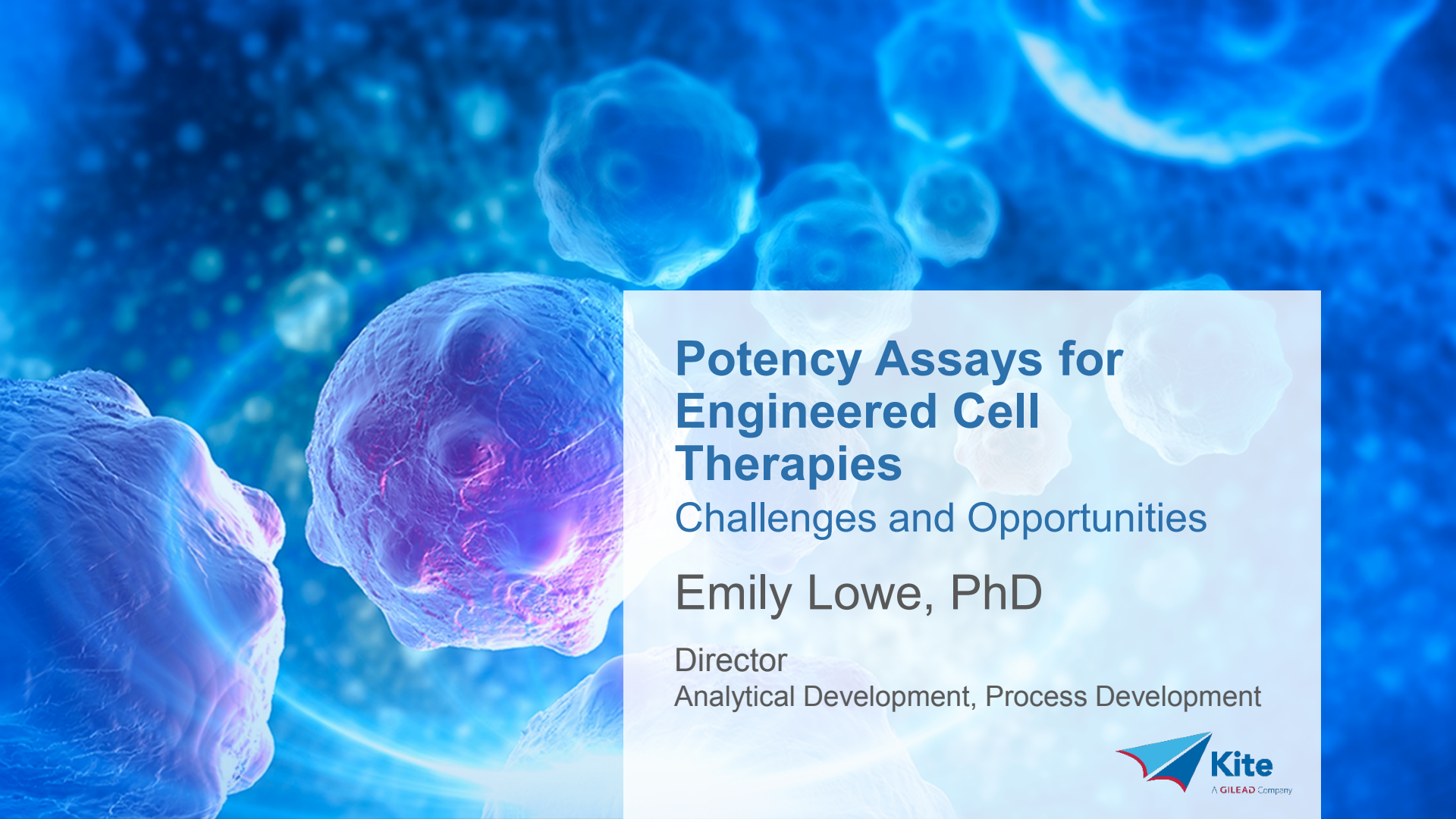

Potency Assays for Engineered Cell Therapies

Challenges and Opportunities

Emily Lowe, PhD

Director
Analytical Development, Process Development

