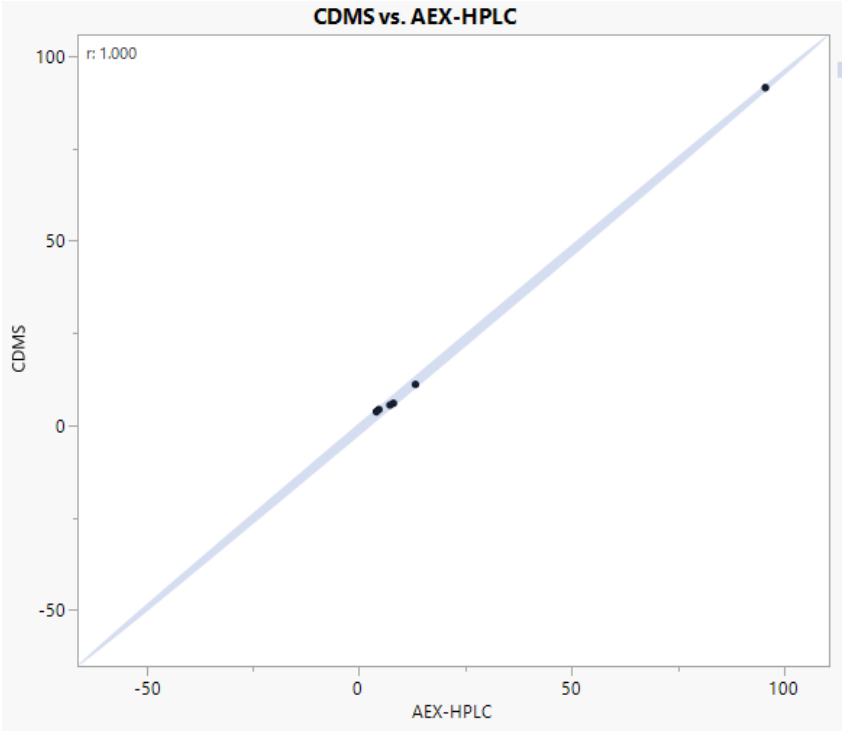


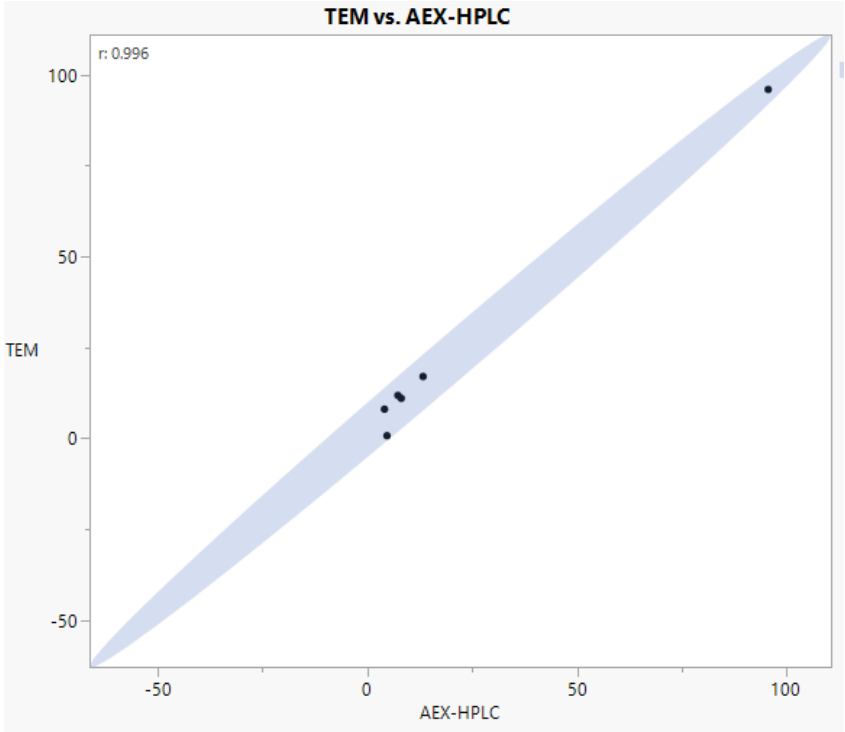
Mean	Lower Bound	Upper Bound	Lower 50%	Upper 50%
7.5	4.2	10.8	6.289238	8.710762

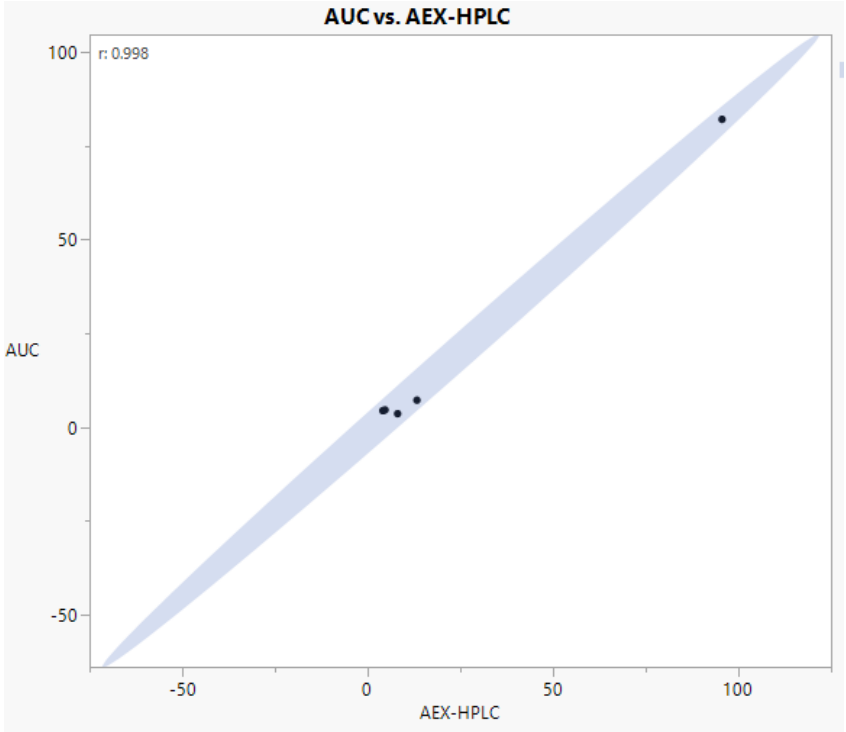
Technology	Lower Bound	Upper Bound	Outlier Lots
AUC	2.5	7.5	PDREC-000313-001
TEM	4.8	14.6	PDREC-000313-001
CDMS	3.0	9.2	PDREC-000313-001
AEX-HPLC	4.2	10.8	PDREC-000313-001



