Leader in AAV gene therapy

Manufacturing of Gene Therapy Products: Advances in Process Development and Scale-Up Methods to Meet Future Demand

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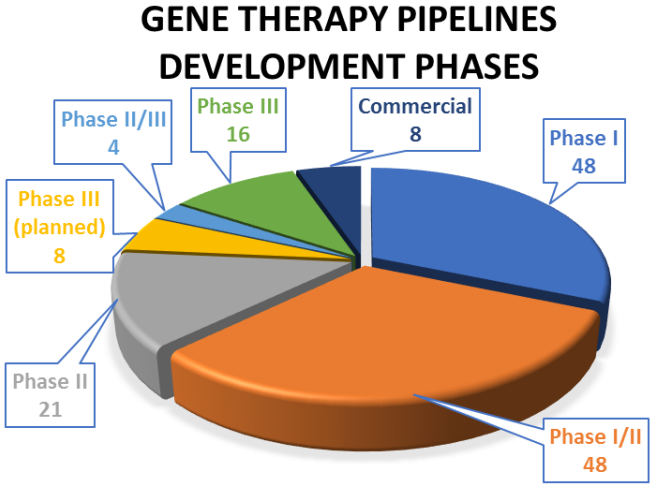
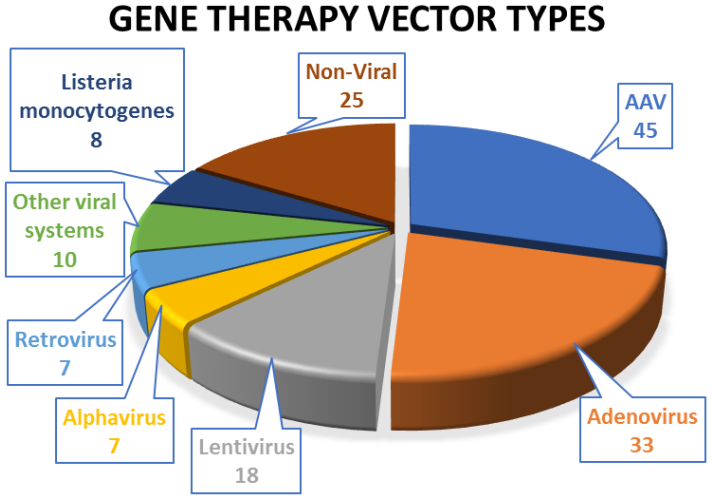
Recent Progress in Gene Therapy

- In 2017, more than 150 Gene Therapy products were in clinical stages or approved.
- Multiple products have moved into late or commercial stages.
- Increased need of development of scalable, robust and cost-effective production





Gene Therapy Market 2018-2030, Roots Analysis Report

Key Drivers for Gene Therapy Process Development and Manufacturing Improvements

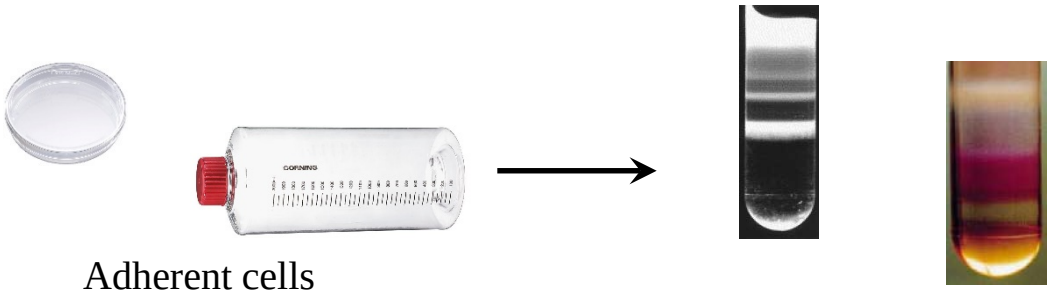
- Overall increase in vector demand
  - Studies in indications with significant patient populations
  - Global approach to commercialization
  - Targeting of Liver for specific indications
- Need for reduction in Cost of Goods
  - Cost-prohibitive processes moving to late-stage development
  - Rising prices for contract manufacturing activities
- Drive for increased purity level of final product and improved analytical methods
  - Improved analytical methods
  - Decreased level of partially full or empty capsids
  - Desire to reduce HC DNA levels

Improvement of Upstream Process for Gene Therapy

- Improvement of Upstream Processes can be broadly characterized in three ways
  1. Brute Force – increased scale of operations with existing process to reduce COGS
     - If using adherent cell lines, methods such as the Pall iCellis system provides this scalability
     - If using suspension cell lines, increased scale of operation via Bioreactors is a logical step
       - Companies progressing from 50 liters to 2000 liters
  2. More productive cell lines and media
     - Switch from adherent cell line to suspension
     - Introduce new cell line
     - Optimize media to produce more vector
  3. A combination of approaches 1 and 2
     - Requires careful integration with clinical plan and regulatory approach
     - Strong understanding of product quality attributes
     - Sound approach to comparability

