Potency Assays for Engineered Cell Therapies
Challenges and Opportunities

Emily Lowe, PhD
Director
Analytical Development, Process Development
Biological Drug Development Timeline

Screen 1000s of molecules
Select drug candidate
Phase I
Phase II
Phase III
Launch
Post-approval research and monitoring
>10 y from discovery to licensure
$2.6B cost to develop a biologic
Clinical studies are large & complex
<12% of these are FDA approved

## Life Cycle Approach to Cell Therapy Product Design

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The QbD Challenge with Engineered Cell Therapies

- **The ATP/CQA Dilemma**: The product’s CQA should drive the design, development and validation of appropriate analytical methods but CQAs are defined fairly late in the process!

- Due to product complexity, CQA’s are often not known in early development & CMC quality control can be challenging (high batch-to-batch variability)

- Thorough Product and Process characterization are key to understand, identify, and develop appropriate quality control strategy for various phases of clinical development through commercial

- Defining CQA’s by correlational analysis requires a broad range of cell characterization and potency assays

QbD: Quality by Design; CQA: Critical Quality Attribute; ATP: Analytical Target Profile; eATP: Early Analytical Target Profile
The Critical Role of Potency Assays For Product Understanding and Final Product Release

- For each specific product, Potency methods need to:
  - reflect the **mechanism of action (MoA)** or relevant biological property/activity of the product
  - preferably be a biological assay (i.e. cell-based)
    - non-biological: immunochemical, biochemical, and/or molecular attributes
  - demonstrate stability indicating properties for the product
  - be fit for purpose, robust and easy to use for QC release testing

- Cell-based potency methods are used for
  - final product release and stability
  - enhancing product knowledge and understanding for products with a more complex MoA
  - qualification of critical reagents, internal positive controls and reference standards
  - support of changes in the Manufacturing Process
Inherent Challenges of Cell Therapy Potency Assays

- Cell-based potency assays are essential for engineered cell therapy products to demonstrate **final product activity is linked to biological CQAs**
- Autologous cell therapies **lack universal controls** and produce relatively **small sized lots**
- Allogeneic cell therapies rely on healthy human donors = **lot-to-lot variability**
- Desire for **fast release** potency assays so product can get to seriously ill patients
- Identifying and **controlling variability** is one of the biggest challenges in designing and executing cell-based potency assays

Poorly controlled and highly variable assays:
- Increase invalid and re-test rates (compliance risks)
- Manufacturing process may appear out of control
- Final product may appear unstable
A QbD Approach to Analytical Method Development Provides Early Opportunities

**ATP**
- What precision is required to understand whether Process changes impact CQAs?

**Design Space**
- Assay type
- Reagents
- Timeframe
- Plate layout
- **Magnitude of response**
- Reference material?
- Equipment (21CFR Part 11 compliance)
- Analysis
- Reportable
- **QC user requirements**

**Assay Development**
- Identify system suitability controls early
- Sample handling & stability
- Reagent and control stability
- Robustness of assay parameters (cell concentration, reagent volumes)
- Reagent robustness (lot to lot variability)
- Stability indicating

**Assay Performance**
- Specificity
- Sensitivity (LOB/machine noise)
- Precision: Intra assay/inter assay repeatability
- Linearity
- Accuracy
- Range, LOQ

QbD: Quality by Design; CQA: Critical Quality Attribute; ATP: Analytical Target Profile; LOB: Limit of Blank; LOQ: Limit of Quantitation
Analytical Methods can be Divided into Unit Operations

Sample Preparation
- Thaw and wash cells
- Solubilize cell pellet
- Dilute supernatant

Sample Measurement
- Cell Concentration
- DNA/RNA Concentration

Plate Preparation
- Aliquot cells, DNA, supernatant
- Aliquot reagents

Run Assay
- Stain cells
- Co-culture, titration
- ELISA
- PCR

Data Analysis
- Analyze raw data
- Export data from equipment
- Reportable generation
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- Control identified sources of variability
- Platform wherever possible (improved method understanding for more successful qualification/validation strategies)
- Simplify steps
- Automation friendly (assay and analysis)
- Assay validity and method monitoring controls
• ~100% of target cells can be killed at “max dose”
• ~0% of target cells are killed at “min dose”
• Small perturbations do not result in large differences

QbD Approach to Cytotoxicity Method Dev
The Design Space: Maximize Robustness, Minimize Variability

- Chromium release: canonical, uses radiation
- MTT, LDH: colorimetric assays, simple equipment, low hands on time, not specific to target cell
- Luciferase: sensitive and low hands on time, requires genetic modification
- Flow cytometry: no genetic modification, increased hands on time, specialized equipment

**Assay Type**

**Assay Conditions**

- **Platformable, automation friendly**
  - Reagents – accessibility, stability, robustness
  - Timeframe – based on QC user requirements: <6 hr, same day/overnight
  - Plate layout – simple order of operations, consistent, “pipettable” volumes

**Target Cells**

- Susceptibility to being killed (caspases-3/7 induction)
- Consistency (ranked order) with orthogonal methods a plus
- Identify healthiest starting conditions

**E:T, Seeding Density**

- ~100% of target cells can be killed at “max dose”
- ~0% of target cells are killed at “min dose”
- Small perturbations do not result in large differences
It’s Never too Early to Think about Method Life Cycle

- Identify critical reagents that have an impact to method performance
- Implement robust incoming critical reagent qualification strategy to reduce invalids due to reagents

**Critical Reagent Qualification**
- Protocols
- Equipment
- Software versions
- I/OQ and PQ

**Standardization Across Sites**
- Reference, positive control and/or appropriate method control with a qualified range
- Timely method performance monitoring and proactive remediation to avoid OOS/invalids

**Method Optimization**
- Identify root causes of invalids for all methods
- Implement and execute an Analytical method optimization/remediation roadmap

**Method Remediation**
- Proactively identify method improvements and remediation to avoid major issues and crises

Late phase changes can impact specifications set during pivotal and require extensive bridging/comparability studies
Development of Cell Therapy Products Requires A Robust Toolbox of Potency Methods

Early Clinical Development
- Proposed MoA
- Ensures Strength for Dosing

Clinical Development
- Process Improvements
- Data from Translational
- Multiple Measures of Potency
- Orthogonal for Chzn

Commercial Release
- Simplify Control Strategy
- Simplest Validated Stability indicating

Your clinical potency methods will likely be more complex than your commercial method – to support equivalence, plan your studies early

MoA: mechanism of Action; Chzn: Characterization
Conclusions

- Engineered cell therapies are still very new modalities, have complex modes of action, and large inherent sources of variabilities.
- Using a QbD approach to Process and Method development ensures the focus is on Product Quality from the beginning.
- A well planned eATP will help to identify the right controls ensuring confidence in data through early and late phase.
- Thorough Analytical characterization is key to understand, identify, and develop an appropriate quality control strategy for various phases of clinical development through commercial.
- The Analytical toolbox of characterization and release potency assays are key to the product and process control.
- The entire scope of the method lifecycle and method impacts needs to be continually considered.
- Analytical method performance monitoring through control trending and invalid rate assessment should be implemented as early as Phase 1.
- Communications with Regulatory Agencies and obtaining scientific advice in advance of IND or BLA submissions is key.
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