Orthogonal Characterization of Empty Capsids - Bivariate fit analysis

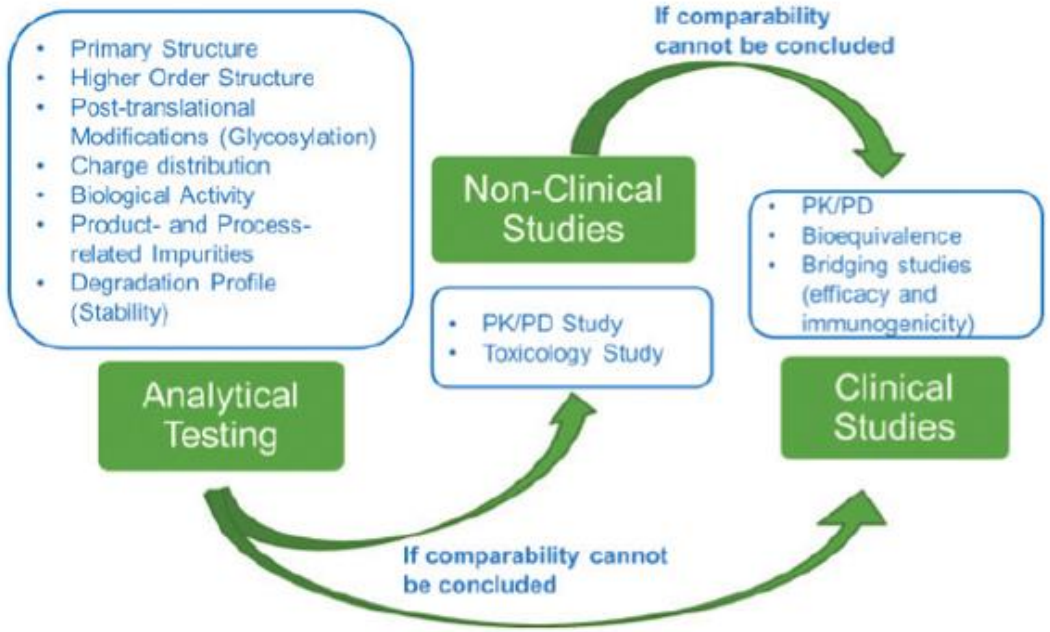






Strong correlation (r>0.99) for AEX-HPLC vs all orthogonal assays with strongest correlation against CDMS

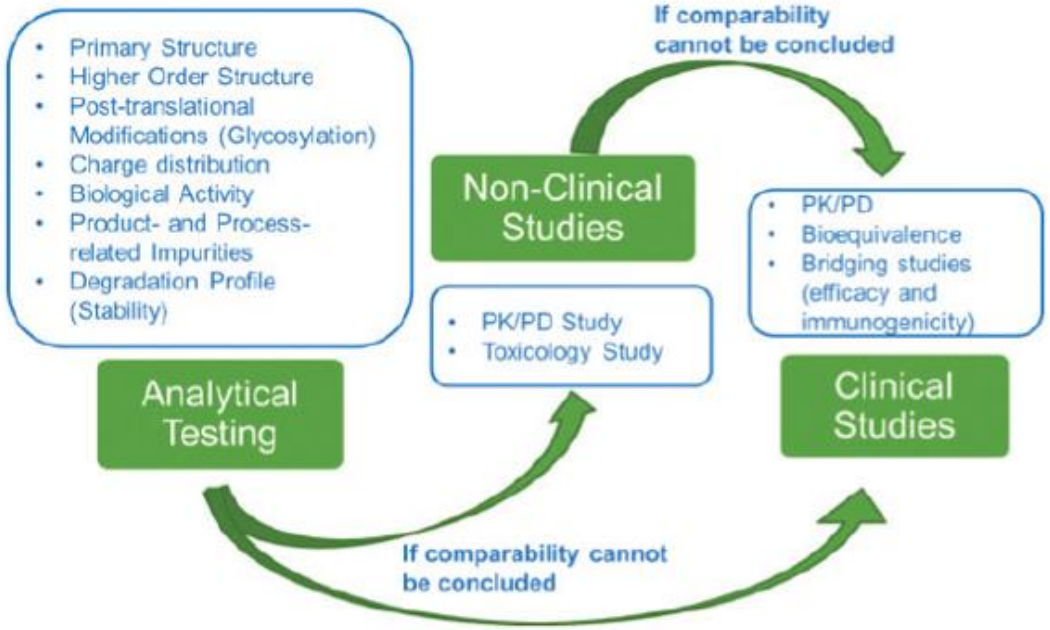
Case Study 2: Control Strategy for Process Change Leveraging Analytical Comparability



Comparability Study

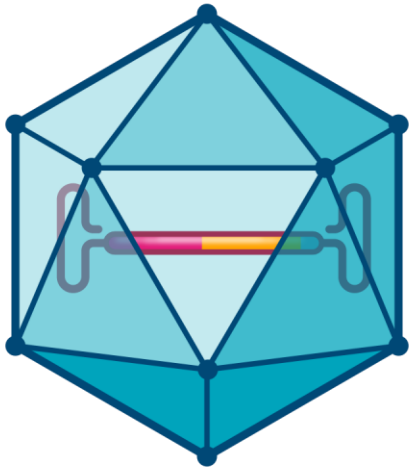
- **What? – Impact assessment of changes in manufacturing processes for any therapeutic modality (i.e., FDA = Major Change, EMA = Type II variation)**
- **Why? - Demonstrate pre- vs post-change materials are comparable**
- **How? - Design varies with the stage of product development (Early, Late, Post approval, and potential impact of changes)**
- **Product Comparability is a sequential process -**
  - **Analytical Comparability – Comparison of Quality attributes**
  - **Non-clinical Comparability – In vitro and In vivo tests, PK/PD**
  - **Clinical Comparability – Clinical bridging**