Cost: Improvements in Manufacturing Processes

- Historically, academic institutions used small scale production methods with limited scalability



Adherent cells

Ultracentrifugation

- For human applications, vector doses can reach up to 3e15 vector genomes per patient – about 1,000 roller bottle equivalents.
- Therefore, the upstream process needs to be improved by use of new scalable production cell systems, and the downstream process by scalable, chromatographic based purification

Pictures from: Corning website; Sandalon, J. Virol. 2000; Zolutukhin, Gene Ther. 1999

Improvement of Downstream Processes and Analytical for Gene Therapy

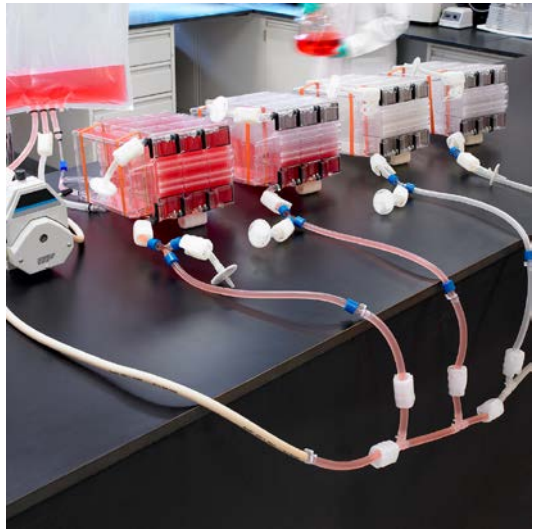
- Improvement of Downstream Processes Brute Force – increased scale of operations with existing process to reduce COGS
  - Utilization of scalable methods – transition to unit operations with linear scale
  - Development of methods and substrates to improve separations
    - Increased resolution
    - Increased throughput

- Improvement of Analytical Methods
  - Increase in product characterization and understanding
    - Stability indicating assays via forced degradation studies
  - Reduce variation and development of stat assays for production
  - Optimize potency and infectivity assays for level of detection and robustness

- Streamline Formulation and Finished Drug MFG
  - Updated vial configurations
  - Reduce the number of vials used per patient administration (single use bag technology)
  - Develop commercial formulations to remove -80 degree C storage and transport

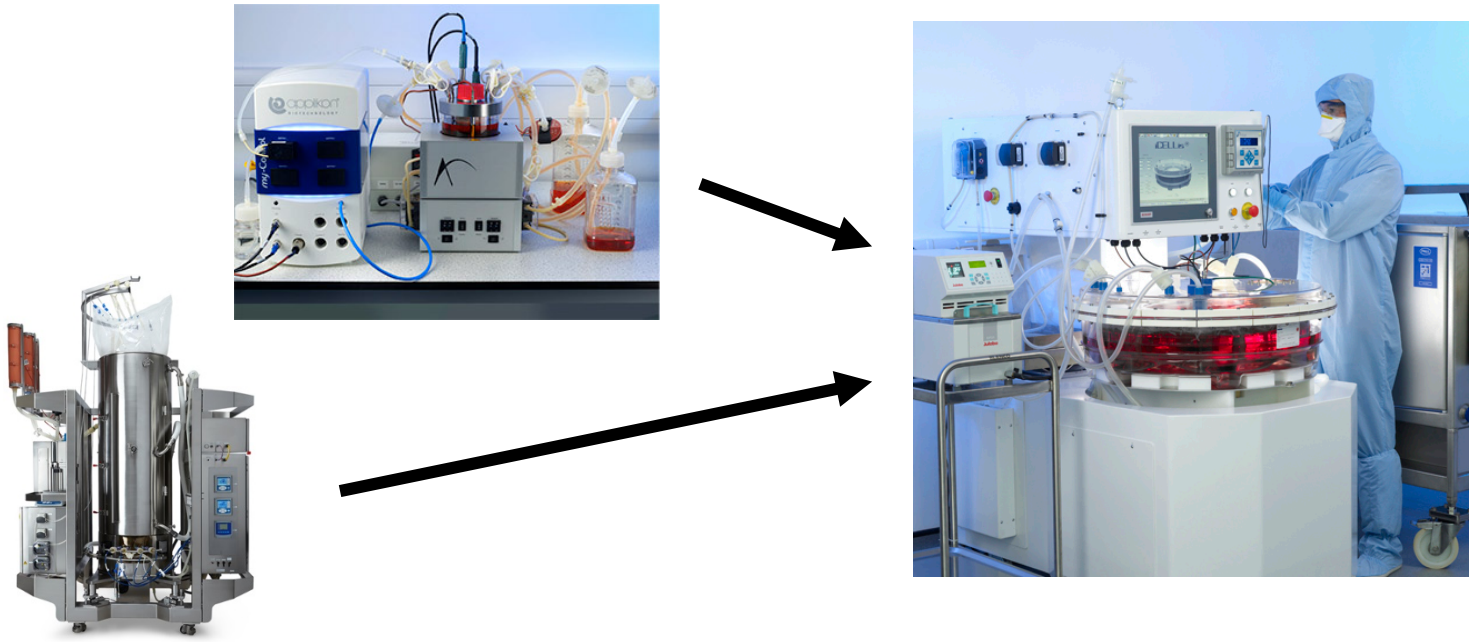
Adherent Cell Systems; Comparison, Challenges and Solutions

