FDA Requirements For Cell & Gene Therapy Characterization

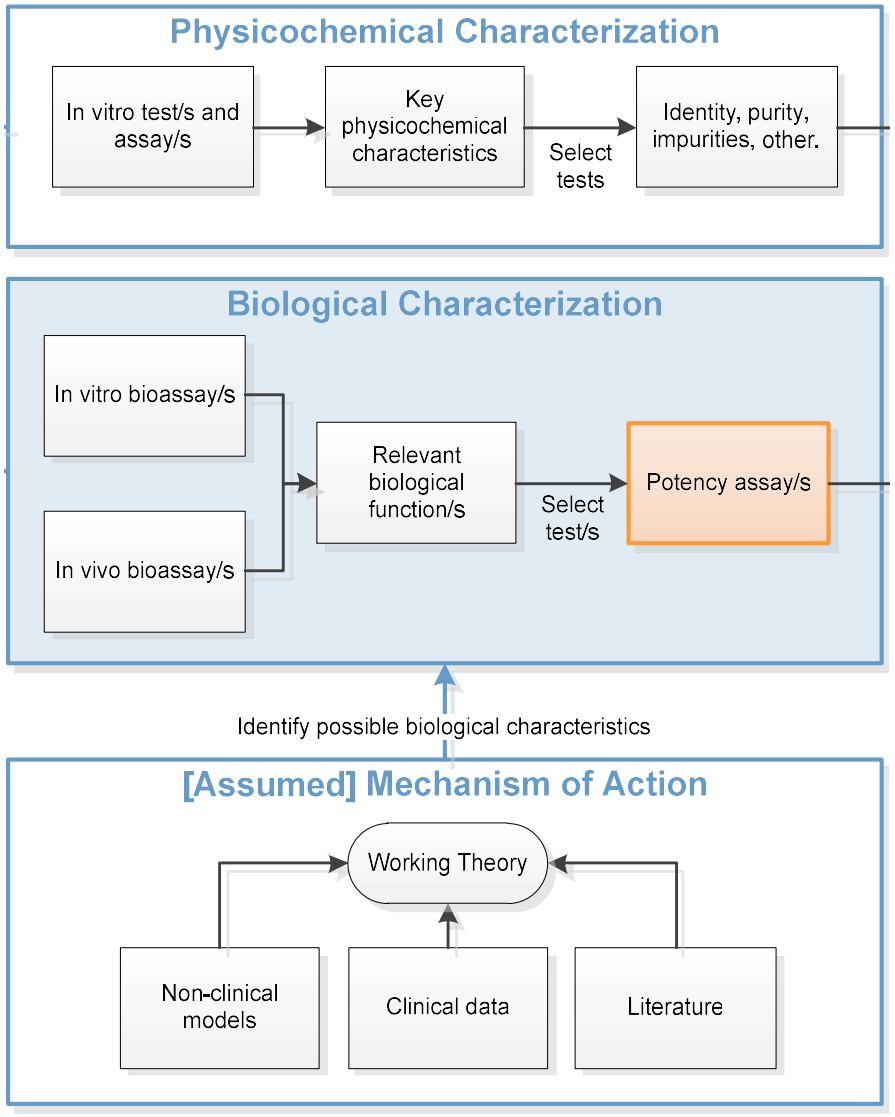
Scott R. Burger, MD

European Cell & Gene Therapy Analytical Development 2024
April 17, 2024

Characterization Testing Framework

- FDA requirements based on 21 CFR 610
- Safety
  - Sterility cultures
  - Mycoplasma
    - PCR-based assay acceptable, if validated against PTC
  - Adventitious agents
    - Blood donor testing (21 CFR 1271) for human-derived cells/tissue
    - More extensive testing if extensively expanded or using animal-derived reagents
    - Replication-competent virus, if relevant
  - Vector copy number
  - Tumorigenicity
- Purity, Identity
  - Measure intended product components, as well as contaminating cells and other undesired agents, including endotoxin.
  - Non-compendial analytical methods commonly used, acceptable if qualified (Phase I-II), validated (Phase III-IV)
- Potency
  - Relevant biological function(s). May require functional and nonfunctional assays.
- Stability

Characterization Strategy



Physicochemical characterization

Use of methods that measure physical and chemical characteristics

- ***Physical:*** size, morphology, light scattering properties, tensile strength, cell number, confluence.
- ***Chemical:*** identification of phenotypic markers and secreted substances, genotype, gene expression profile.