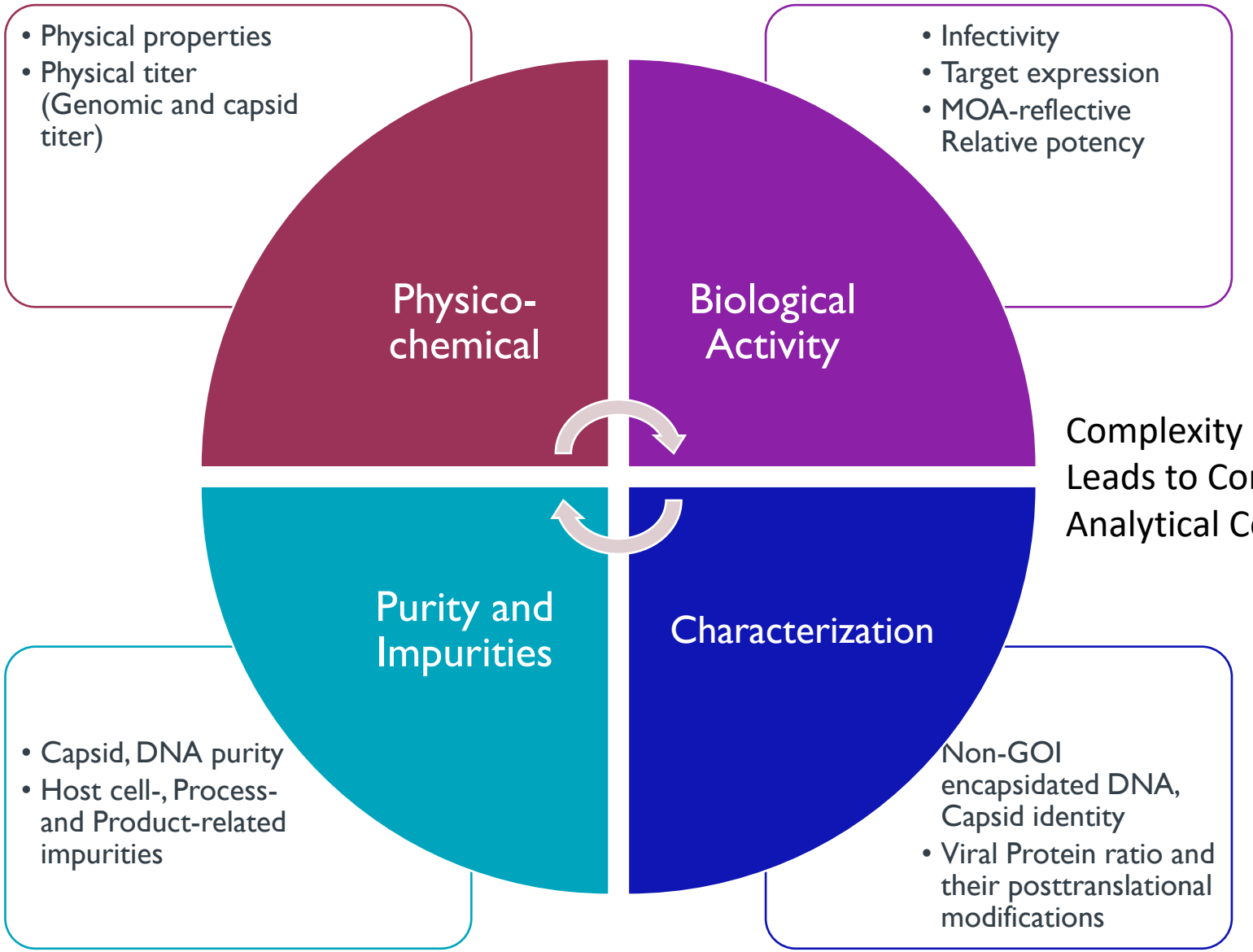
Risk Based Comparability for Complex Molecules during Expedited Development: Leveraging Enhanced Technology and Regulatory Mechanisms – Armando et al; Am. Pharm. Review, 2018



References:
*ICHQ5E and
*Doc. Ref. EMEA/CHMP/BMWP/101695/2006

Adeno-associated Virus - Potential Critical Quality Attributes (pCQAs)