- Scale-out solutions to address limitations of scalability of adherent cell culture systems
  - Labor intensive
  - Limit to batch size



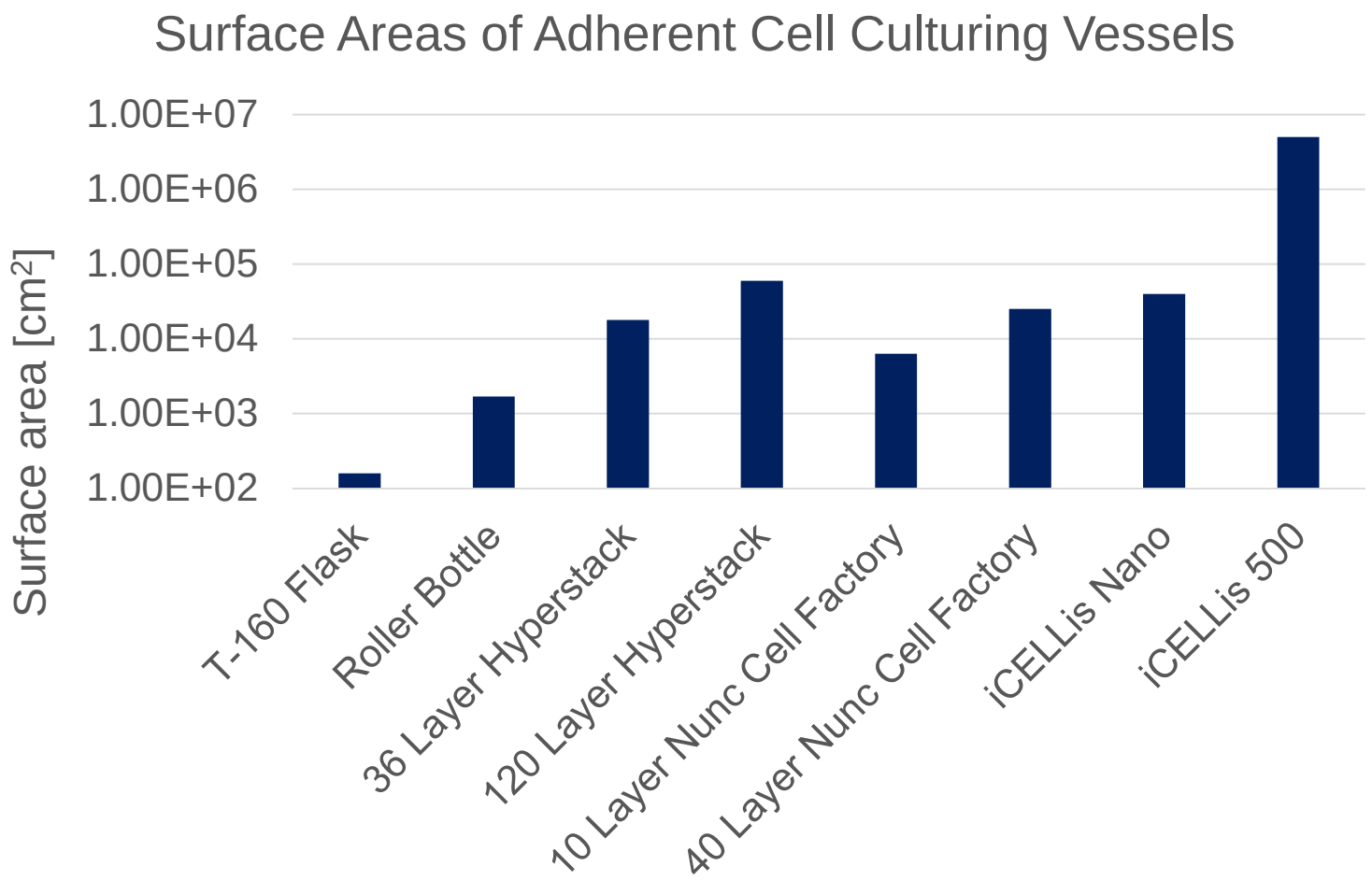
Adherent Cell Systems; Comparison, Challenges and Solutions

- Further scalability for adherent cell culture systems
  - Microcarrier systems (iCELLis) require adaptation of cells: use of scale down iCELLis Nano and leveraging on experience in adaptation of suspension bioreactors