Biological characterization

Use of methods that measure biological function, such as how physicochemical characteristics influence biological systems.

- ***Biological:*** *in vitro* and/or *in vivo* measurements of cytotoxicity, cell growth, de/differentiation, proliferation, migration, immunomodulation.

Characterization Testing Development

- Establish patterns for purity/identity, function
  - Cell-surface marker expression, humoral factor production, functional assays, gene arrays…
  - Novel methods welcome, if scientifically valid and controlled
  - Multiple, orthogonal methods increase sensitivity, robustness
- Assess variability of raw material and product
  - Identify sources of variability, distinguish controllable *vs.* intrinsic/biological variability
  - Donor-to-donor, product-to-product, lot-to-lot variability
- Develop analytical standards and controls. Over time, qualify and validate analytical methods.
  - 21 CFR 610 methods, ***or demonstrate equivalence***
  - At BLA, 21 CFR 610 methods ***or validated alternative methods***

Characterization is expected to improve as clinical development progresses, but analytical rigor is needed from the outset

FDA Expectations for Characterization (I)

- Appropriately controlled assays for Phase I. Safety testing should be qualified prior to Phase I.
- Determine suitability of the analytical method for providing useful data
  - Quantify assay performance characteristics
  - Identify potential sources of error
  - Quantify potential errors
- ICH Q2 method validation required by BLA stage
  - Accuracy, Precision
  - Limit of Detection, Limit of Quantitation
  - Specificity
  - Linearity and Range
  - Ruggedness, Robustness
  - Suitability

FDA Expectations for Characterization (II)

- FDA Guidance on CMC Information for Human Gene Therapy INDs, 2020
  - Description of viral capsid composition and envelope structures, as appropriate, any modifications to these structures (e.g., modifications to antibody binding sites or tropism-changing elements)
  - Biophysical characteristics (e.g., molecular weight, particle size) and biochemical characteristics (e.g., glycosylation sites).
  - Describe viral vector genome – SS, DS, or self-complementary, DNA or RNA, copy number (vg/particle).
  - Identity -- vector genome restriction digest and protein capsid analysis
  - Process-related impurities -- residual cell substrate proteins and DNA, plasmid DNA, helper virus contaminants, ancillary reagents.
  - Product-related impurities -- defective interfering particles, non-infectious particles, **empty capsids**, Report full:empty capsid ratio, ratio of physical:infectious titers.

Consider assay variability and "worst case" in designing assays and setting criteria

Assays are sometimes qualified/validated under ideal or best case conditions, and may factor only one variable at a time. This can lead to overconfidence of an assay. Real world use may involve:

- **Different QC analysts**
- **Different batches of reagents**
- **Different equipment**
- **Samples held for different lengths of time**
- **Different interpretation of procedures due to vague SOPs**
- **Subjective parameters (such as flow cytometry gates, background cut offs, dilutions, etc.)**

Assay variability can confound efforts to demonstrate manufacturing consistency, comparability, or stability.

Potency Testing

- Monitoring product quality and lot-to-lot consistency
- Provides data to establish specifications for lot release
- Supports:
  - Product stability studies
  - Comparability studies
  - Clinical data interpretation
- Requires a flexible, progressive approach, begin *early*
- Any assay used for biological characterization could be a potency assay if it gives a meaningful indication the product will be 'potent'.
- One assay alone is unlikely to reflect all the relevant biological effects.
- One or more biological assays may be needed together to define potency.
- Biological characterization allows identification of which assays are candidate 'potency assays'

Core Requirements for Potency Testing

- Potency testing must
  - Indicate product-specific biological activity/activities
  - Measure identity and activity of active components
  - Provide test results for product release
  - Meet pre-defined acceptance and/or rejection criteria
  - Include appropriate reference materials, standards, controls
  - Be feasible to validate (eventually)
    - Established, documented accuracy, sensitivity, specificity, reproducibility of test methods
  - Provide data to establish stability specifications
  - Provide quantitative data
  - Comply with biologics regulations and GMPs
  - Meet labeling requirements
- Correlation with clinical outcome important, but not required

At the Beginning…

- Evaluate multiple characteristics and candidate assays, guided by proposed MOA and data from POC studies

  "…you should measure a wide range of product properties in addition to those performed for routine lot release. …Assess which product attribute(s) best correlate(s) with potency."