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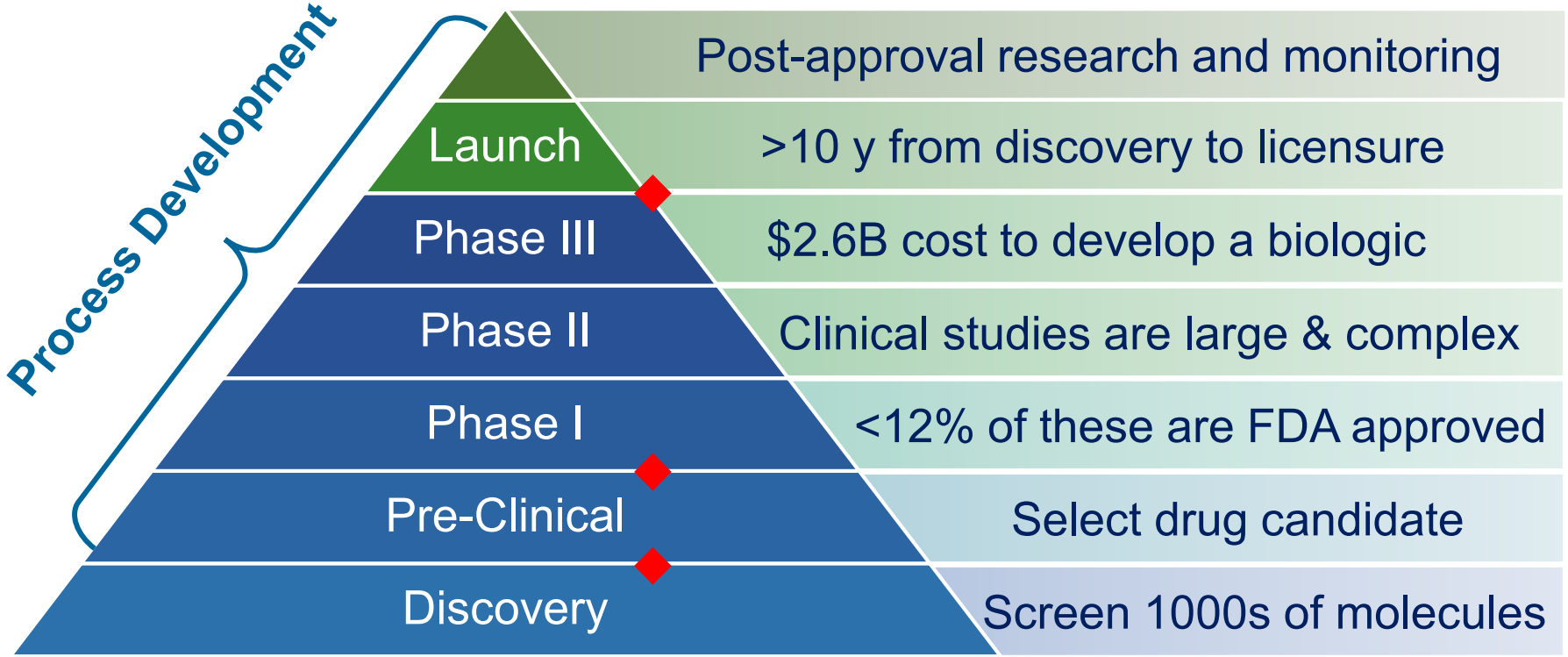
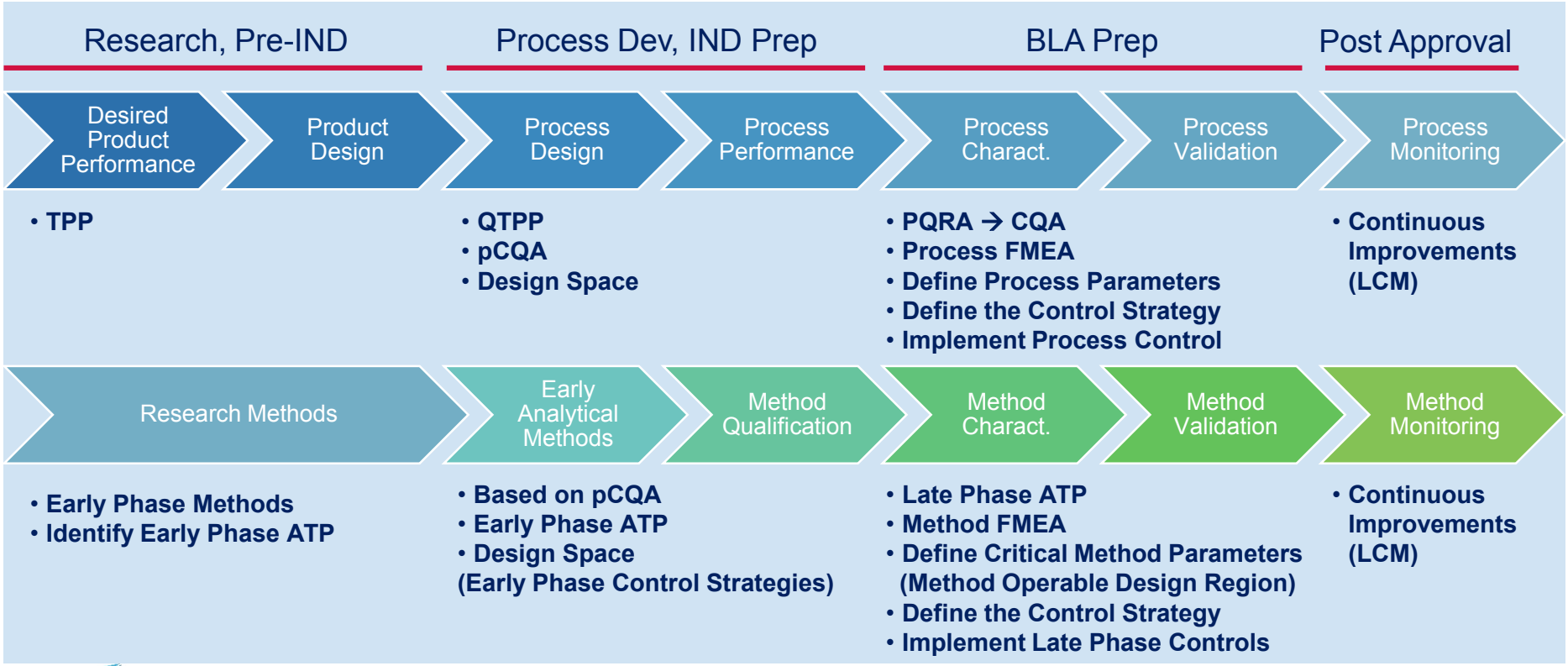
Biological Drug Development Timeline



