Establishing Analytical Tools for an AAV Platform

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Agenda

- High similarity in AAV processes, structure, tests
- Analytical developments – new and better methods
- Opportunities for leveraging platform knowledge
  - Process characterization
  - Method validation
  - Release testing
  - Product stability
- Future directions for reducing redundant testing

Complexity of Gene Therapy Products

"In traditional drug review, 80% of the review is focused on the clinical outcome and 20% on the product issues. This is almost completely inverted when it comes to cell and gene therapy where more of the challenging questions relate to product manufacturing and quality."

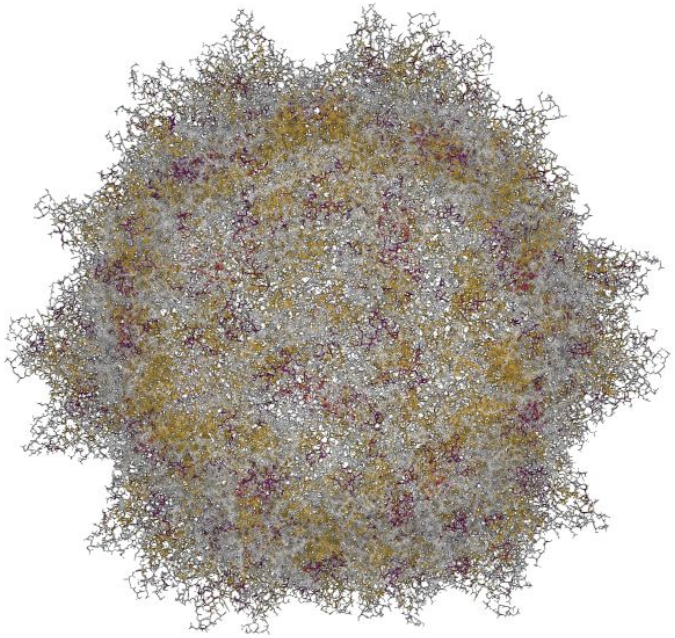
-- Scott Gottlieb, 23rd FDA Commissioner, May 22, 2018

Why the paradigm is different:

- Clinical outcomes often very clear
- Product manufacturing and quality complex
    - Multi-component products (DNA and protein)
    - Large size
    - Complex manufacturing (transduction)

But there is good news…

- High similarity across products → platform



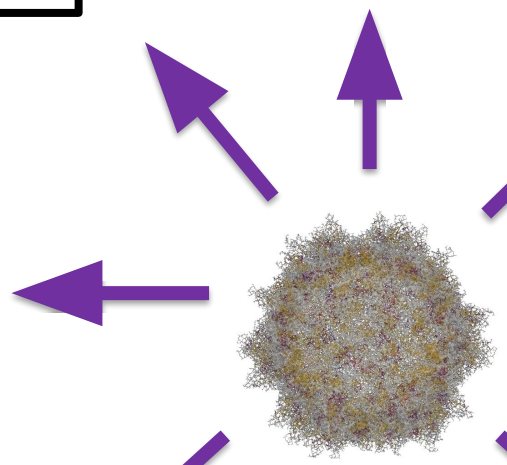
AAV9
MW = ~5.1 MDa

Overview of AAV Lot Release / Characterization Assays
(rBV / Sf9 Process)

Genome Assays
- Identity
- Quantity (ddPCR)
- Purity / Integrity (agarose gel, CE)

Potency Assays
- Cell-based potency
- Infectious titer

AAV Assembly Assays
- Full/partial/empty (AUC, IEX, cryoEM)
- Aggregates / fragments (SEC)
- Particle size and distribution (DLS)



Capsid Assays
- Identity
- Quantity (ELISA)
- Purity and VP ratio (CE-SDS)
- Peptide map and PTM (MS)

Non-viral process residuals
- Sf9 HCP (ELISA) and DNA (qPCR)
- BV DNA (qPCR)
- Benzonase (ELISA)
- Affinity ligand (ELISA)
- Rhabdovirus RNA

Viral process residuals
- Replication-competent AAV
- Residual baculovirus
- Residual rhabdovirus
- On banks and in-process, adventitious viruses and mycoplasma

Contaminants
- Endotoxins
- Bioburden
- Appearance

Other Assays
- Appearance
- Sterility
- pH
- Sub-visible particulates
- Excipients
- Osmolality

