Unleashing the Power: Assessing Cell Therapy Potency and Maximizing Lifecycle Management

June 27th, 2023

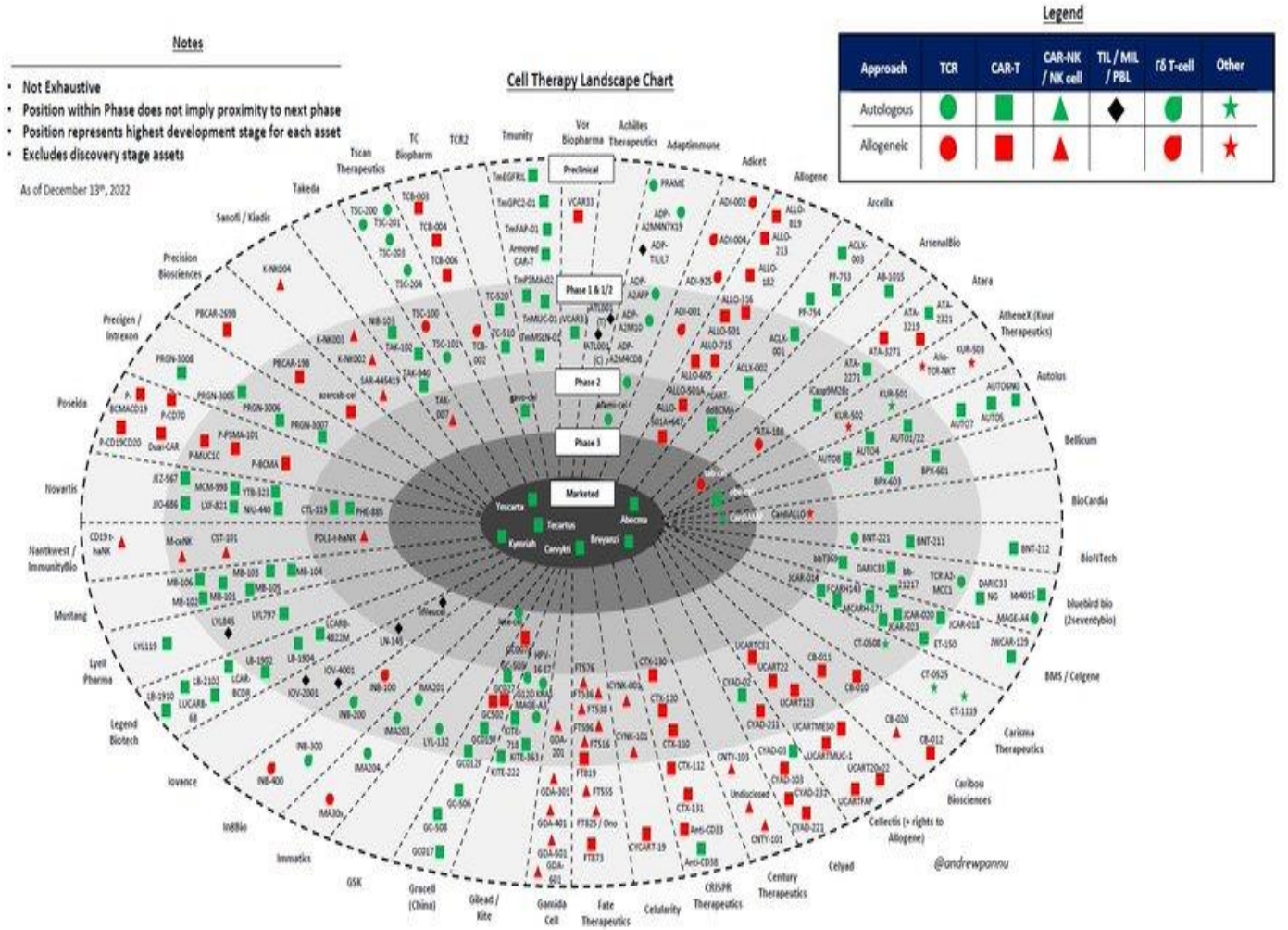
Seema Bansal, Associate Director, Gene Delivery Process and Analytical Development, Cell Therapy Development

