U.S. FOOD & DRUG ADMINISTRATION

Regulatory Considerations for Analytical Development of Gene Therapy Products

Angela Whatley, PhD
CBER/FDA

Cell & Gene Therapy Products (CGTP): Manufacturing, Quality and Regulatory Considerations

June 10, 2019

Human Gene Therapy (GT) Products

"mediate their effects by transcription or translation of transferred genetic material, or by specifically altering host genetic sequences"

- Viral vectors
- Bacterial vectors
- Oncolytic viruses and bacteria
- Plasmid DNA, mRNA
- Human genome editing products
- Ex vivo genetically modified cells

GT Product Approvals by FDA

Oncolytic herpes simplex virus (HSV)

Imlygic (talimogene laherparepvec)

- Treatment of patients with melanoma (local treatment of unresectable cutaneous and nodal lesions).

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CD19-directed genetically modified autologous T cell immunotherapy

Kymriah (tisagenlecleucel)

- Treatment of patients up to 25 years of age with B-cell precursor acute lymphoblasticleukemia (ALL) that is refractory or in second or later relapse.
- Adult patients with relapsed or refractory (r/r) large B-cell lymphoma after two or more lines of systemic therapy including diffuse large B-cell lymphoma (DLBCL) not otherwise specified, high grade B-cell lymphoma and DLBCL arising from follicular lymphoma.

Yescarta (axicabtagene ciloleucel)

- Treatment of adult patients with relapsed or refractory large B-cell lymphoma (DLBCL) not otherwise specified, primary mediastinal large B-cell lymphoma, high grade B-cell lymphoma, and DLBCL arising from follicular lymphoma.

GT Product Approvals by FDA

Adeno-associated virus (AAV) vector-based

Luxturna (voretigene neparvovec)

- Treatment of patients with confirmed biallelic RPE65 mutation-associated retinal dystrophy.

Zolgensma (onasemnogene abeparvovec)

- Treatment of children less than 2 years old with spinal muscular atrophy.

Oncolytic herpes simplex virus (HSV)

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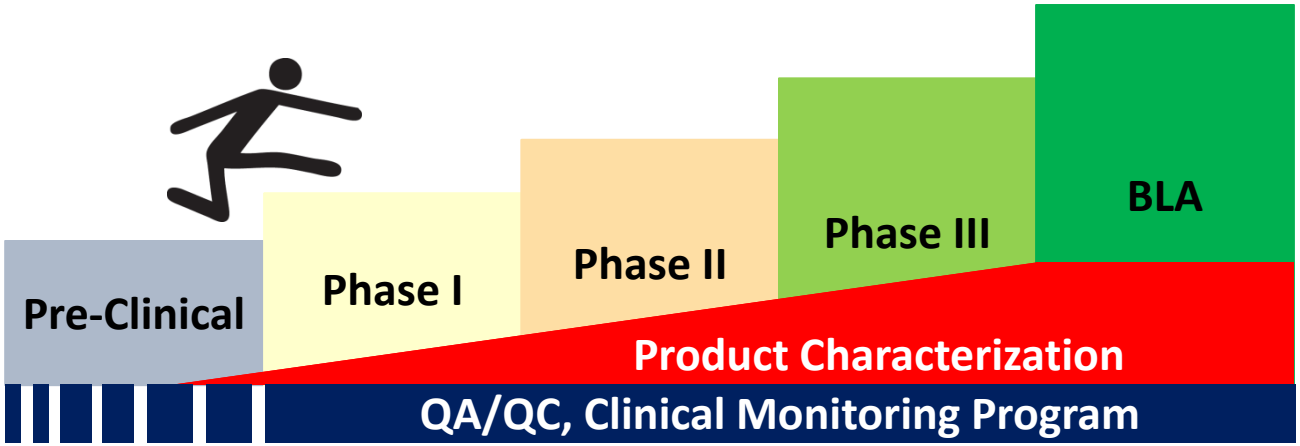
"…In contrast to traditional drug review, some of the more **challenging questions when it comes to gene therapy relate to product manufacturing and quality**, or questions about the durability of response which often can't be fully answered in any reasonably sized pre-market trial.."