Methods, as Part of a Platform

- Platform
  - Genome design elements
  - Expression
  - Purification
  - Formulation
  - Presentation and container closure
  - Analytical methods
- Platform methods
  - Empty / partial capsids – AUC/AEX
  - Particle distribution – DLS/SEC
- Non-platform methods
  - Genome identity – sequencing (NGS/Sanger) or ddPCR
  - Genome quantity
  - Cell-based potency

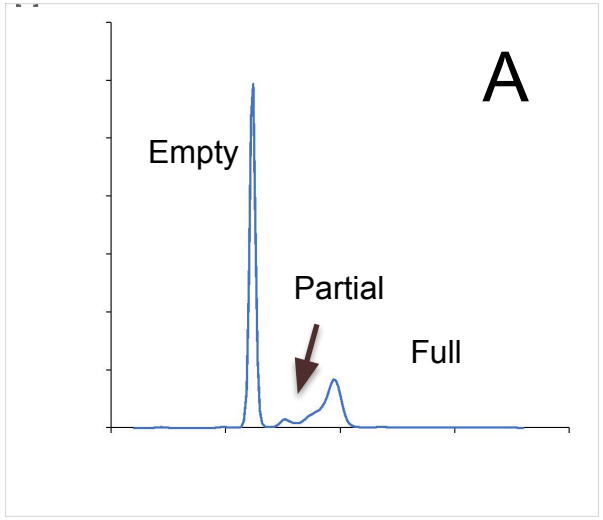
Empty (and Partially Full) Capsids

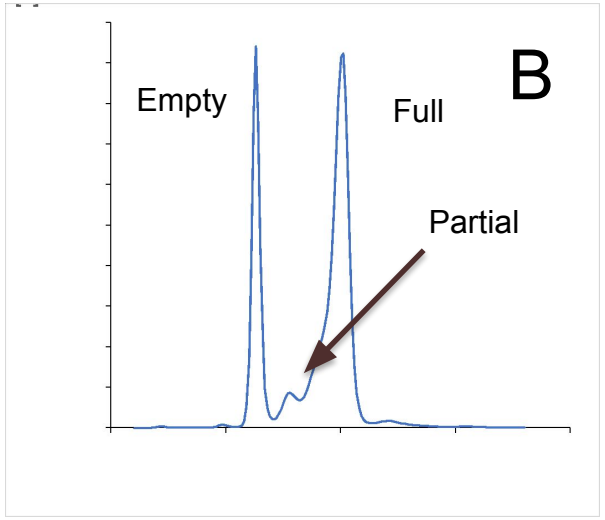
- Analytical Ultracentrifugation (AUC) and alternatives
  - The “gold standard”
    - Bulk measurement
    - Good separation
    - Low throughput
  - The alternatives are challenging
    - CryoTEM – small fraction of AAV measured, image analysis difficult
    - CDMS – analysis of individual particles, specialized equipment
    - SEC-MALS – without physical separation, limited utility
    - AEX – promising, but so far, limited separation of partially full capsids
  - Orthogonal testing of empty, full, partial, and stressed samples will demonstrate suitability
- During evaluation of an alternative test,
  - AUC may remain a spec test
  - The alternative test may be a routine characterization test
- In a potential future state,
  - An alternative may be a spec test
  - AUC (and others) may be EoS
  - AUC may also be used for method characterization and comparability

Comparison of analytical techniques to quantitate the capsid content of adeno-associated viral vectors
A.K.Werle et al., Mol Ther Methods Clin Dev. 2021 Dec 10; 23: 254–262.