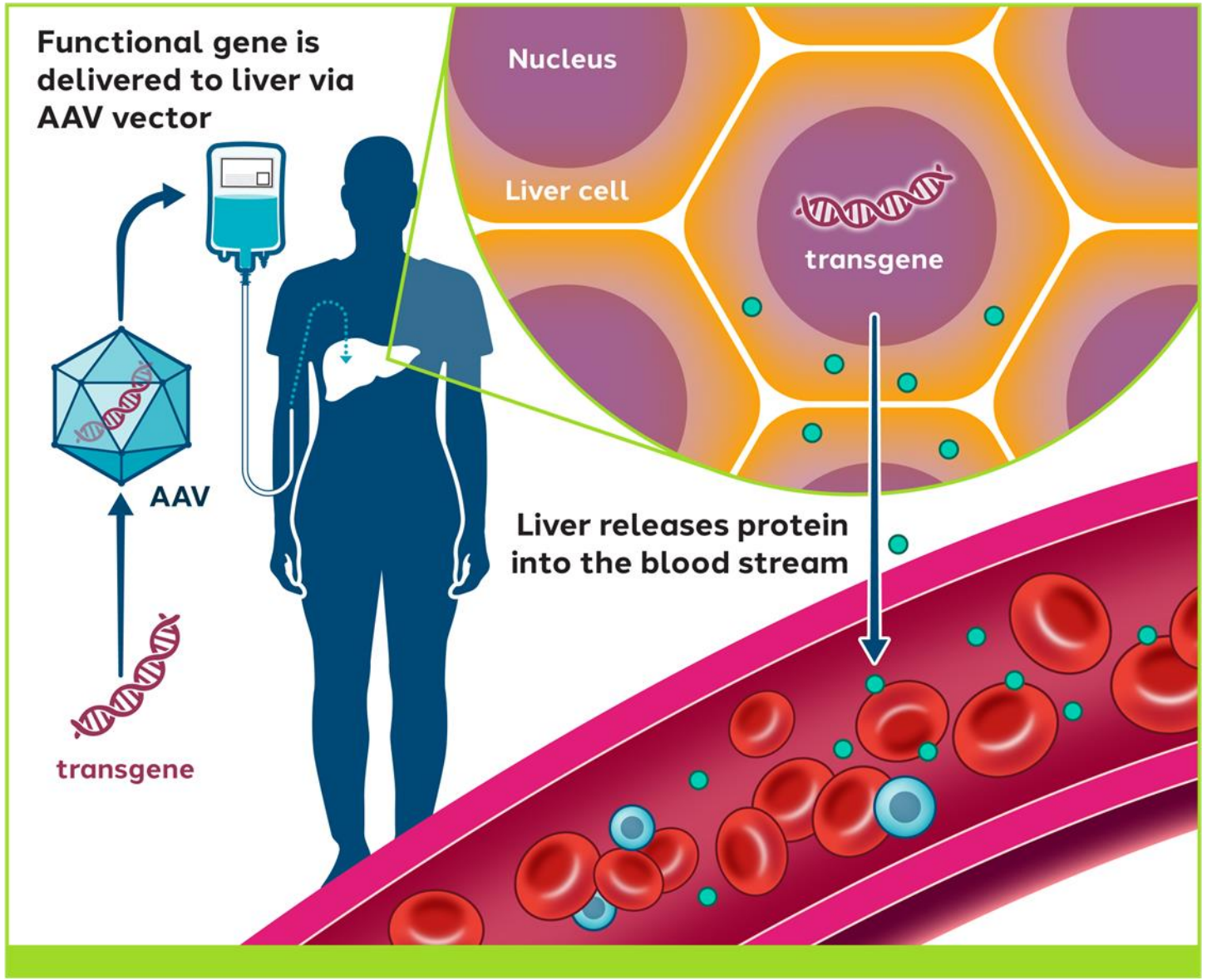
Complexity of the Molecule Leads to Complexity of the Analytical Control Strategy

Ramsey, Khatwani *et al* Current Opinion in Biomedical Engineering, **20, 2021**

Sangamo THERAPEUTICS

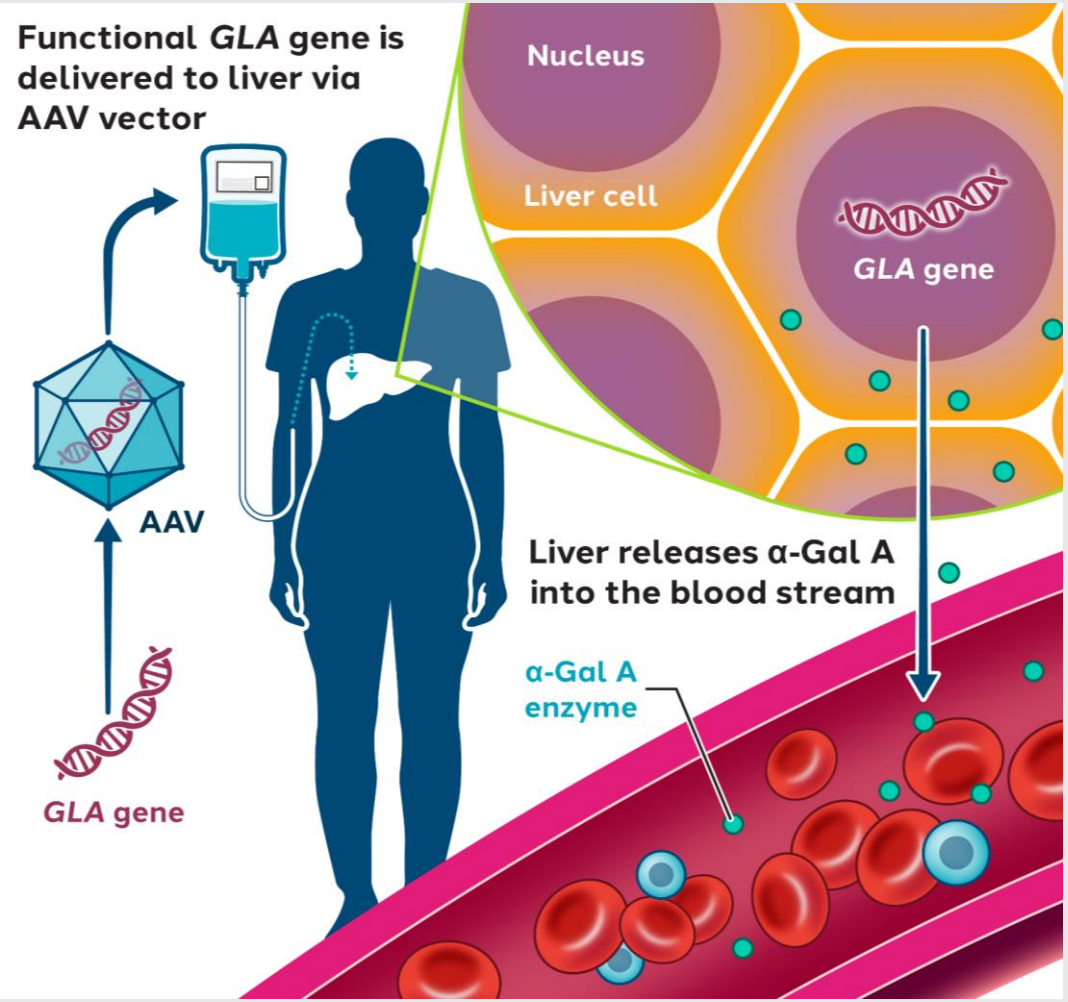
Case Study for Late-Stage Product

Systemic delivery of AAV vectors allows *in vivo* correction of monogenic disease