- *Scott Gottlieb, M.D., Commissioner of FDA*

on FDA's efforts to advance development of gene therapies
July 11, 2018

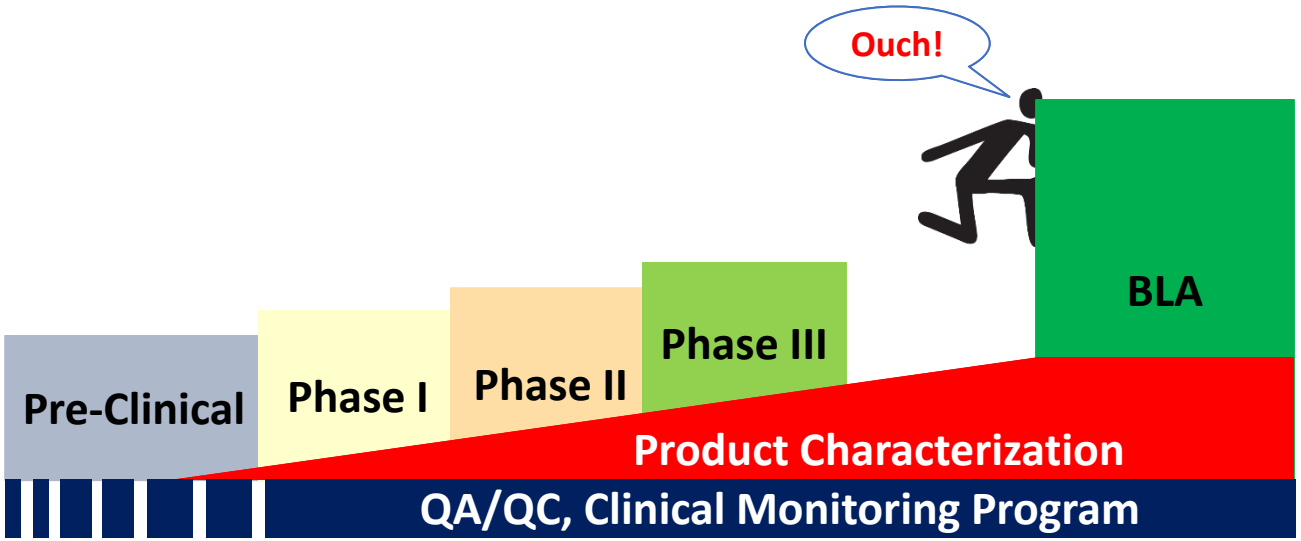
Product Lifecycle Approach to Analytical Development

- Stepwise assay development
  - Investigation of biological activity
  - Development of relevant assay

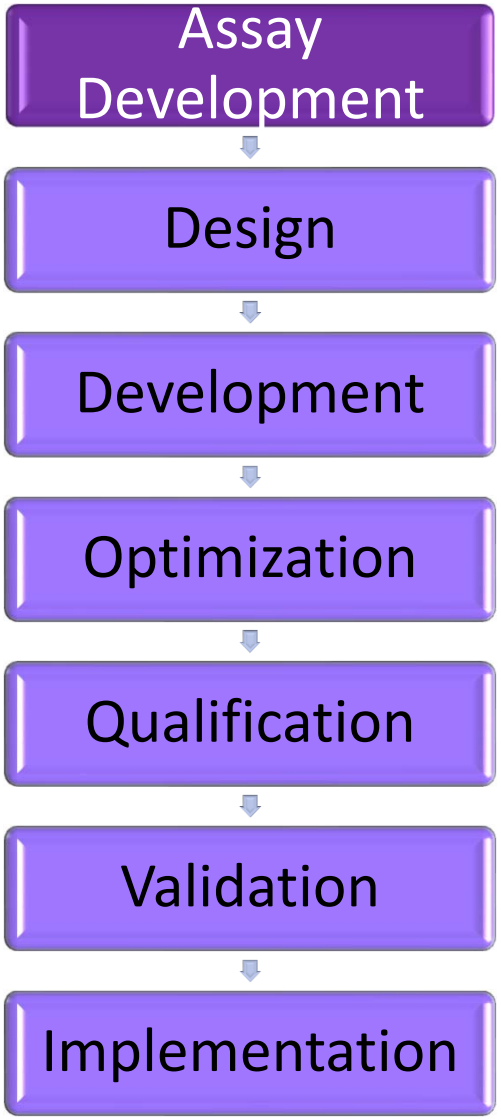


Expedited Development Does Not Change Regulatory Requirements

- Validated before clinical studies to support safety and efficacy for licensure



Align Assay & Product Lifecycle



Plan assay development timelines carefully

- Consider overall product lifecycle
- Consider regulatory program

Challenges Affecting GTPs on Expedited Programs

- Limited manufacturing experience:
  - Not enough retention or test samples available
- Limited in-process testing:
  - process variables and critical process parameters (CPP) not known
- Limited product characterization:
  - Critical Quality Attributes (CQAs) not known
- Limited knowledge of product- and process-related impurities
- Limited product stability data collected
- Limited assay development (potency, purity):
  - assays not qualified
  - reference standards not established or adequately characterized