Source: Tufts Center for the Study of Drug Development (CSDD) Briefing: "Cost of Developing a New Drug," Nov. 2014. Tufts CSDD & School of Medicine., and US FDA Infographic, "Drug Approval Process, "https://www.fda.gov/downloads/Drugs/ResourcesForYou/Consumers/UCM284393.pdf

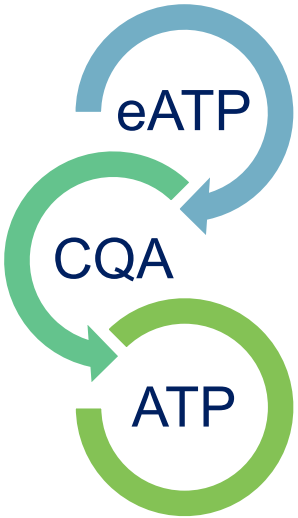
Life Cycle Approach to Cell Therapy Product Design



IND: Investigational New Drug; BLA: Biologics License Application; TPP: Target Product Profile; QTPP: Quality Target Product Profile; CQA: Critical Quality Attribute; pCQA: potential Critical Quality Attribute; FMEA: Failure Mode Effects Analysis; LCM: Life Cycle Management; ATP: Analytical Target Profile

The QbD Challenge with Engineered Cell Therapies

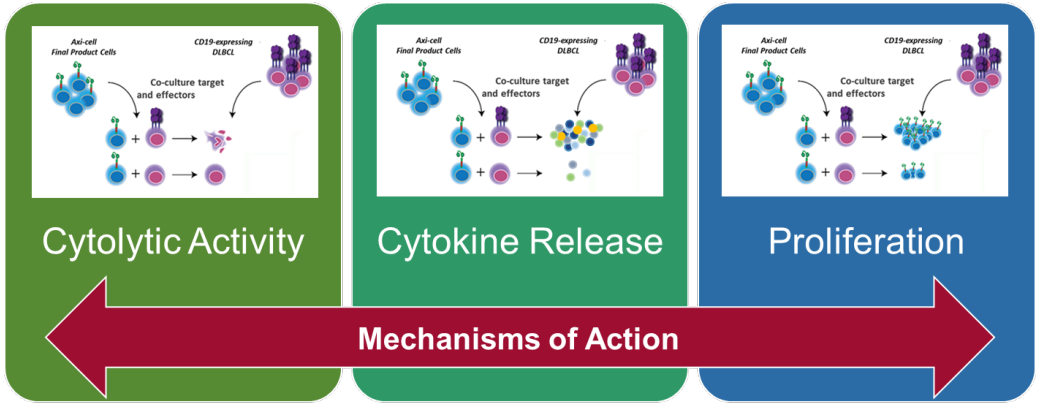
- **The ATP/CQA Dilemma**: The product's CQA should drive the design, development and validation of appropriate analytical methods **but CQAs are defined fairly late in the process!**
- Due to product complexity, CQA's are often not known in early development & CMC quality control can be challenging (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**



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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

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CQA: Critical Quality Attribute