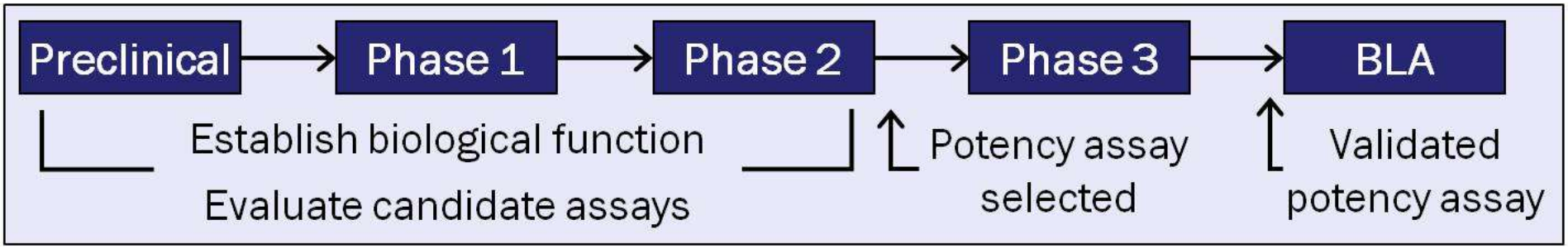
- What product characteristics and biological activities contribute to function?
  - Pre-clinical studies, proof-of-concept studies
  - Early clinical data
  - Historical experience
  - Reference materials and controls

Selecting Potency Assay(s)

- Functional assays may not be practical for use in product release
  - Turnaround time, sample size requirements may be problematic
- Surrogate assays can be used for release testing, if qualified against functional (biological) assays, and/or relevant *in vivo* effects

Progressive Implementation of Potency Testing

- In the course of Phase I and Phase II trials, evaluate multiple candidate assays for *proposed* product function(s)
  - Cytotoxicity, cytokine release, antigen presentation, proliferation, differentiation…
- Adjust assay acceptance criteria throughout product development to reflect manufacturing and clinical experience



Summary

- Cell and gene therapy products present unique challenges for characterization, largely due to complexity and variability of these living biological products and raw materials.
- Analytical methods must be refined over the course of preclinical and clinical development.
- Early in development, gaps in understanding of product CQAs, manufacturing CPPs, and MOA increase risks of manufacturing changes. Lack of analytical methods, particularly for testing potency testing, can hamper comparability studies.

References and Resources

- FDA Guidance - CMC Information for Human Gene Therapy INDs, 2020
- FDA Draft Guidance - Potency Assurance for Cellular and Gene Therapy Products - 2023
- ASTM F3368-19 - Standard Guide for Cell Potency Assays for Cell Therapy and Tissue Engineered Products
- FDA Guidance - Testing of Retroviral Vector-Based Human Gene Therapy Products for Replication Competent Retrovirus During Product Manufacture and Patient Follow-up – 2020
- ISO 20391-1:2018 – Biotechnology - Cell counting - Part 1: General guidance on cell counting methods
  - ISO/CD 20391-1 is in development and, when finalized, will replace ISO 20391-1:2018
- ISO 20391-2:2019 – Biotechnology - Cell counting - Part 2: Experimental design and statistical analysis to quantify counting method performance
- NIST - Cell Counting for Cell Therapies
  - COMET (Counting Method Evaluation Tool)
- NIST - Evaluating the Quality of Cell Counting Methods: Experimental Design and Statistical Analysis
- PIC/S Guide to GMP for Medicinal Products (PE 009-15), Annex 2A: Manufacture of ATMPs for Human Use - 2021
- FDA Draft Guidance - Manufacturing Changes and Comparability for Human Cellular and Gene Therapy Products, 2023
- FDA Guidance - Considerations for the Development of Chimeric Antigen Receptor (CAR) T Cell Products - 2024
- BSI PAS 83 - Developing human cells for clinical applications in the European Union and the United States of America, 2012
- FDA Guidance - Studying Multiple Versions of a Cellular or Gene Therapy Product in an Early-Phase Clinical Trial - 2022
- ICH Q9(R1) - Quality Risk Management
- ICH Q5E - Comparability of Biotechnological/Biological Products Subject to Changes in Their Manufacturing Process, 2005
- ISO 20399:2022 - Biotechnology - Ancillary materials present during the production of cellular therapeutic products and gene therapy products

Scott R. Burger, MD
Principal
Advanced Cell & Gene Therapy, LLC
+1 (919) 414-6947 - Mobile
celltherapy@ac-gt.com
www.ac-gt.com