Encourage Early Product Characterization

A Critical Quality Attribute (CQA) is a physical, chemical, biological, or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality. *- ICH Q8 (R2)*

- Explore many CQAs during early development
  - Report results early in development
  - Choose relevant tests for late phase studies
- Evaluate multiple measures of CQAs, especially potency
  - Matrix of assays
  - Orthogonal methods
  - Stability indicating
- Support comparability studies

Concurrent & Early Assay Development

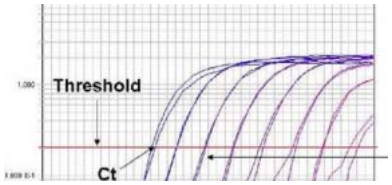
Example: AAV

Product attribute: Potency

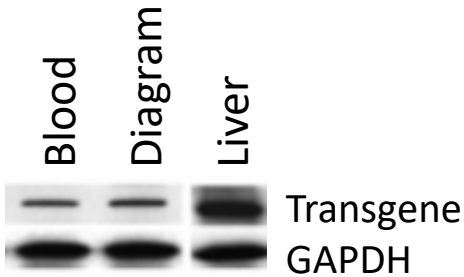
Evaluating a variety of methods during development supports:

- Product characterization and stability
- Understanding effects of manufacturing changes
- Choice of potency assay and relationship to clinical outcome for licensure

TCID 50
With qPCR detection

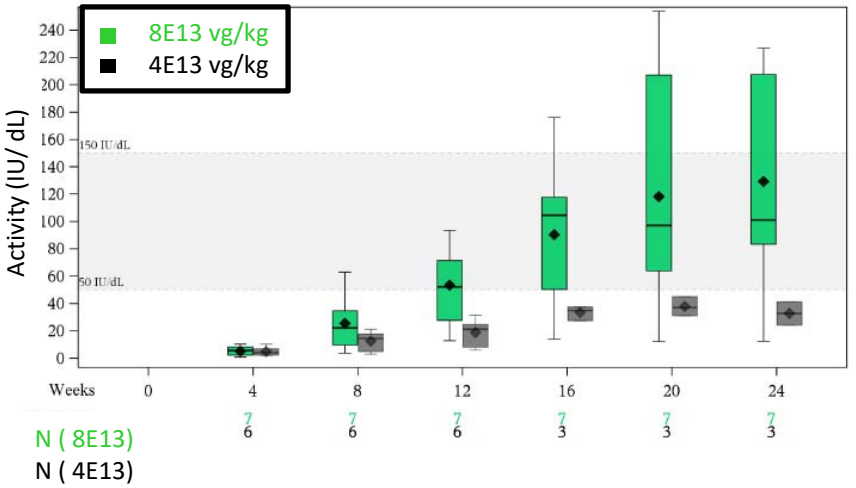


Transgene expression



Biological activity

Dose response Curve

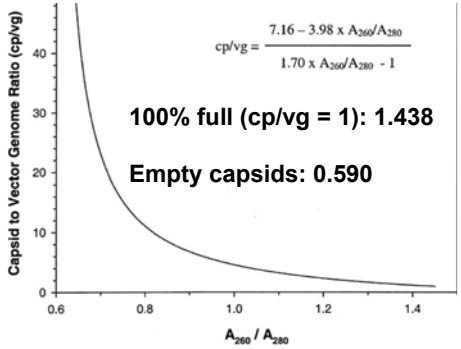


Evaluate Current Technologies to Characterize Product Attributes

Example: AAV gene therapy vector
Product attribute: Particle content (empty-to-full ratio); measure of purity

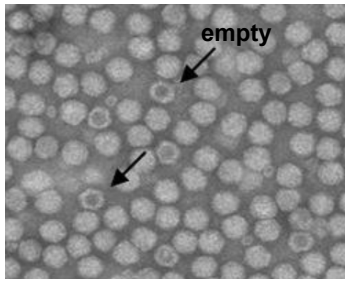
Are the assays sensitive, specific, quantitative, and well-controlled?