Today's Presentation

- Cell therapy landscape and CMC challenges
- Assessing cell therapy potency; step wise/matrix approach
- Viral vector potency assay development
- Considerations during assay development, optimization and validation
- Life cycle management

Cell Therapy Landscape and Challenges of Autologous CAR-T

- Variability in starting cell composition
- Limited availability of starting material for process, product, and test method development
- Increased demand for qualified reagents and materials
- Unique scale, formulation, cryopreservation, storage, shipping and drug delivery challenges – *manufacturing technologies are in development*
- Characterization and comparability – novel/sophisticated analytical methods ***(Potency assays)***
- Regulatory guidance for cellular therapies is evolving and differs between jurisdictions and across life cycle of the product



Source: Downloads - Andrew Pannu

Delays Arising from Lack of CMC Information (Potency!)

DIVE BRIEF

FDA delays decision on Bristol Myers cell therapy, putting Celgene deal payouts in jeopardy

Published Nov. 17, 2020

May 19, 2021 06:26 AM EDT | Cell/Gene Tx, FDA+

CMC holdup delays Iovance filing again — to 2022 — as FDA seeks more assay data for TIL therapy

"One of the biggest things we see at the current time is the success of some of these products in the clinic has led to really rapid development, and the CMC doesn't always keep up with that pace."

- FDA regulator

October 6, 2020 07:04 AM EDT | Cell/Gene Tx

Iovance shares hammered on TIL therapy filing delay — although analysts aren't as bothered

Sarepta Duchenne Muscular Dystrophy Candidate Faces U.S. Food and Drug Administration Assay Request, Patent Suit

Alex Philippidis

Published Online: 16 Oct 2020 | https://doi.org/10.1089/hum.2020.29137.bfs

"One of the things that we see is that [developers] get ready to do a pivotal or phase 3 trial and they haven't developed a potency test to demonstrate that the lots that are going to be used in the clinical trials are going to have similar potency for all the different patients. And yet, that's one of the requirements for getting into a pivotal trial."

- FDA regulator

Potency Assay Expectations

- Potency is “the specific ability or capacity of the product, as indicated by appropriate laboratory tests or by adequately controlled clinical data obtained through the administration of the product in the manner intended, to effect a given result.” (21 CFR 600.3(s))
  - Strength is "[t]he potency, that is, the therapeutic activity of the drug product as indicated by appropriate laboratory tests or by adequately developed and controlled clinical data.” (21 CFR 210.3(b)(16))

- Considered an essential aspect of the quality-control system for a Cell Therapy (CT) drug substance and drug product

- **Performed to assure identity, purity, potency (also called *strength* in FDA documents), and stability of products used during all phases of clinical study as well as for licensed products**

- Inherent complexity of CGT products creates challenges to adequately assess potency
  - Complex or not fully characterized mechanism of action (MoA), multiple active elements and/or biological activities, limited product stability, biological assay is not robust, quantitative or precise

Steps in Potency Assay Development Throughout Life Cycle

a) **Understand the disease mechanism and therapeutic product**
- pathophysiological mechanism of disease/implicated pathways
- product composition, biological properties pre-clinical & and clinical investigations
-make hypothesis on Mechanism of Action [MoA (s)] and design appropriate methods for measuring and correlating MoA to efficacy

Candidate discovery

Clinical stage process & analytical development

Pivotal/LCM

b) **Collect sufficient characterization data through multiple assays to establish correlations**
- using qualified assays, characterize product to gain information about potential MoA/s
- DP (cell viability, purity and proliferation)- correlation to biological activity
-measure potency through different methods related to hypothesized MoA to support co-relation to efficacy
-refine choice of potency methods throughout development
-try surrogate if possible (fast/simplify/functional); define release spec