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

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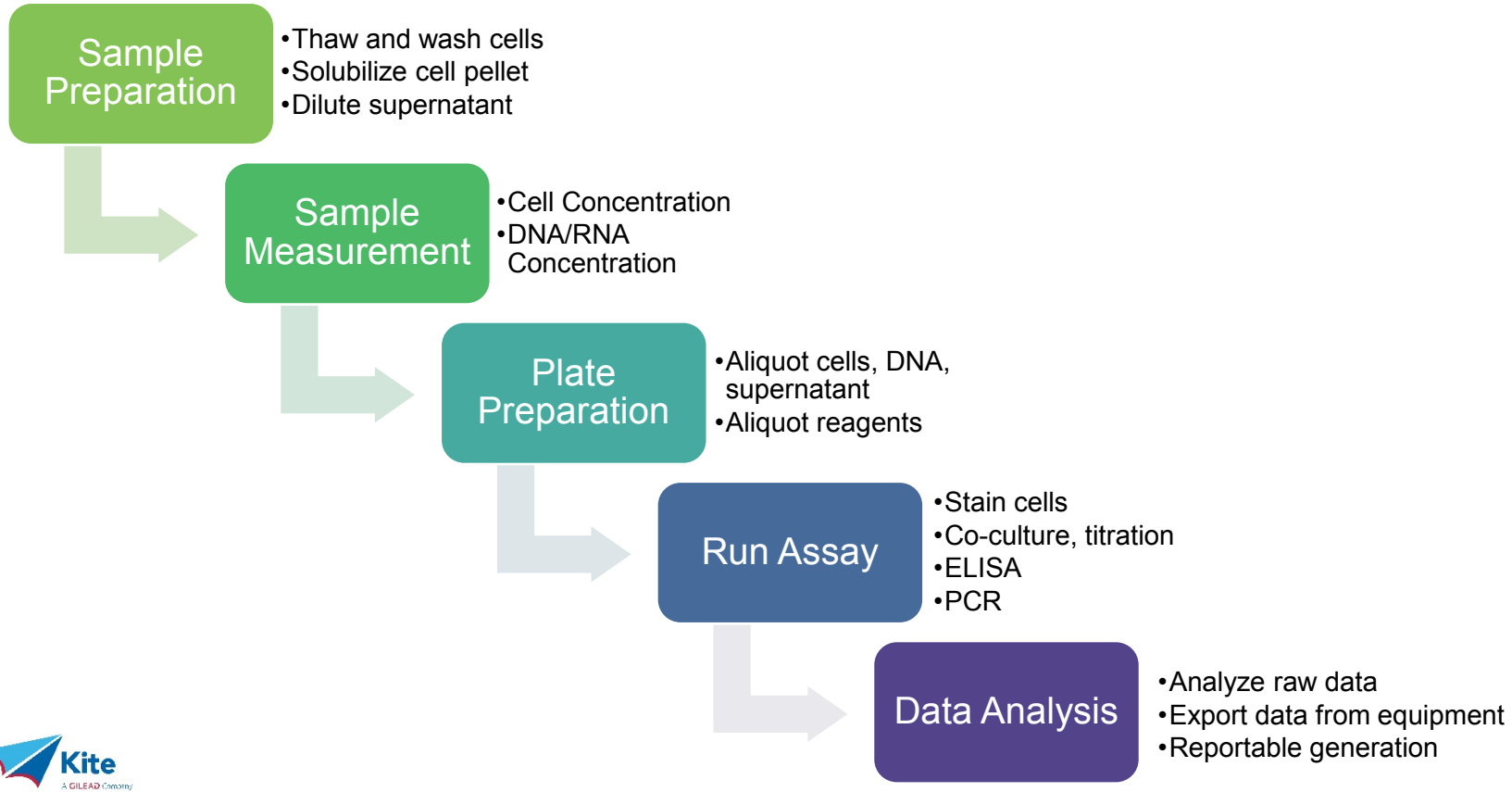
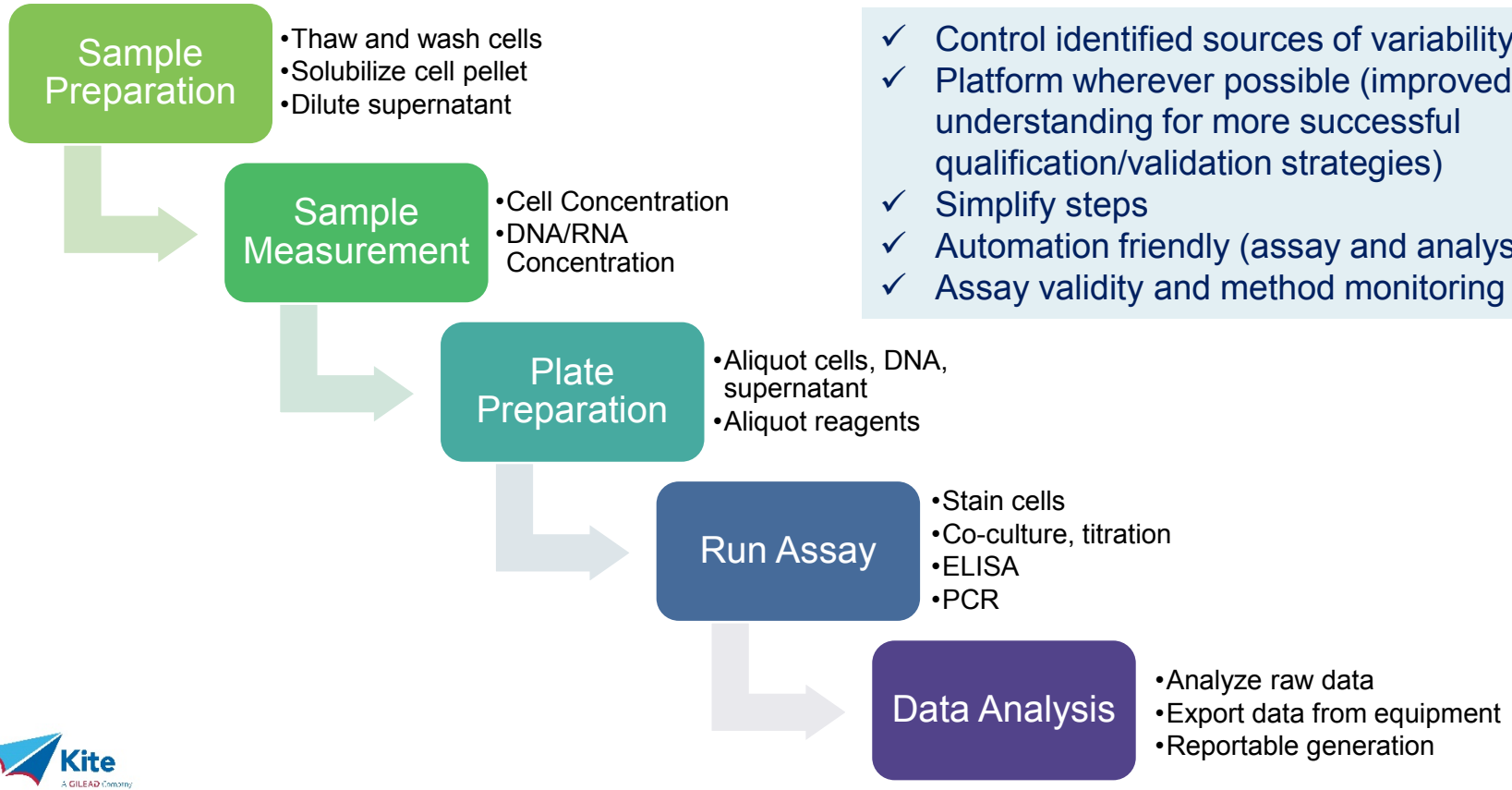
QbD: Quality by Design; CQA: Critical Quality Attribute; ATP: Analytical Target Profile; ATP: Analytical Target Profile; LOB: Limit of Blank; LOQ: Limit of Quantitation