Absorption: $OD_{260:280}$ ratio



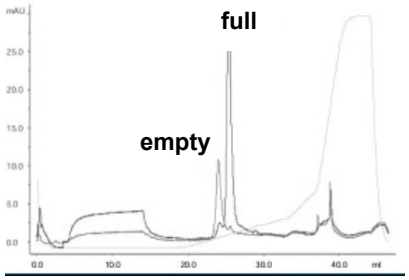
Sommer et al., 2003

Transmission Electron Microscopy

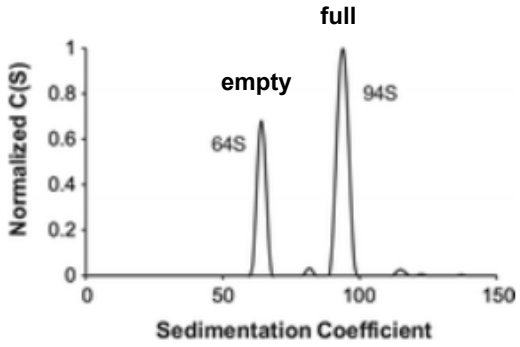


Lock et al., 2012

Ion Exchange Chromatography

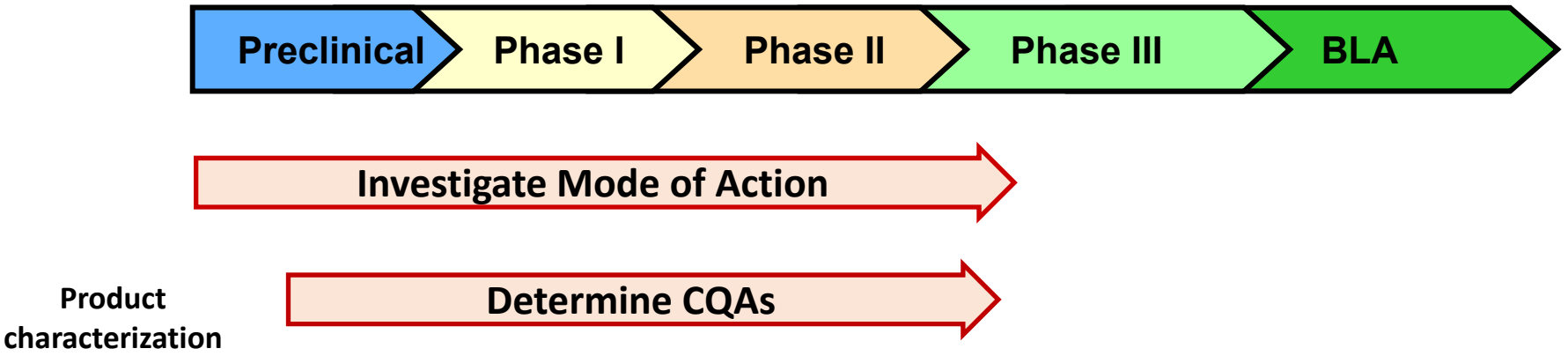


Analytical Ultracentrifugation



Burnham et al., 2015

Assay Development Timeline



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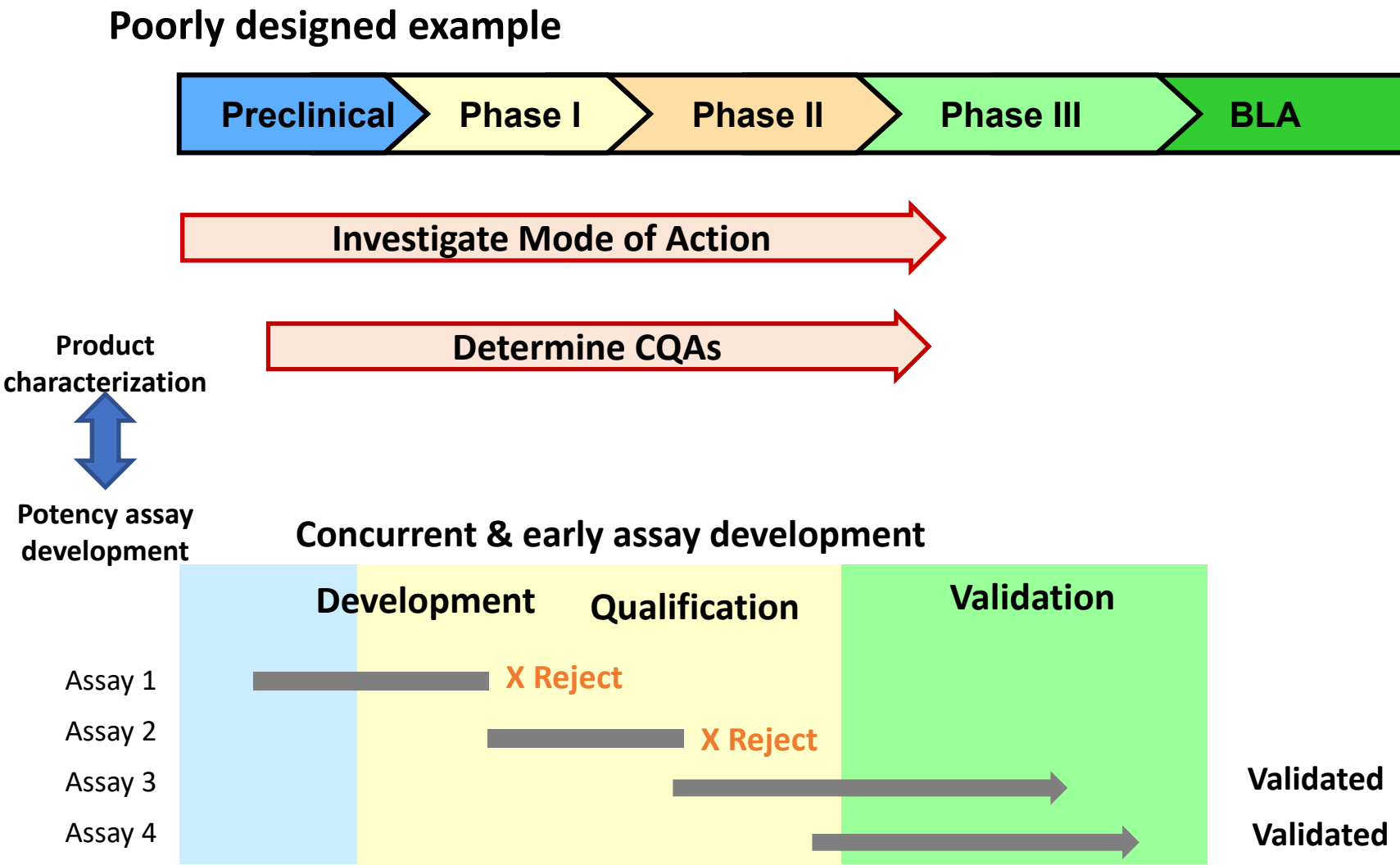