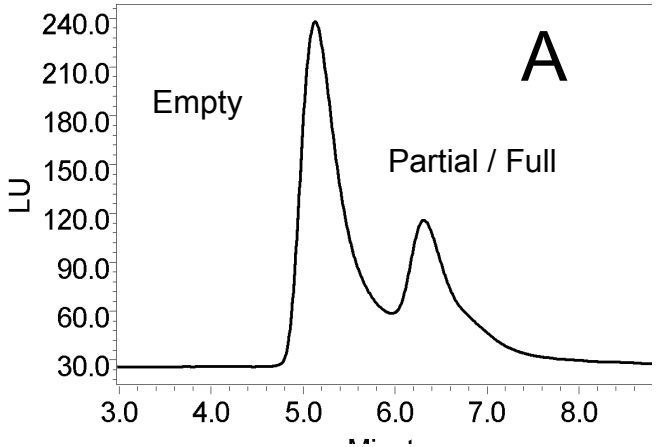
Quantitation of Empty Capsids

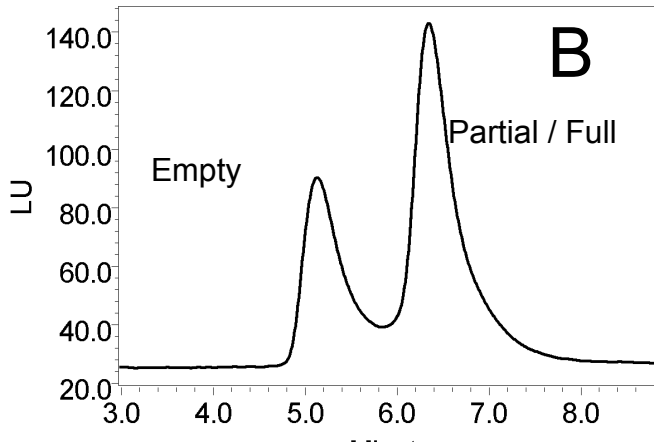
AUC





AEX





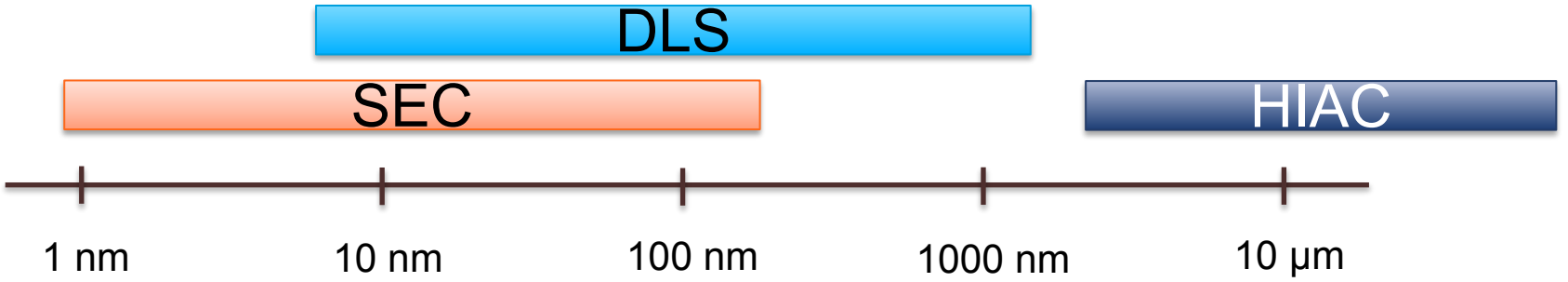
Empty (%)

Technique	Sample A	Sample B
AUC	66.4	28.3
AEX	65.1	32.9

Particle Size and Aggregates: DLS and SEC

- Dynamic Light Scattering (DLS):
  - Measures hydrodynamic diameter by the decay of the autocorrelation function
  - Resolves mass differences > 5-fold
  - Information on absolute particle size and polydispersity
- Size Exclusion Chromatography (SEC):
  - Separates based on sieving
  - Resolves small aggregates (dimers, trimers) and fragments
  - Particle size from internal or external standards
- SEC compared to DLS for product control
  - Easier to run
  - More straightforward data analysis
  - Better separation of certain degradation products

- Selection of control methods depends on:
  - Product profile
  - Degradation pathways
- Multiple techniques in early development
- As development proceeds,
  - Select “simplest” tests as spec tests
  - Reserve orthogonal tests for characterization of spec methods and occasional use (comparability, PPQ)



ID Testing of Viral Genome

- Common practice and current recommendation from A-gene case study: Deep sequencing for Identity
- Drivers for alternatives
  - Technically challenging for QC labs
  - Massive amounts of data – a compliance challenge
- FDA gene therapy guidance (emphasis mine)
  - ID testing of DS

    Test for vector identity by methods such as restriction enzyme mapping with multiple enzymes or **PCR** should be performed on the drug substance (see 21 CFR 610.14). In the case of a facility making multiple constructs, it should be verified that the identity testing is **capable of distinguishing the constructs** and detecting cross-contamination.
  - Testing of vectors and banks

    Early in product development, vector characterization consisting of sequence data of appropriate portions of vectors and/or restriction mapping supplemented by protein characterization is acceptable. For later phases of product development and licensure, **more extensive sequencing** information should be provided.