Fabry Disease: isaralgagene civaparvovec (ST-920)

Abbreviated clinical pathway supports efforts to secure a collaboration partner



- Largest gene therapy program in Fabry disease
  - Enrollment, screening and dosing complete in Phase 1/2 STAAR study – 33 patients total
  - 17 of 18 patients off Enzyme Replacement Therapy (ERT)*
- Compelling clinical data
  - Continue to amass encouraging clinical data, including evidence of improvements in kidney function.
  - In18 patients treated >1yr, observed a statistically significant rise in both mean and median eGFR levels.
  - Updated clinical data expected in the coming months.
- FDA alignment on abbreviated regulatory pathway
  - Aligned on a single-arm study with up to 25 patients, alongside confirmatory evidence, as an acceptable pathway to BLA
- Held productive meeting with EMA on regulatory pathway
- Received EMA PRIME eligibility and UK MHRA ILAP status

Sangamo THERAPEUTICS

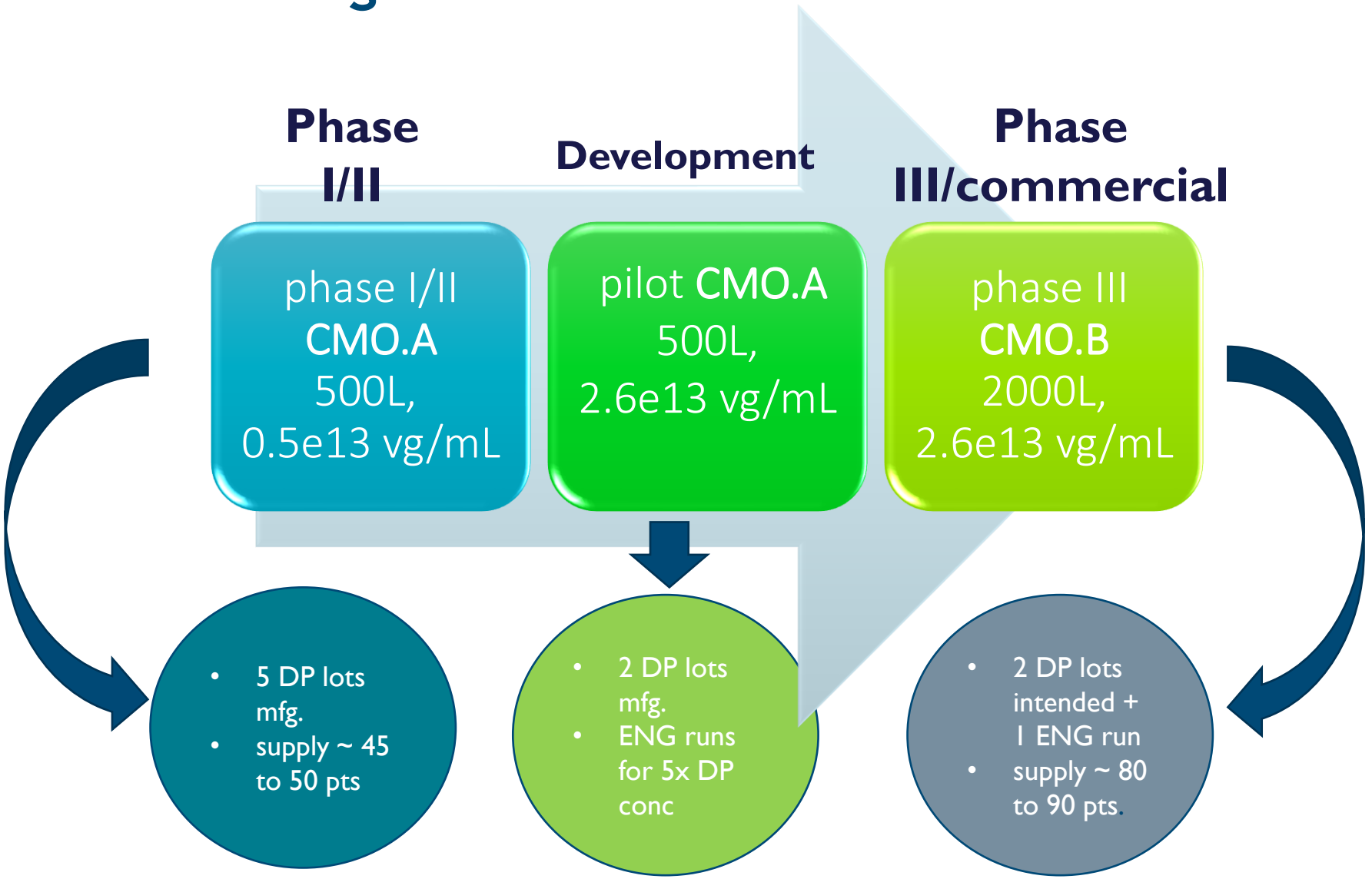
*As at August 6, 2024

Challenges in CMC Development

Understanding the Target Product Profile

Value proposition	• **e.g. what differentiates the therapeutic from current Standard of Care?**
Indication	• Disease or condition to be addressed
Studied populations	• Male and female • Adults or adolescents • Prior therapy or orphan indication
Efficacy: primary	• Clinical end-points
Efficacy: secondary	• Clinical end-points
Safety & Tolerability	• Adverse events, infusion reactions
Dose, ROA, Regimen	• Target dose X E13 vg/kg • Administered by IV infusion in outpatient hospital setting
Storage & Handling	• Shipped and stored at ≤-65^{0}C • Vials thawed at RT or at 4^{0}C for duration • ≤ # of vials per patient dose