Adherent Cell Systems; Comparison, Challenges and Solutions

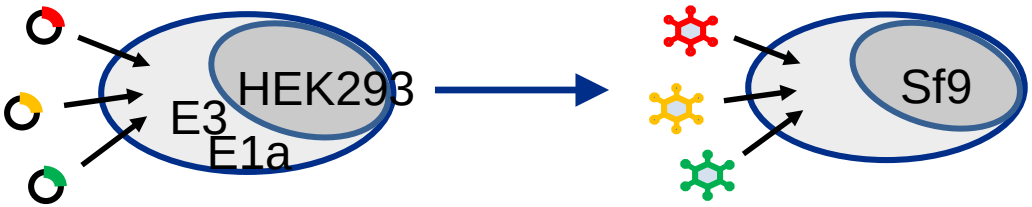
- Scale up of viral gene therapy production using adherent cells



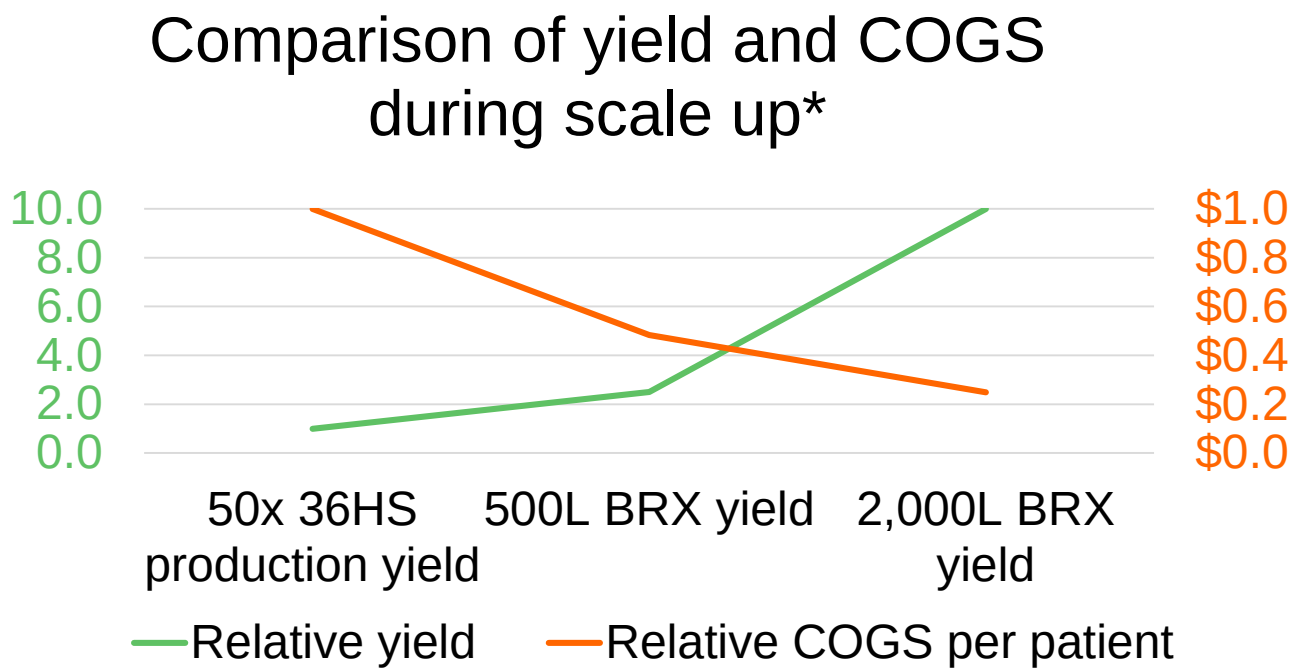
Need of Further Scalability

- Approaches for change from adherent to suspension cell process
  - Adaptation of adherent cell line to suspension
    - Example: adherent HEK293 to suspension HEK293

  - Replacement of current production system with new system
    - Example: adherent HEK293 to suspension Baculovirus system



Reduction of COGS utilizing scalable methods



Increasing the batch/run size increases the yield per batch and decreases the COGS per patient

* Assuming same yield per cell

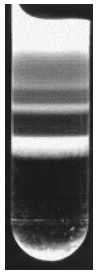
Improved Scalability of Downstream Process

- Higher yield in upstream processes as well as high labor requirement of original purification methods demands improved purification method, addressing scalability, cost and reliability
- Separation of non-product related as well as product related impurities needs to be addressed
  - Non-product related impurities:
    - Host-cell proteins
    - Host-cell DNA
    - Plasmid DNA or helper virus DNA/protein
  - Product-related impurities
    - Empty/full capsid ratio