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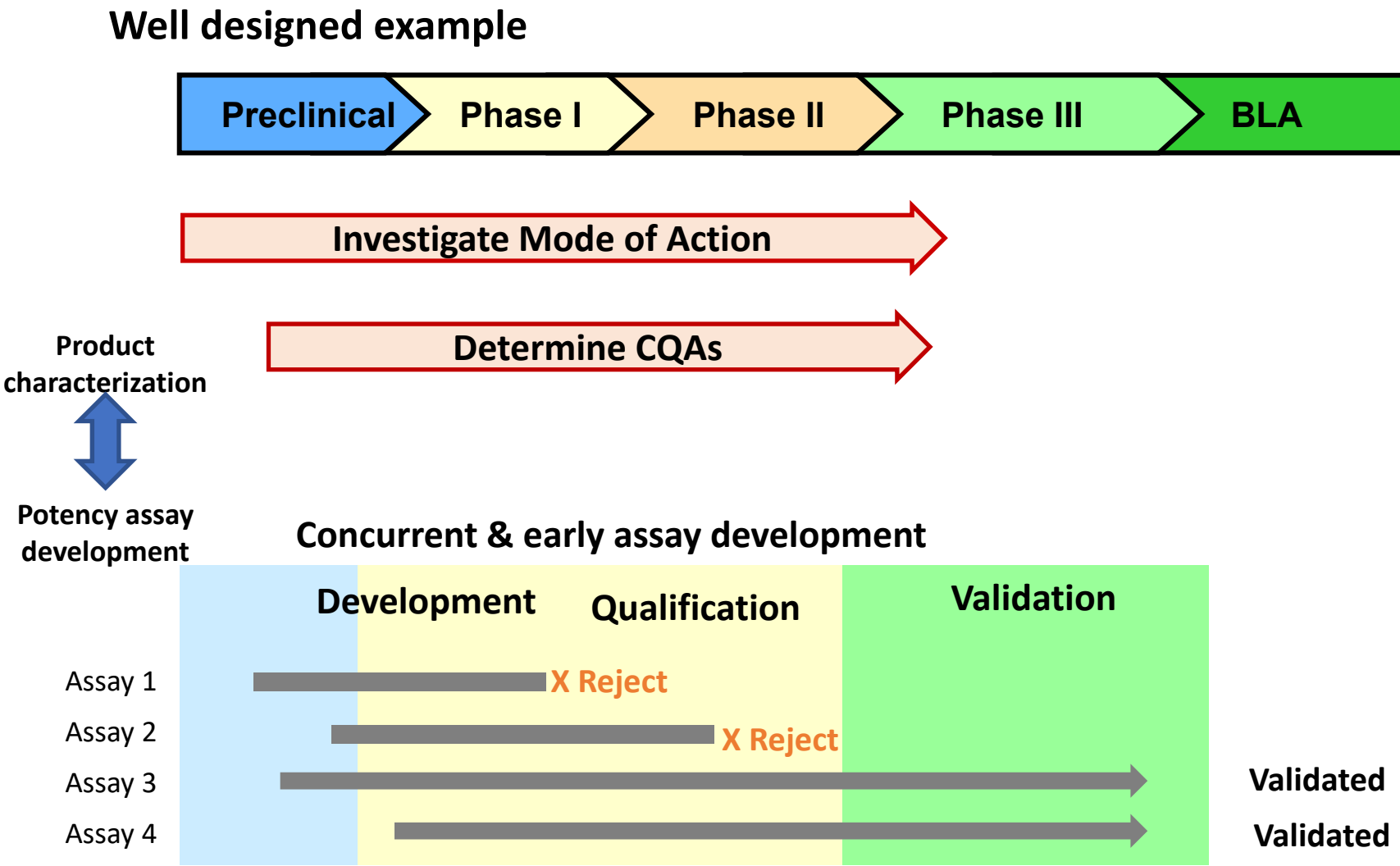


Consider Development Plan

- Consider analytic platform approaches
  - A single assay can be used to support more than one CQA
  - A assay matrix may be required to assess a single CQA
- Cross reference new submissions to original submissions that utilize the same assay
- Assay Timing
  - Not all tests are required on DS and DP
  - Conduct adventitious agent tests at stage most likely to detect contamination

Required Tests

- Safety tests
  - Well controlled for early studies
  - Provide justification and supporting data for non compendial and rapid methods
- Other Required tests
  - Identity, Purity, Potency
  - Removal of process related impurities
  - Animal derived reagent testing
- Characterization
  - Acceptance criteria may have wide ranges for early studies

Specific Challenges for AAV Vector Products

- Empty vs full capsids
- Host / plasmid DNA
- Purity of starting materials including plasmids
- Infectious titer
- Potency
- Dose determining assay
  - FDA workshop
    - https://www.fda.gov/vaccines-blood-biologics/workshops-meetings-conferences-biologics/quantitation-aav-based-gene-therapy-products-12072018-12072018#event-information

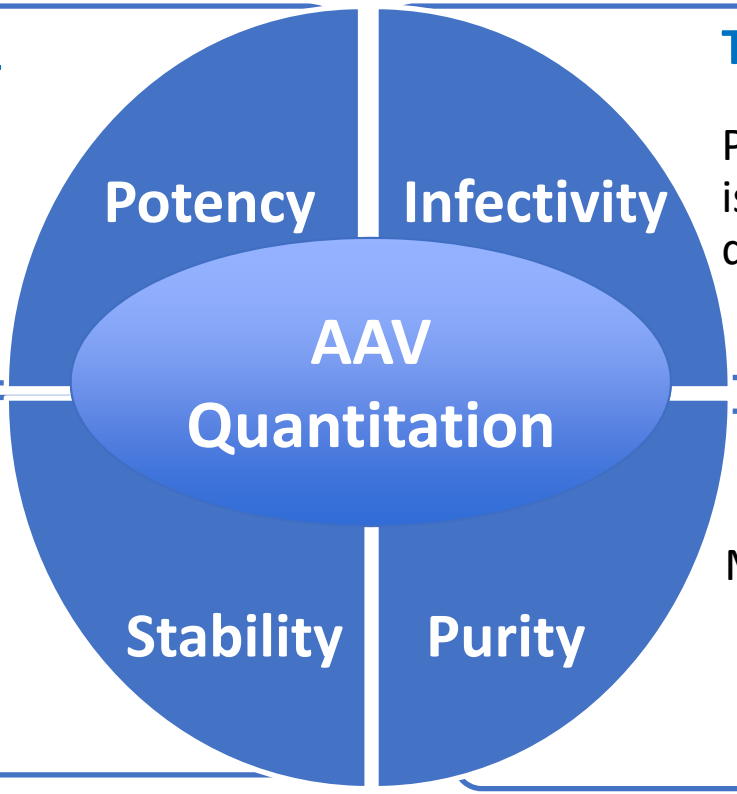
AAV Quantitation Assay is Critical for Measuring Key Product Attributes