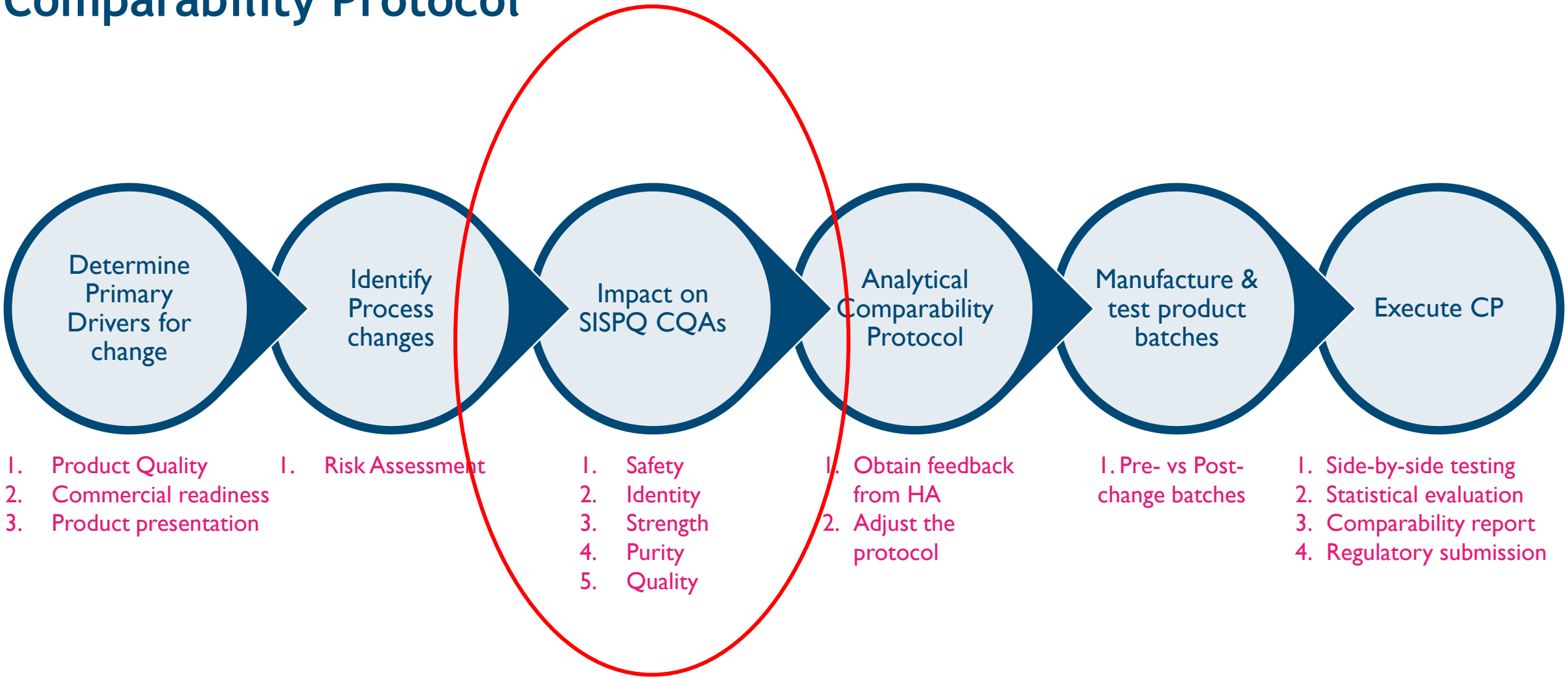
Transitioning from Phase I/II to Phase III



- **5x increase in DP conc.**
- **Formulation change**
- **Scale increase from a 500 L to 1700 L bioreactor to meet phase III/commercial needs**
- **Site-change from CMO.A to CMO.B**

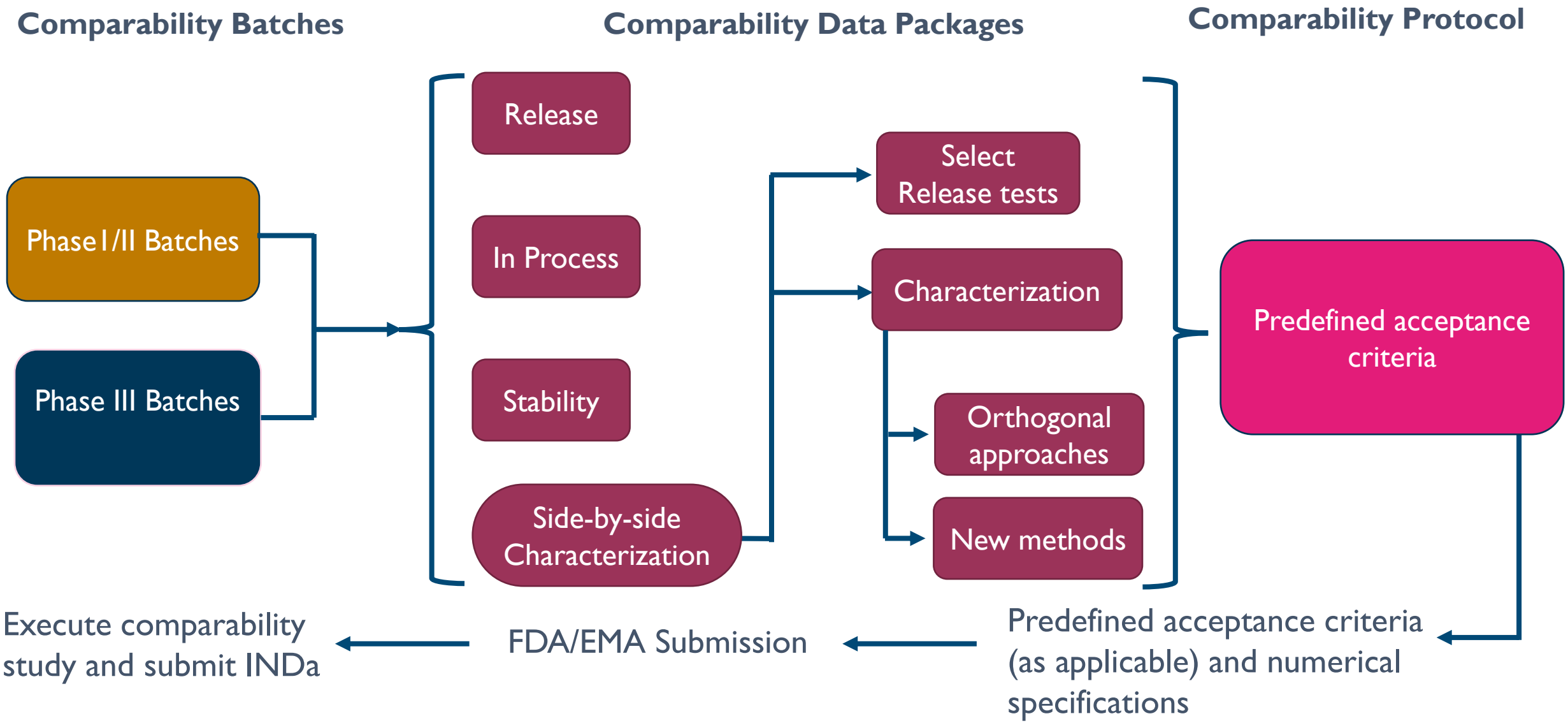
successful type C meeting on comparability and phase III plans with the FDA; clear guidance was obtained