Analytical Methods can be Divided into Unit Operations



Analytical Methods can be Divided into Unit Operations



- ✓ 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

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QbD Approach to Cytotoxicity Method Dev

The Design Space: Maximize Robustness, Minimize Variability

Assay Type

- 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 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 (caspas-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

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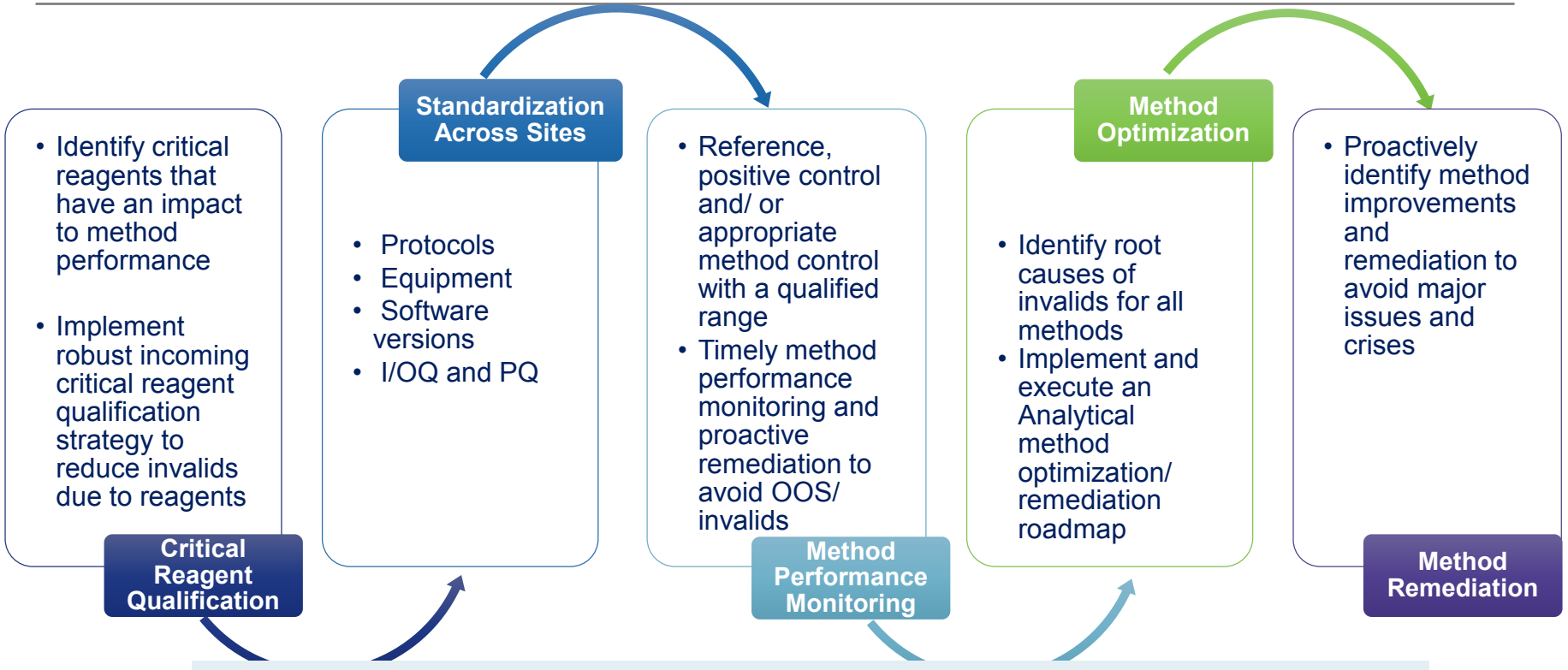
It's Never too Early to Think about Method Life Cycle