Improved Scalability of Downstream Process

Linearly scalable process methods

Difficult to scale process method



Ultracentrifugation

Non-product related impurities

Affinity Resins

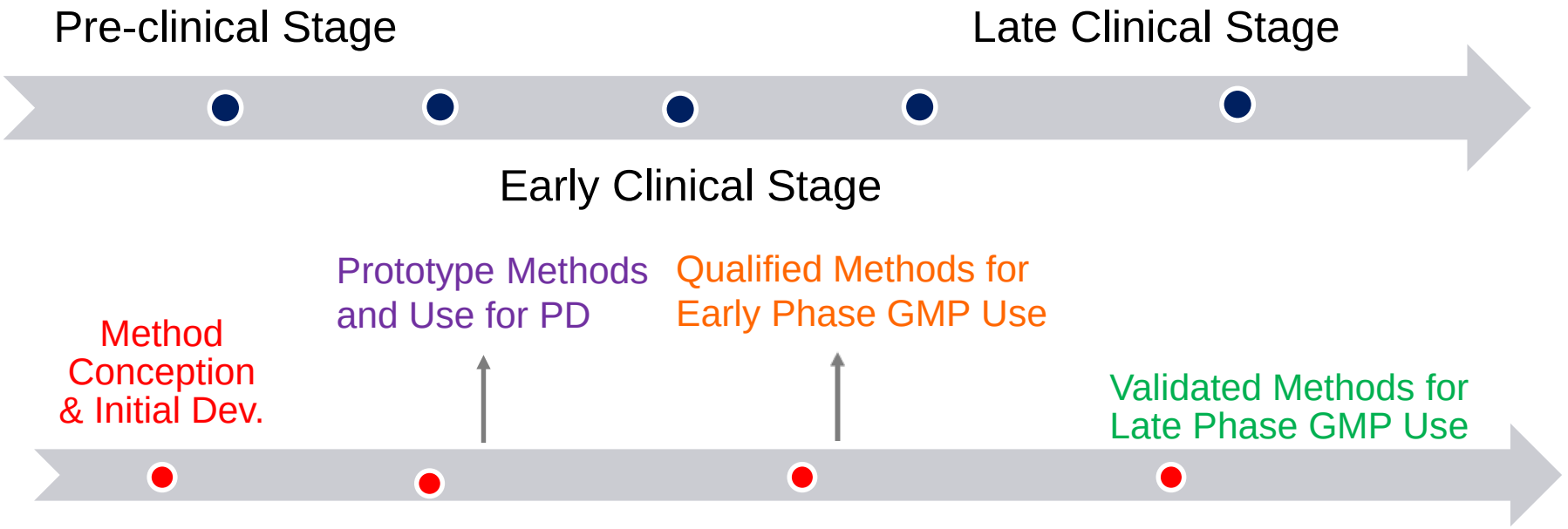
Product related impurities

Anion Exchange Resins/Columns

Pictures from: ThermoFisher, Biaseparations, and GE websites; Sandalon, J. Virol. 2000; Zolutukhin, Gene Ther. 1999

Analytical Method Development During the Lifecycle of a Program

Good analytics is a key enabler to product development and advancement to commercialization of any therapeutic



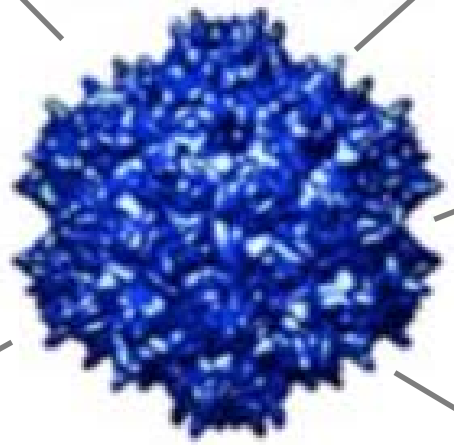
Major pitfalls can be created if we do not drive the analytics to a quality level that is sustained and relevant to our product development, delay or failure to reach commercialization can be huge/real risks.

Key Analytical Characteristics of AAV Product

Is it the right AAV capsid? → **Capsid ID**

Will the vector function and transduce target cells? → **In Vitro Potency**

Does it contain the right transgene? → **Gene Sequencing**



Does the product have the right dosage strength? → **GC titer (ddPCR)**

Are the millions of capsids filled with the transgene? → **Empty/Full Ratio**

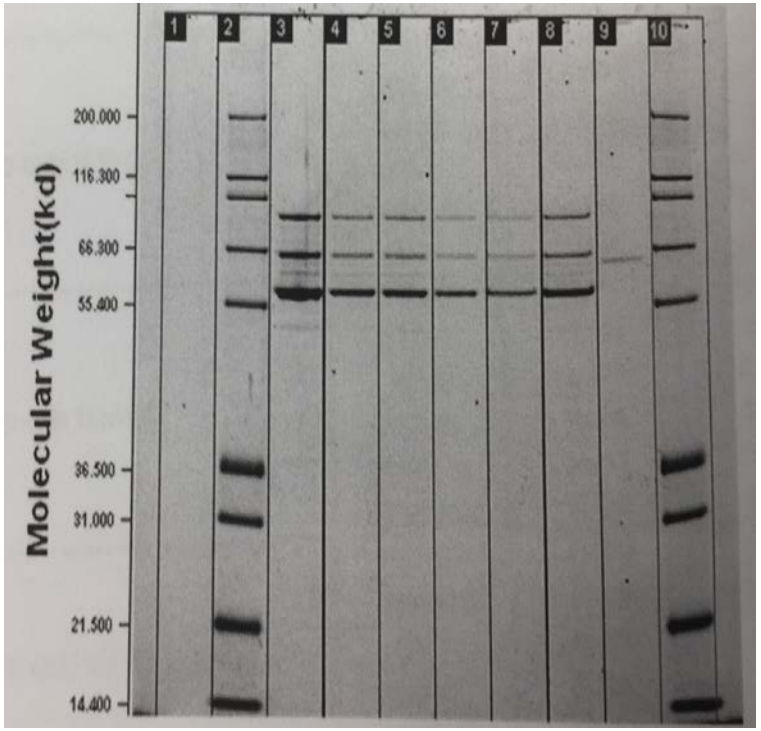
Is the product free of contaminates from process? → **Purity Methods**