Transgene Expression & Biological Activity

Potency measured as a factor of dose

TCID50

Particle to infectivity ratio is a measure of vector quality



Stability

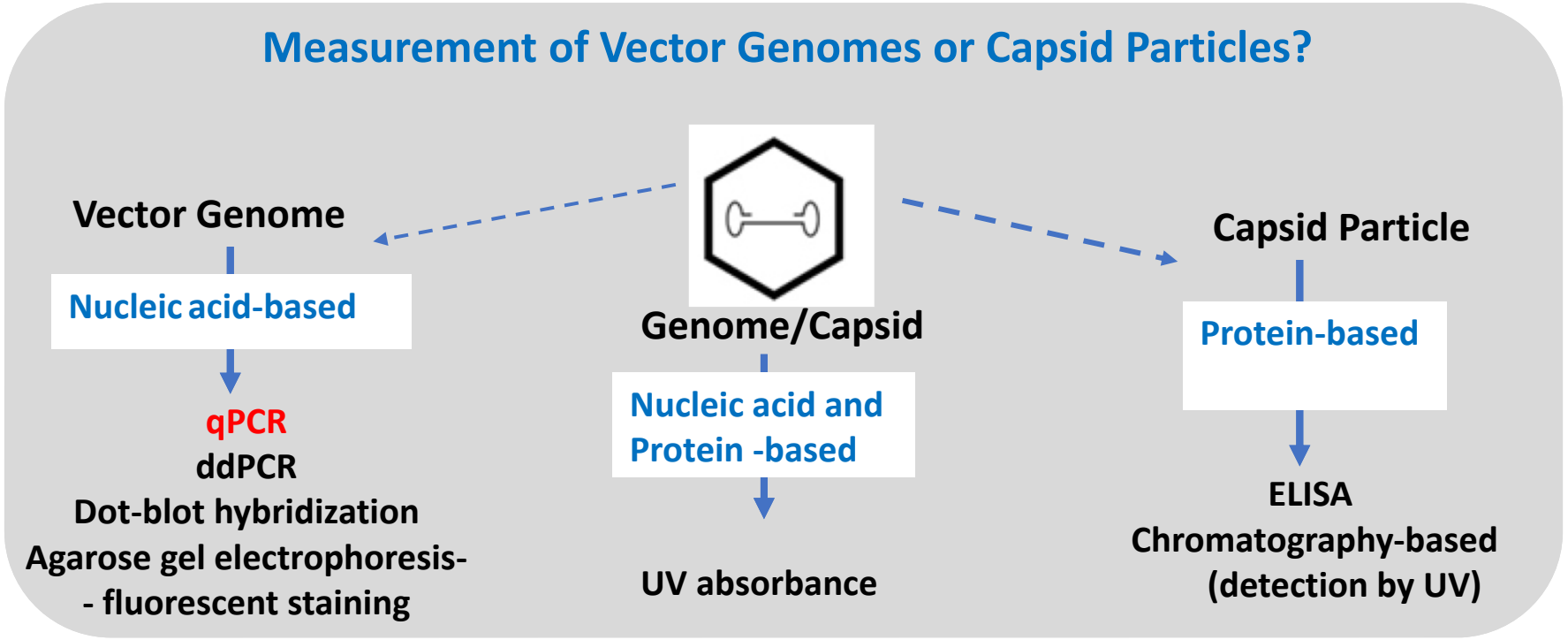
Long term, short term, stress conditions, or

In the delivery device.

Empty to full ratio

Measure of vector purity

Methods Used to Quantitate AAV Vectors



- Most commonly measured in terms of viral genomes using PCR-based assays.
- Many manufacturers are developing innovative methods to quantitate AAV vectors.