Key Steps in Generating and Executing an Analytical Comparability Protocol



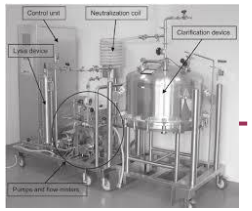
SISPQ – Safety, Identity, Strength, Purity, Quality

Comparability Protocol - Overall Roadmap



slide courtesy Santosh Khatwani and Michael Molony

Sangamo AAV Manufacturing Process Platform via Sf9



AAV Vector Drug Substance

Upstream

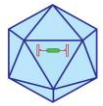
- Working Cell Bank
- Thaw WCB
- Start Cell Expansion
- End of Cell Expansion
- Infect with WBIICs
- Harvest
- Lysis & Clarified Harvest
- Filtration

Helper WBIIC Bank

Transgene WBIIC (ZFP-TF, cDNA)

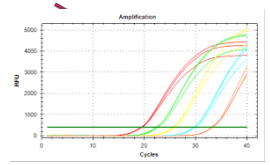
Downstream

- Detergent Treatment
- Affinity Chromatography
- Ion Exchange Chromatography
- Nanofiltration
- Concentration
- Formulation & Sterile Filtration
- Bulk Fill & Freeze
- AAV Vector Drug Substance



AAV Vector Drug Substance

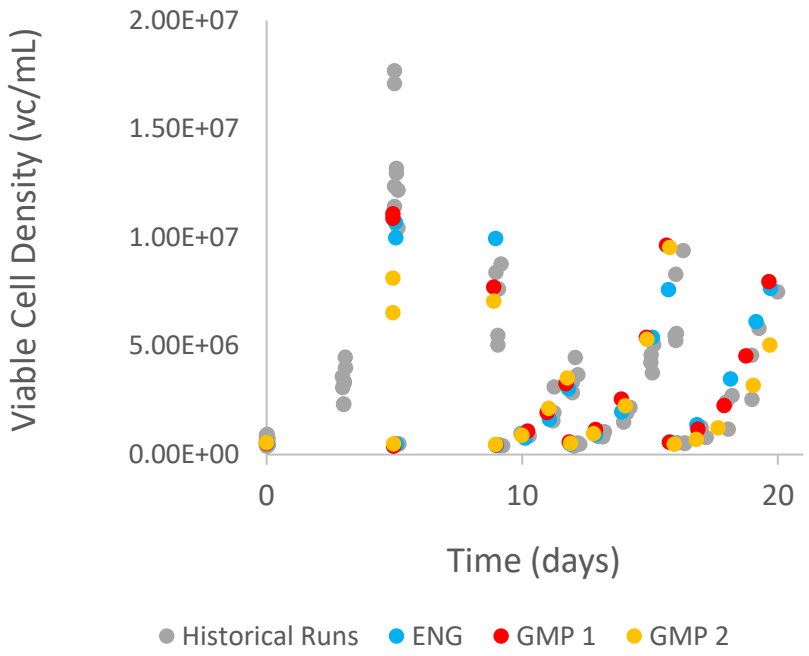
- AAV Vector Drug Substance
- Thaw
- Sterile Filtration
- Fill & Freeze
- Test & Release
- AAV Vector Drug Product