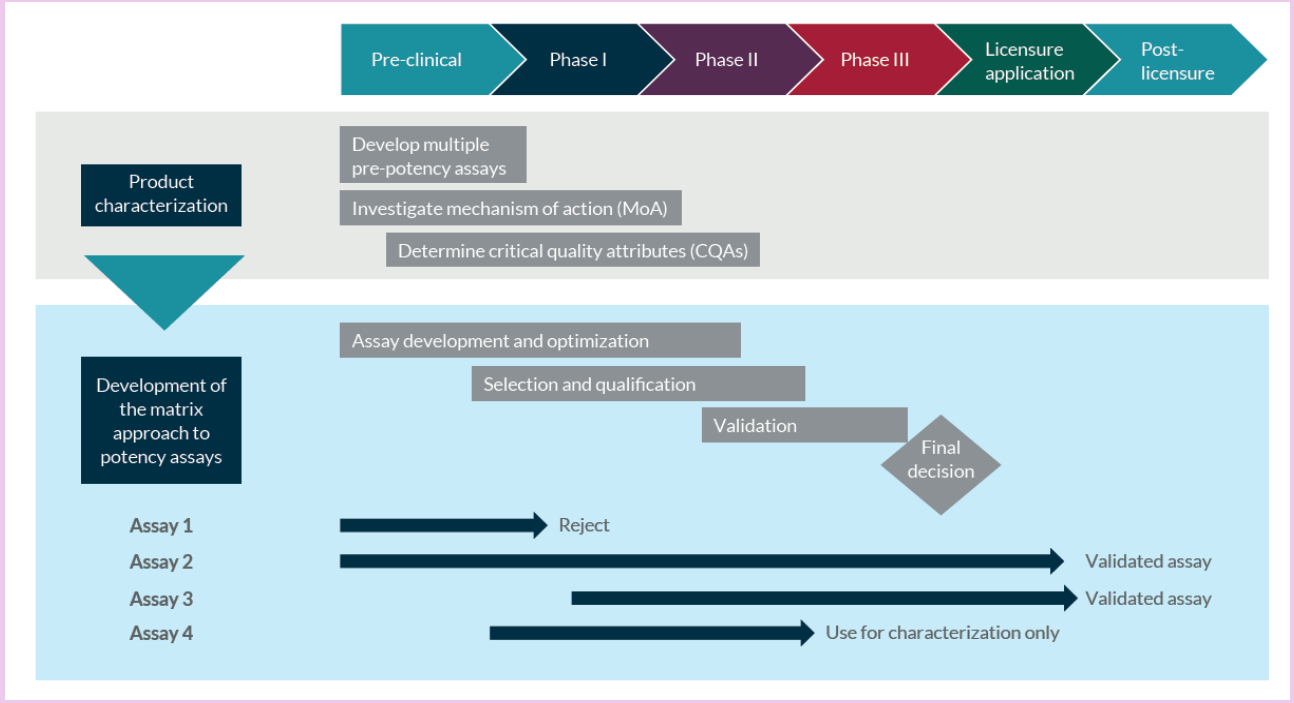
c) **Analyze and validate assay parameters**
- Ensure assay is practical/QC friendly
-accurate, precise, specific and robust
-tighten specifications
-validated with clinical outcome
-monitor performance (SPC, trending)

Matrix Approach to Assay Development

- A quantitative validated potency assay is required for BLA; however, assay should be established in GMP labs for pivotal trials
- At minimum, a qualitative assay is required for initiation of clinical trial