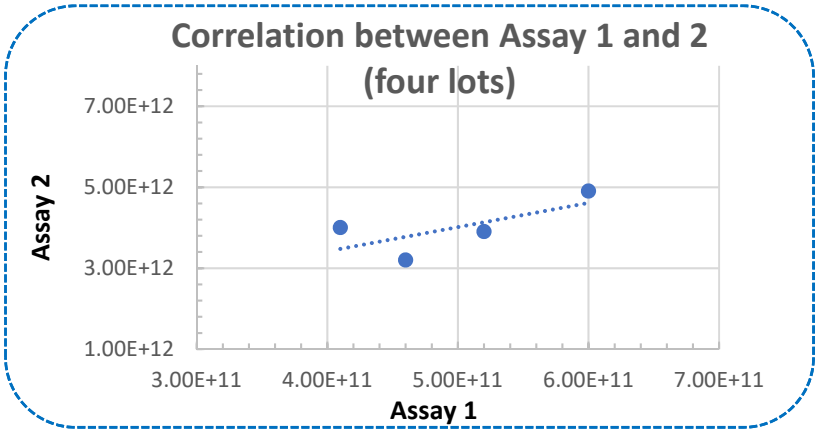
Choose the most suitable technologies and methodologies for the product under study to develop a reliable, precise and accurate assay

Case Study 1

Different assay used in preclinical studies than in clinical studies

Titer of multiple lots were compared with Assay 1 (preclinical assay) and Assay 2 (clinical assay) to show correlation.

Example



Lots	Assay 1	Assay 2	Fold difference
Tox	6 x 10^{11}	4.9 x 10^{12}	8.2
Eng. 1	4.1 x 10^{11}	4.0 x 10^{12}	9.8
Eng. 2	4.6 x 10^{11}	3.2 x 10^{12}	7.0
Clinical	5.2x 10^{11}	3.9 x 10^{12}	7.6
Average			8.1

Best practices: Plan early; use the same AAV quantitation assay in preclinical and clinical studies

Case Study 2

Assay is not reproducible

- When the assay has poor intermediate precision, subjects may not receive the dose as planned in the study

  Example: Qualified PCR assay with a intermediate precision of ~ 50% is not acceptable

- Degree of variability overlaps with the safety margin
  - Is the planned dose-escalation safe?

Best practices:

- **Understand assay variability and set up adequate controls.**
- **Precision of ≤ 15% CV is reasonable for early phase studies**
- **Select less variable and more sensitive AAV quantitation assays, and new assay technologies early in development**

Case Study 3

Poor Operator training

- Variability, commonly at the step of sample preparation & serial dilution; inadequate controls.
- Procedures do not allow for additional dilutions when the vector concentration is higher than expected/planned for.
- Not enough replicates at each dilution of the test article sample
- As operator's experience grows, there is less assay variability

Example

Initial assays performed by analyst 1 and 2

N= 59 Analyst 1	**%CV 17.05**
N=37 Analyst **2**	%CV 20.60

Last 10 assays performed by analyst 1 and 2

N=10, Analyst 1	**% CV 6.85%**
N=10, Analyst 2	% CV 7.81%

Protocol development and operator training are key to developing a well-controlled, reliable assay for AAV quantitation

Summary

- Align assay and product lifecycle development
  - Product type
  - Manufacturing plan
  - Regulatory timeline
- Begin assay development early in product life cycle
- Evaluate concurrent assays and consider current technologies
- Utilize analytical platforms
  - Single assay can be used to support more than one CQA
  - Matrix of assays may be used to define a single CQA
  - Method can be cross referenced for multiple products, but assay qualification may be needed for each specific product

CBER Recruitment

Medical Officers/Physicians	Consumer Safety Officers
Biologists/Microbiologists	General Health Scientists
Chemists	Program Analysts
Biomedical Engineers	Program Support Specialists
Pharmacologists	Toxicologists

https://www.fda.gov/about-fda/center-biologics-evaluation-and-research-cber/jobs-center-biologics-evaluation-and-research-cber

Contact Information

- **Angela Whatley PhD**

  angela.whatley@fda.hhs.gov

- **Regulatory Questions:**

  OTAT Main Line – 240 402 8190

  Email: OTATRPMS@fda.hhs.gov and

  Lori.Tull@fda.hhs.gov

FDA Headquarters
Federal Research Center at White Oak
10903 New Hampshire Avenue
Silver Spring, MD 20993-0002

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