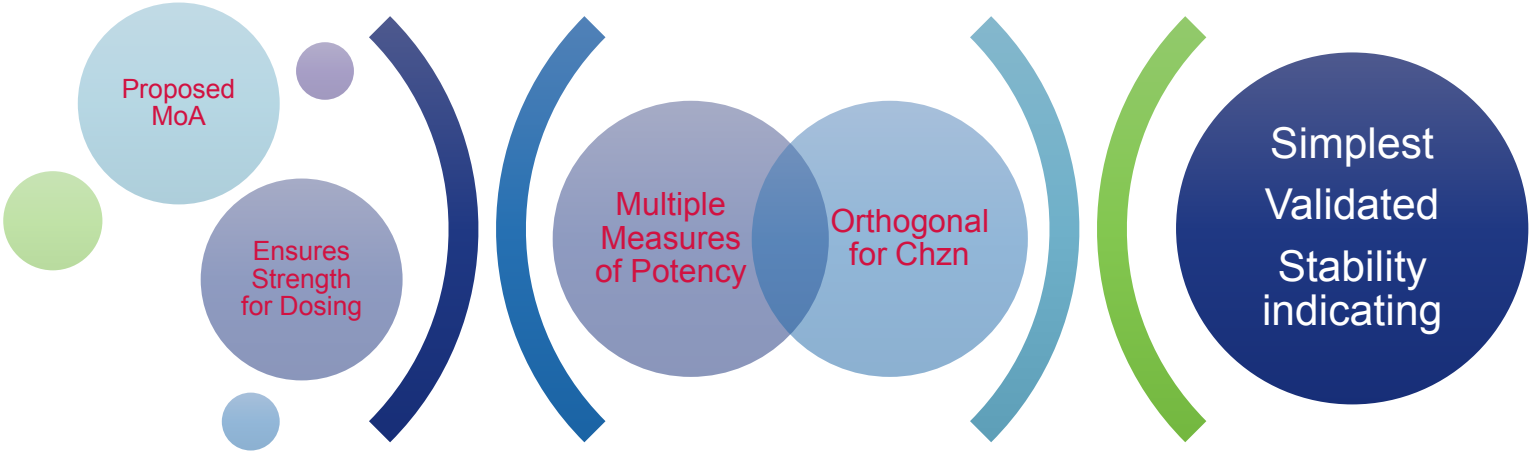
Late phase changes can impact specifications set during pivotal and require extensive bridging/comparability studies

Development of Cell Therapy Products Requires A Robust Toolbox of Potency Methods

Early Clinical Development

Clinical Development
Process Improvements
Data from Translational

Commercial Release
Simplify Control Strategy



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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Acknowledgements

- The patients, family, friends, and caregivers
- The Process Development Department under the leadership of Sugu Patro
- The Analytical Development Group under the leadership of Max Tejada

Questions?


May your ligands bind, sequences amplify and proteins fluoresce
-Dream Team 2016