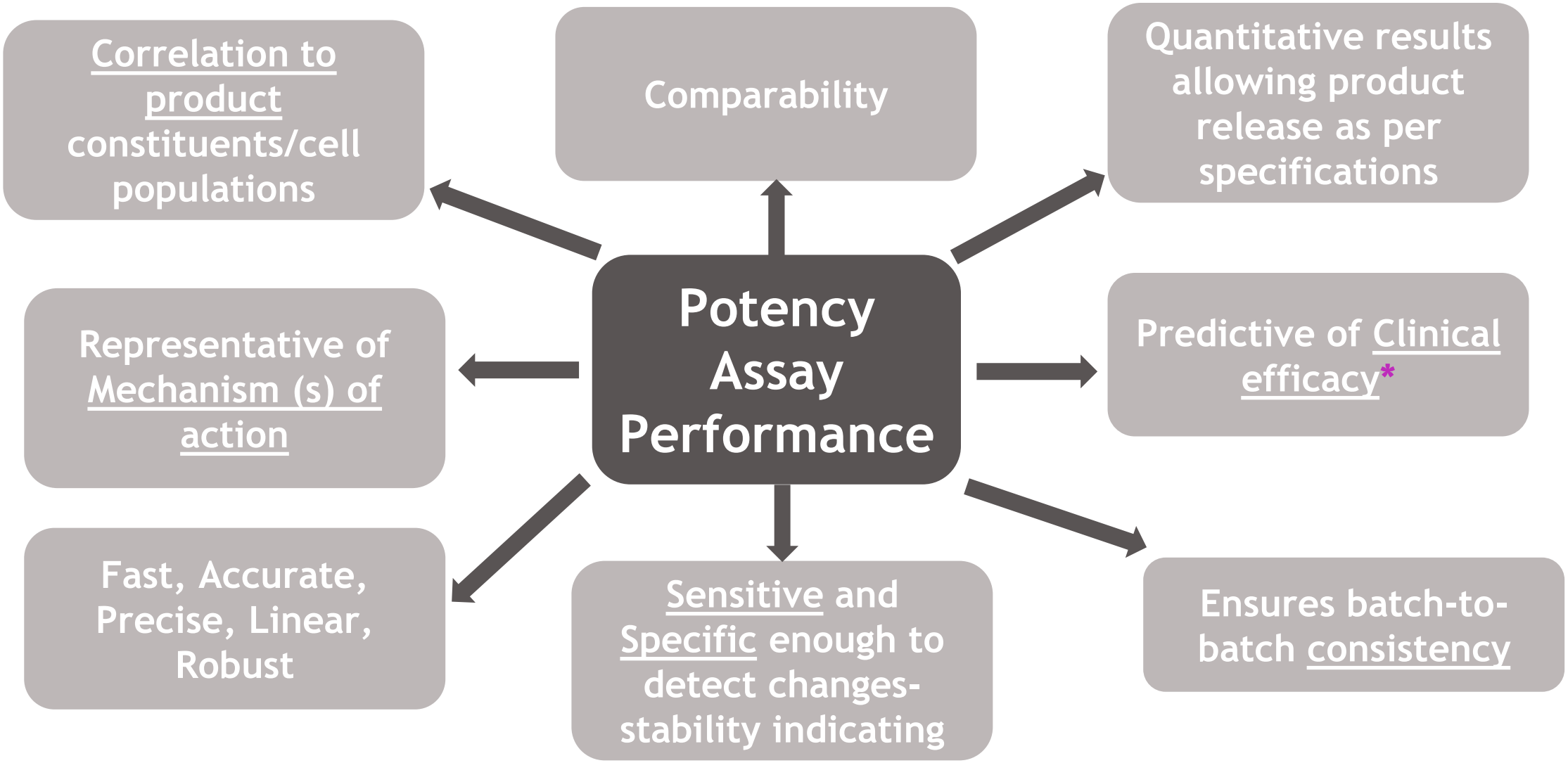
Utilize a matrix approach to adequately assess potency

- Development of multiple assays early in development (sum of assays/assay matrix)
- Not mandatory for development, however benefits of matrix approach include:
  - Enable range of quantitative, semi-quantitative and qualitative assays
  - Quantitative assay (required for BLA) may be developed iteratively
  - Orthogonal methods support product understanding
  - Support comparability studies during assay & process changes



Source: A-CELL, Alliance for Regenerative Medicines 2022; publicly available

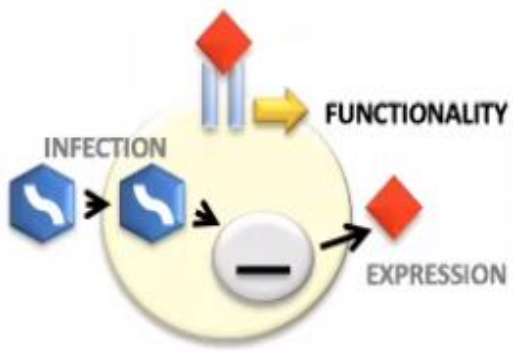
Desired characteristics of a potency assay