    When a virus, with or without a therapeutic gene, is used as a seed in the manufacture of a therapeutic vector, it is recommended that a Master Viral Bank be created and characterized.
- Alternative approach
  - NGS for plasmids and/or viral banks and for EoS for DS
  - ddPCR for ID testing of DS and DP – target unique part of GOI at a minimum
  - Deep sequence data more appropriate to Elucidation of Structure than release testing

Method Validation

- Phase-appropriate validation
  - ICH validation, except robustness and intermediate precision
- Robustness
  - ICH Q2R1 (similar language in Q2R2 draft)

    “It should be noted that robustness is not listed in the table but should be considered at an appropriate stage in the development of the analytical procedure.“
  - ICH Q14 (draft)

    "Robustness is typically conducted during development … [and] does not necessarily need to be repeated during validation."
- Timing for ICH validation
  - FDA: potency methods for pivotal
  - EU: all methods for pivotal
  - ICH: validation for BLA/MAA submission

Reduced Validation for Platform Methods

- Narrowest definition of platform: only the GOI changes
- Deviations from “platform”
  - Change to process: production system, purification and viral clearance (**A**)
  - Change to presentation: formulation, AAV concentration (**B**)
  - Different capsid (**C**)
- Impact to platform methods
  - Process: new production system will require new trace methods
  - Capsid: new capsid will require new methods for identity, quantity, assembly, and purity
- Qualification/validation
  - Evaluation of method suitability
  - Risk-based assessment

Analyte	Specificity	Sensitivity	Accuracy	Precision	Linearity	Sol’n Stability
Trace residuals	Always	**B**	**B** or **C**	--	May be needed depending on scope of process differences (**A**)	**A**, **B**, or **C**
Capsid ID / quantity	**C**	N/A	**B** or **C**	--	**B** or **C**	**A**, **B**, or **C**
Full / empty AAV (AUC, IEX)	**C**	Always	Always	--	**B** or **C**	**A**, **B**, or **C**
Assembled AAV (DLS, SEC)	**C**	**C**	**C**	--	**B** or **C**	**A**, **B**, or **C**
Protein purity	**C**	**C**	**C**	--	**B** or **C**	**A**, **B**, or **C**
Non-Platform Methods						
Genome ID / quantity	Always	N/A	Always	Always	Always	Always
Potency methods	Always	N/A	Always	Always	Always	Always

In the Future…?

- Material limitations: need to avoid redundant testing
  - Opportunity cost of material – loss of therapeutic doses for patients
  - Cost of testing
- Data and risk assessments to define integrated control strategy
  - Patient safety: DP
  - Similarity of DS and DP
  - Storage condition (typically deep frozen)
  - Tests at earlier points for technical reasons (e.g., micro)
  - New ICH Q1 guidance being drafted (Concept Paper)

	Current State:	Future State:
Release Testing	In-process, DS and DP	In-process and DP
Stability Testing	Both DS and DP	DP only
	Resupply batches	Only site or process changes
	Full ICH for each product	Leveraging prior knowledge and matrixed designs

Conclusions

- Current testing expectations
  - Redundant
  - High material demand
- High similarity in AAV processes, structure, tests → platform
- Opportunities for leveraging platform knowledge
  - Simplified process characterization
  - Flexibility to use prior data for platform method validation
  - Non-redundant release testing
  - Drug product, but not drug substance, stability
- Future:
  - Reduced testing
  - Maximized material for patients
  - Meaningful impact to bringing quality medicines to patients

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Questions?

AAV9 Image References

- AAV9 image derived from RCSB PDB: 3UX1, structure from: Dimattia, M.A., Nam, H.J., Van Vliet, K., Mitchell, M., Bennett, A., Gurda, B.L., McKenna, R., Olson, N.H., Sinkovits, R.S., Potter, M., Byrne, B.J., Aslanidi, G., Zolotukhin, S., Muzyczka, N., Baker, T.S., Agbandje-McKenna, M. (2012) J Virol 86: 6947-6958; viewer from D. Sehnal, S. Bittrich, M. Deshpande, R. Svobodová, K. Berka, V. Bazgier, S. Velankar, S.K. Burley, J. Koča, A.S. Rose (2021) Mol* Viewer: modern web app for 3D visualization and analysis of large biomolecular structures. Nucleic Acids Research. doi: 10.1093/nar/gkab314)