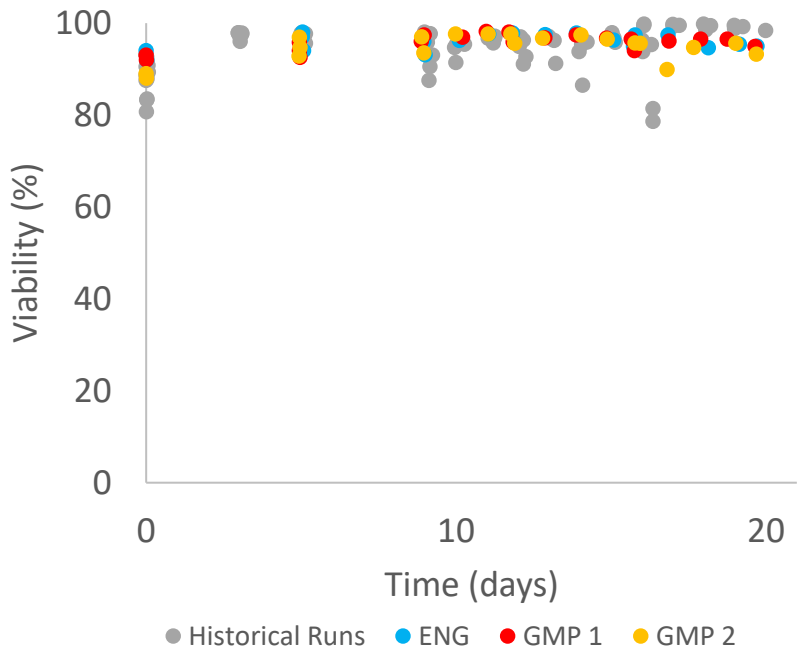


Sangamo THERAPEUTICS

Process Performance Evaluation - Phase I/II vs. Phase III

Seed Train Process Performance



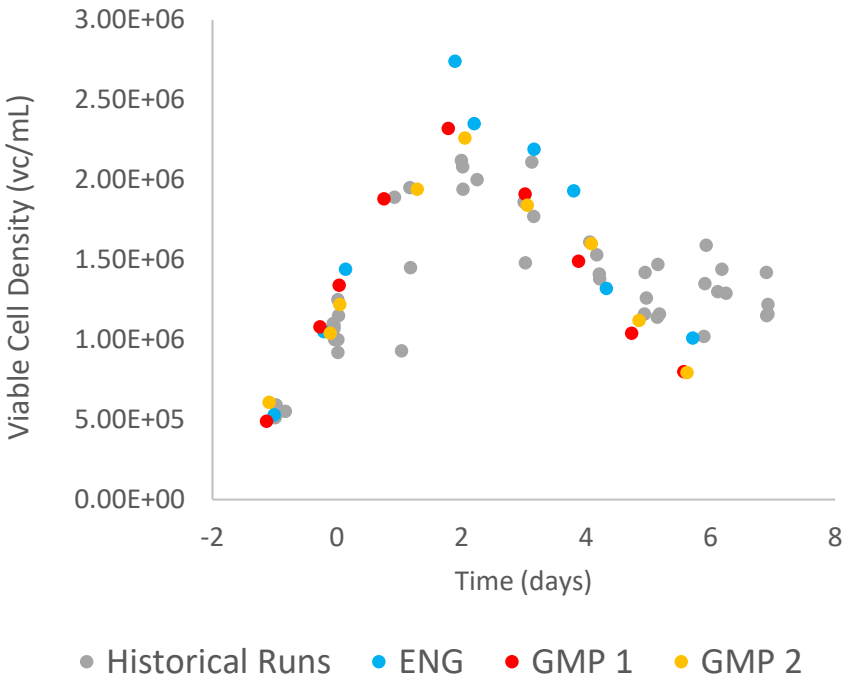


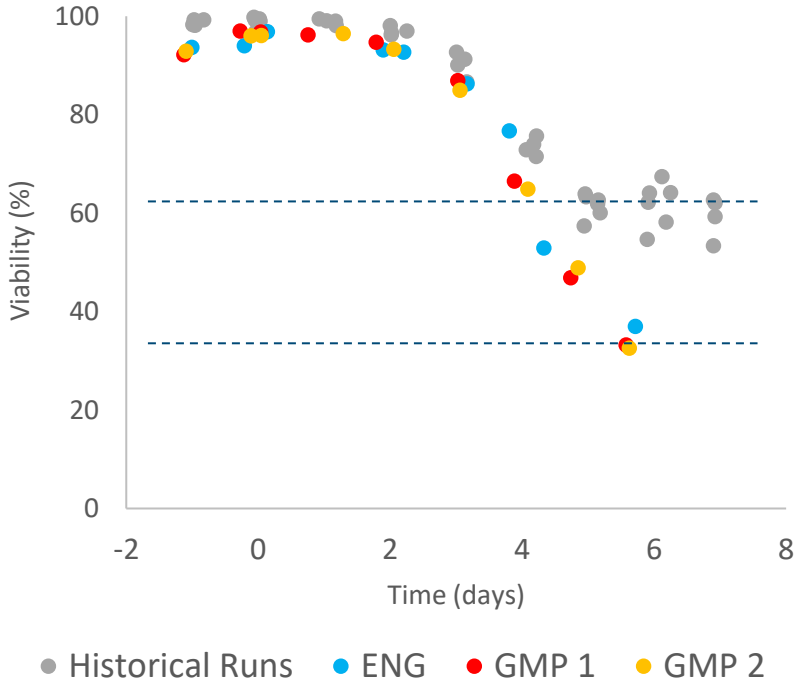
- Process performance across historical phase I/II runs at CMO.A demonstrated process consistency with phase III runs at CMO.B
- VCD and Viability trends in production bioreactor similar between CMO.A and CMO.B

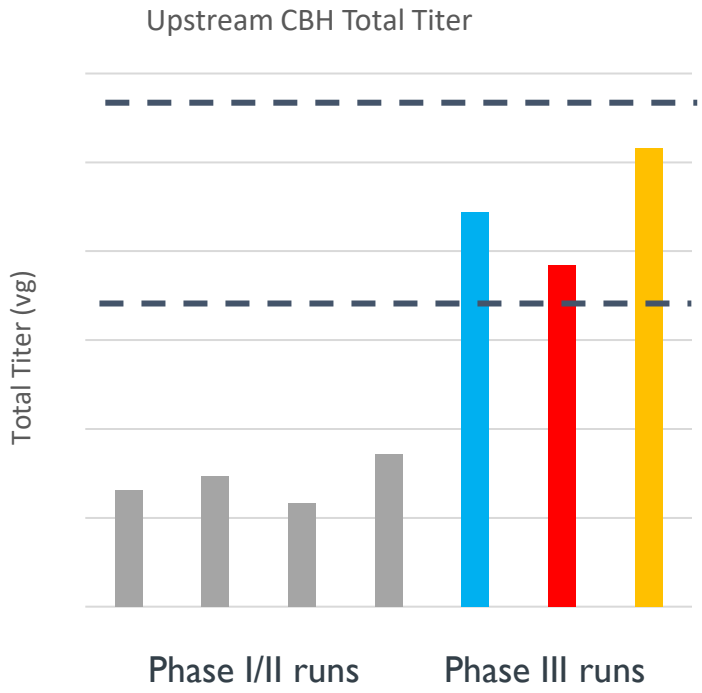
slide courtesy Anthony Chikere

Process Performance Evaluation - Phase I/II vs. Phase III

Production Reactor Process Performance







- Cell viability at harvest consistent between CMO.B during Eng., GMP 1 and 2
- The intended titer increase with phase III development was achieved

slide courtesy Anthony Chikere

Summary – CQAs enable Method Change and Comparability

- Analytical Methods along with Process should evolve during development.
- Method changes and comparability are integral processes to drug development and are supported by clear CQAs.
- Multiple assays for a single CQA can be necessary.
- Seek help from experts in the field to get it right.
- Consider platform data if available.

Acknowledgements

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James Miller

Andy Ramelmeier

Aditya Wakankar

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