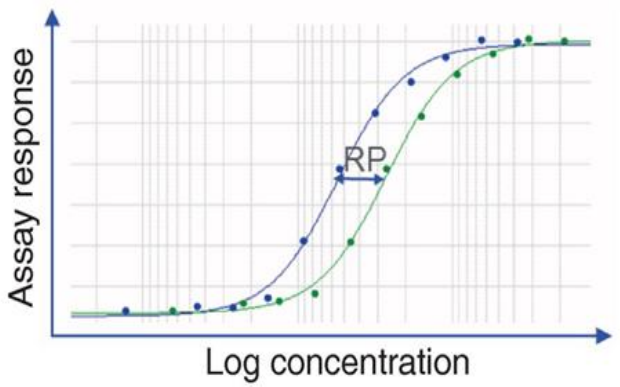


Developing Potency Assay (s) for Viral Vector

- Viral Vector, critical drug substance – potency needs to be controlled

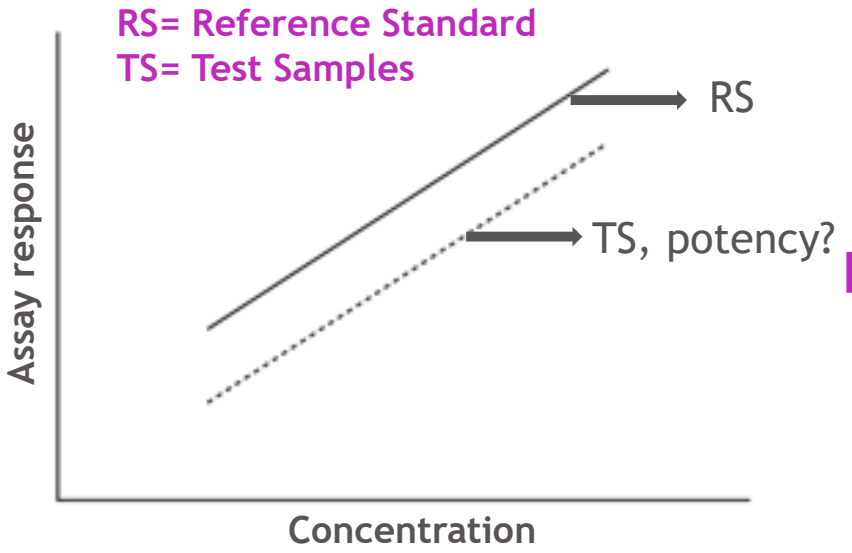
- Development of a bioassay that measures the ability of the viral vector to transduce a cell during DP manufacturing:
  a) Delivers transgene; viral vector is intact (integrating DNA sequence into genome)
  b) Confers expression of the gene of interest
  c) The gene is functional; measure its biological activity

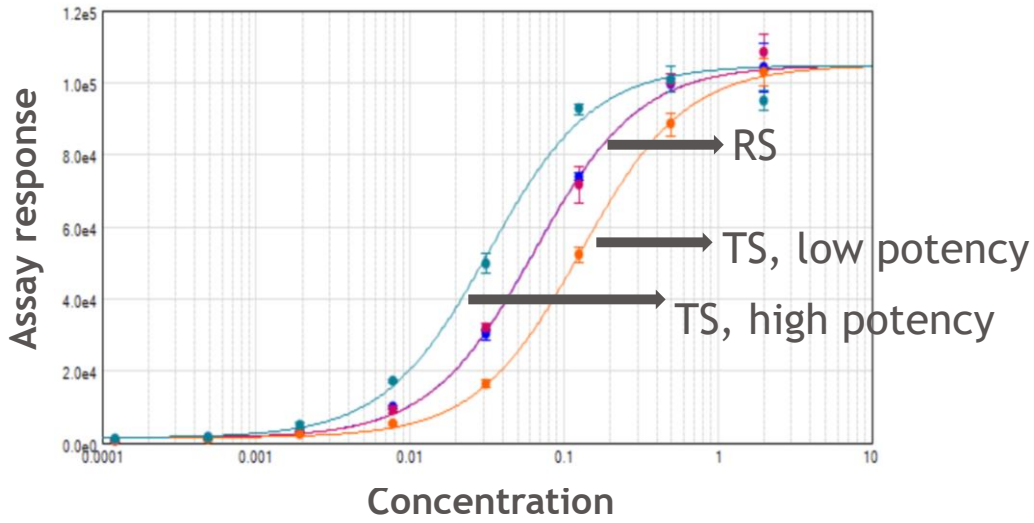




- During development:
  - focus on cell selection, appropriate infection conditions, signal through DR curve, meaningful potency results, can ensure product consistency, can help understand linkage to DP performance and most importantly reflective of MoA
  - develop correlation between an infectious titer assay and other potency assays during development to determine if one orthogonal assay can be used during later phase/BLA