What is the stability of the capsid? What affects the potency? How much residual elements are inside/outside of the capsids? → **Deep Characterization**

Key Methodologies to Assess AAV Product

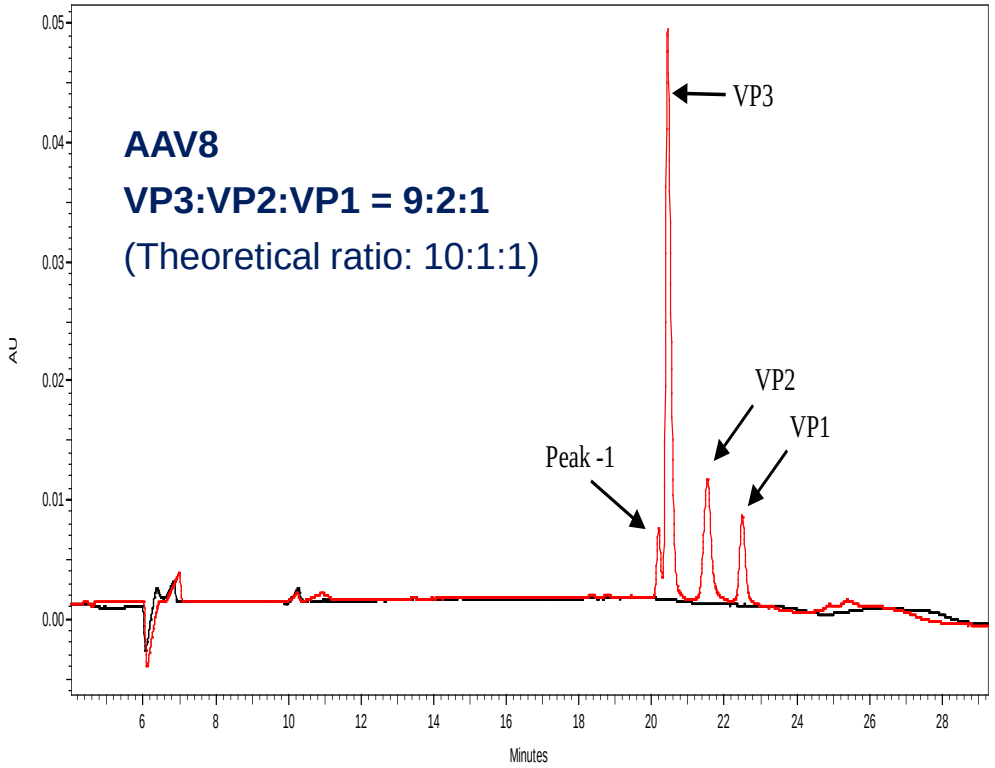
Conventional Method

Vector Protein Purity by SDS-PAGE



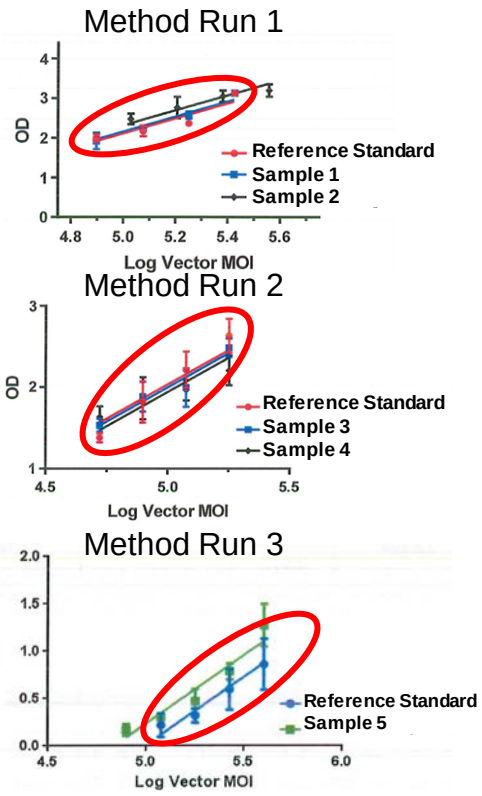
Updated Method

Capsid Protein Purity by SDS-CGE

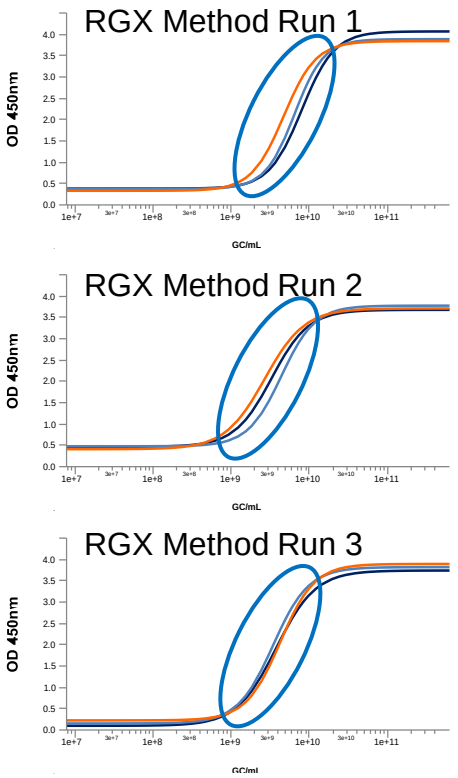


Development of robust potency methods

- Example: optimization of *in vitro* relative potency method:
  - Improved run-to-run variability and model fit for the ELISA response signal
  - Improved ELISA sensitivity to facilitate testing lower concentrations (i.e., FDP)
  - Accommodated common major sources of cell-based potency assay variability (i.e., plate, position, sample preparation)

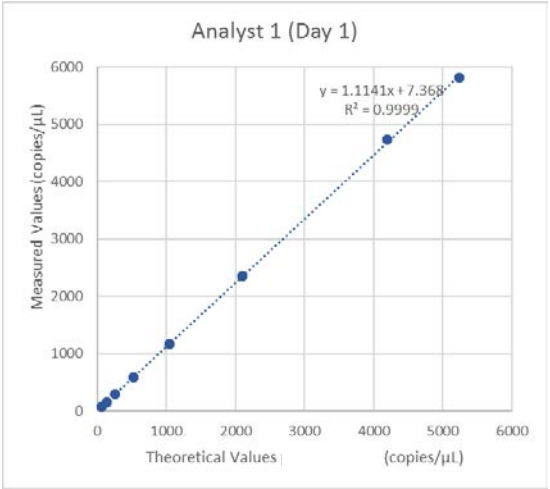


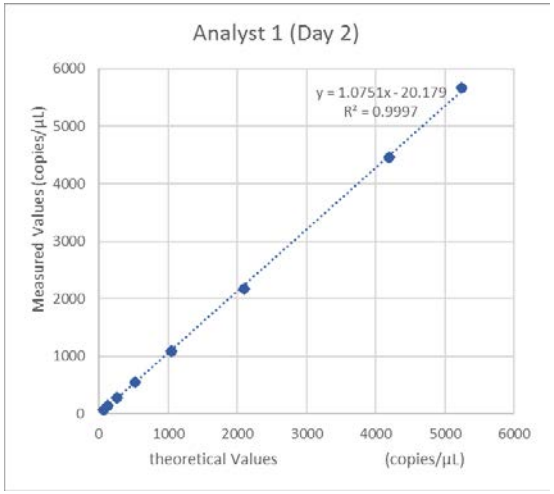
☒ Highly variable response between runs

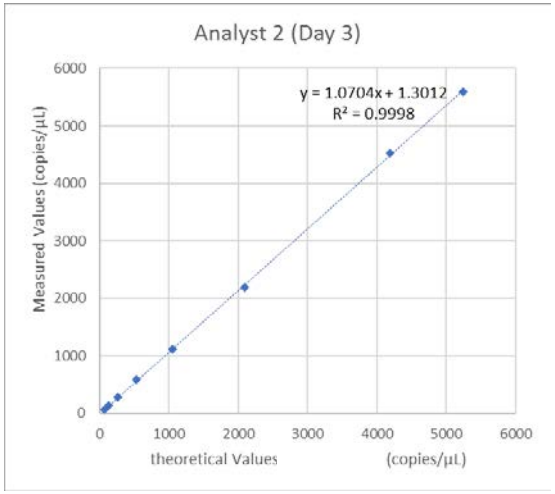


☑ Consistent response between runs

Significant improvement in estimation of titer (reduction of variation) (for Quantitation of Genome Copy)