Continuous Improvement as Part of LCM



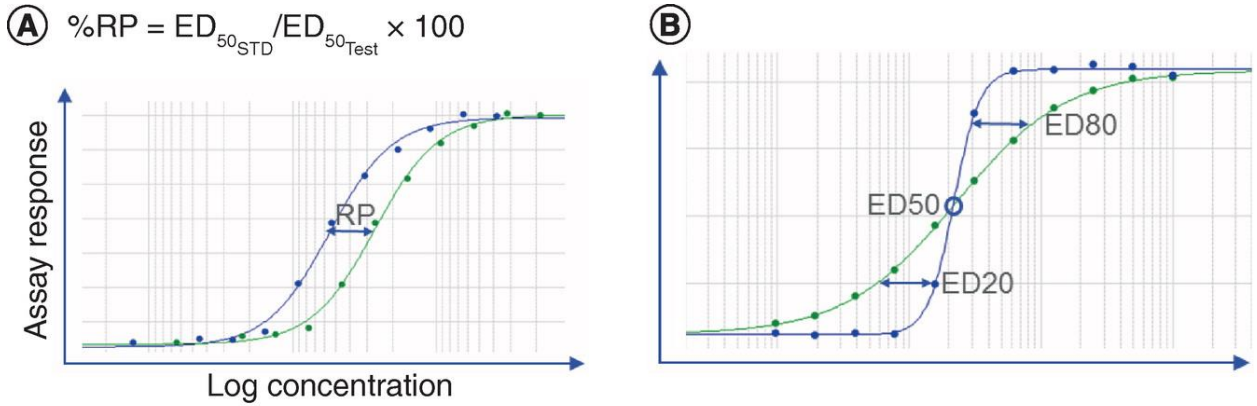


- Full dose response curve; no errors in identifying hyper/hypo-potent lots
- Reduced method duration (hours vs. days)
- Reduced dependency on critical reagents
- Reduced variation due to less complex operational steps
- Improved precision and accuracy
- Improved reliability and high sensitivity
- High dynamic ranges
- Higher throughput
- Easy to manage transfers/establishment at multiple QC labs
- Faster tun-around on investigations; easy LCM

Assay Development, Optimization and Validation

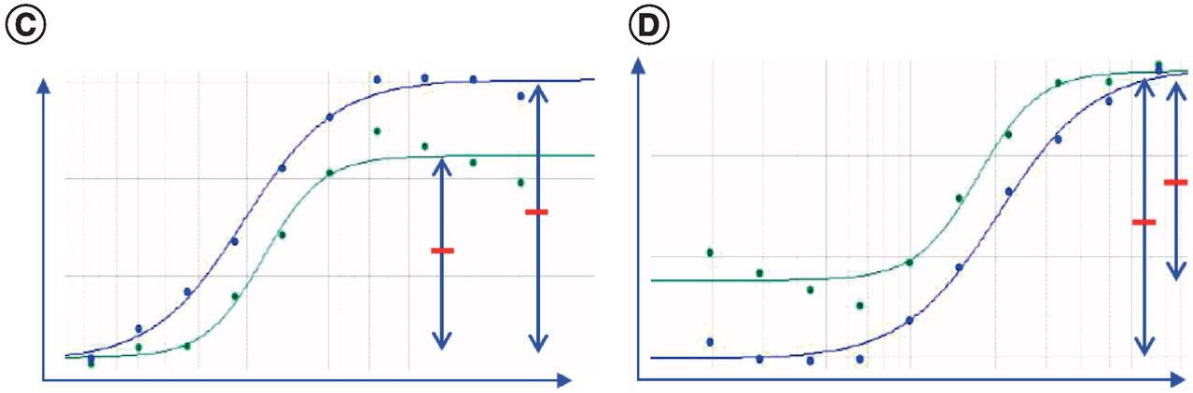
- ✓ Cell banks- Master & Working, Ready-to-plate vs continuous culture
- ✓ Controls: Negative vs positive, stimulated vs unstimulated, PBMCs, FMO, staining controls etc.
- ✓ Inter-assay control and well characterized reference standard
- ✓ Dose response curve: Linear vs non-linear model (4-PL to achieve upper and lower asymptotes), EC50 and slope
- ✓ Plate: Edge effect, dilutions, number of wells and samples/plate
- ✓ Assay conditions: Cell density, passage number, E:T ratios, incubation time and temperature
- ✓ Phase appropriate validation: Specificity, linearity, limit of detection and quantitation, range, accuracy, robustness precision
- ✓ Acceptance criteria: Assay vs the product
- ✓ Cryopreservation: Formulation, container, viability, recovery, resting cells over night (Lei wang et. al 2019)
- ✓ Walk the Health Authorities through your thought process, rationale for the assay, use of controls, preliminary data, bridging strategy if changing an assay during LCM