Linearity

Acceptance Criterion: $R^2 \geq 0.95$

Result: $R^2 = 1.00$

Accuracy

Acceptance Criterion: % Recovery = 70 to 130

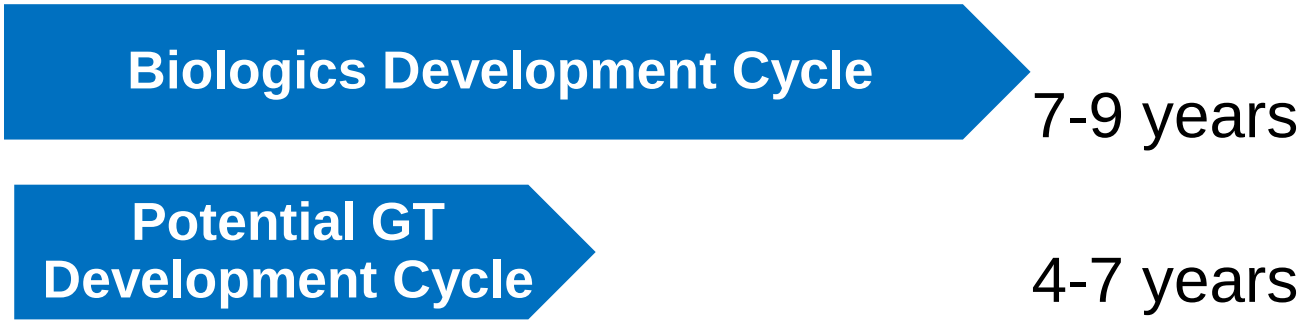
Result: % Recovery = 103 to 114

Intermediate Precision and Robustness

Acceptance Criterion: %CV ≤ 20

Result: %CV ≤ 6

Comparability Approach to Gene Therapy



- Critical to develop a standard approach to comparability given the potential for a fast-track program and likelihood of post-approval change
- Requires significant characterization of product in early stages of development
- Potential to leverage information from other related programs

Comparability of Manufacturing Processes in Gene Therapy

Potency	Genomic titer
	Infectious titer
	In vitro potency
Purity	Empty particles
	Capsid purity
Identity	Vector genome identity
	Capsid identity
Safety	Appearance
	pH
	Osmolarity
	Sterility
	Endotoxin
	Mycoplasma
	In vitro adventitious agents
	Replication competent virus
	Residual protein
	Residual DNA
	Residual helper virus activity
	Residual DNase
	Residual Chemicals (PEI, etc.)
	Residual chromatography leached ligands

Drug Product Manufacturing through Patient Administration

Fill-Finish

Thaw frozen Bulk Drug Substance

Pooling & Mixing

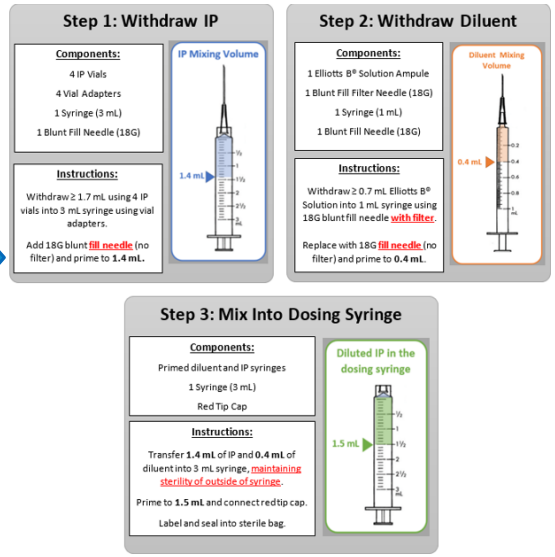
Sterile Filtration

Aseptic filling

Final Drug Product Inspection, Labeling, Storage

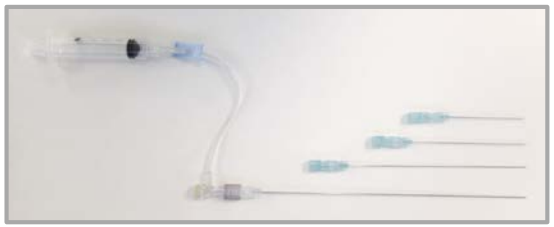
Product Administration

Pharmaceutical Dose Preparation



Product Delivery

Intrathecal



IV

Subretinal

REGENXBIO

Significant advances required in Formulation and Drug Delivery Methods

- Often patients are dosed with 20-50 vials of dilute product as part of treatment
  - Higher concentration formulations required
  - Improved delivery systems (single bag design for IV)
  - Improved storage conditions (-80 deg C common)
- Potential to leverage learnings across serotypes exists
  - Bioavailability
  - Standard formulations across indications
  - Creative stability and sampling methods to preserve product
  - Increased scale ensuring reduced volumetric loss in processing

Summary

- Due to the complexity of the product, the development of a manufacturing process for a viral gene therapy product adds additional challenge
- Scalable methods in upstream and downstream need to be further developed to address the supply for clinical and commercial applications
- The development of new assays is crucial for identifying impurities and feedback for process improvements, as well as the basis for understanding the impact of process changes through the product lifecycle
- Development of a manufacturing platform when working in rare disease is an important aspect of the business model, enabling the potential to leverage learnings across programs efficiently without repetitive testing