Considerations for Biological Similarity for Relative Potency



Ideal situation where dose–response curves share similar parameters. ED_{50} is influenced by the horizontal shift only.

The horizontal shift is not constant across the dose–response relationship and ED_{50} is consequently not representative of the bioactivity.



ED_{50} is influenced because of the difference between the upper **(C)** or lower **(D)** asymptotes and not because of the horizonal shift only (half of the effect is represented by the horizontal red line).

Relative potency should only be considered if **similarity** between dose-response curves is demonstrated

a) Select an appropriate curve fit model
b) Select relevant measures of similarity
c) Define acceptance criteria

Source: Best practices in bioassay development to support registration of biopharmaceuticals | BioTechniques (future-science.com)

Importance of Reference Standard for Potency assays

Why

- Need as a benchmark for DS/DP to a known sources of characterized final product
- Allows comparison of the biological activity of the active components
- Ensures lot-to-lot consistency
- Determine acceptance criteria for release

Risk

- No reference commercially available from USP or any other global source
- No specific guidance on characterization of Reference material- “insufficient; poorly defined’
- Limitation of the yield of product; source material is limited and critical for patient supply

How

- Generate RS using **“representative process”**
  - “At-scale” run
  - GMP material for BLA
  - Meets release criteria
- Generate RS being **“representative product”**
  - Well characterized by different assays
  - Stringency on performance; centerline

Management of RS through LCM

- Ensure to keep sufficient inventory of the RS or first primary RS (our *gold* standard) for calibration purposes during the whole product life cycle
- Keep RS **replacement to a minimum** in order to ensure that the historical link to the clinical material is maintained without disruption
- Keep the RS or primary RS (if stability is ensured) as the calibrator each time another secondary/working material is developed (2-tier):

compare A→B, A→C, A→D and **not** A→B→C→D to prevent **drift**

- Recommend a meeting with agency to gain agreement on RS or primary RS replacement, if necessary

-qualify RS with predefined acceptance criteria
-bridge old vs new
-critical reagent strategy

Method Change During Life Cycle Management (Surrogate)

- Changes to analytical method should be submitted as an INDa or PAS under BLA
- Detailed description of method, rationale for change, risk assessment to understand impact on **product quality**
- Assay validation package, proposed acceptance criteria & justification of success
- Criterion and results of **method bridging** to legacy method (equivalent or better performance)
    - Concurrent testing of multiple lots reflecting product's lifecycle
    - Statistically powered study based on method Intermediate Precision (IP)
    - Important parameter to be compared –linearity, range (edge of spec), stability
    - Confirmation that the specifications support claims of safety and efficacy similar to during BLA

- Major changes should not be implemented late in development
- Establishing assays early supports process development comparability

Wrap-Up

- Invest in potency assay development **early**
  - Qualified potency assays are a requirement to start pivotal studies intended to evaluate efficacy
  - Many to one approach will enhance product knowledge and support transition to late phase

- Effort should be to develop potency assay that reflects the product's relevant biological properties and ideally the MoA

- Think ahead : plan for process changes and life cycle management throughout process/product development
  - Appropriate reference standards and assay controls (bioassay suitability)
  - Retains, retains, retains
  - Robust method bridging plan
  - Monitoring of methods, reagents
  - Data trending
  - Continuous improvement

- Keep abreast of regulatory guidance & requirements, participate in OTAT/FDA meetings(Type C), engage with agency through communications, ask specific questions to receive feedback


Bristol Myers Squibb™

Thank you

Seema.bansal@bms.com

Bristol Myers Squibb™