Autologous Cell Therapy Phase Appropriate Control Strategies from Early Clinical Development to Commercialization

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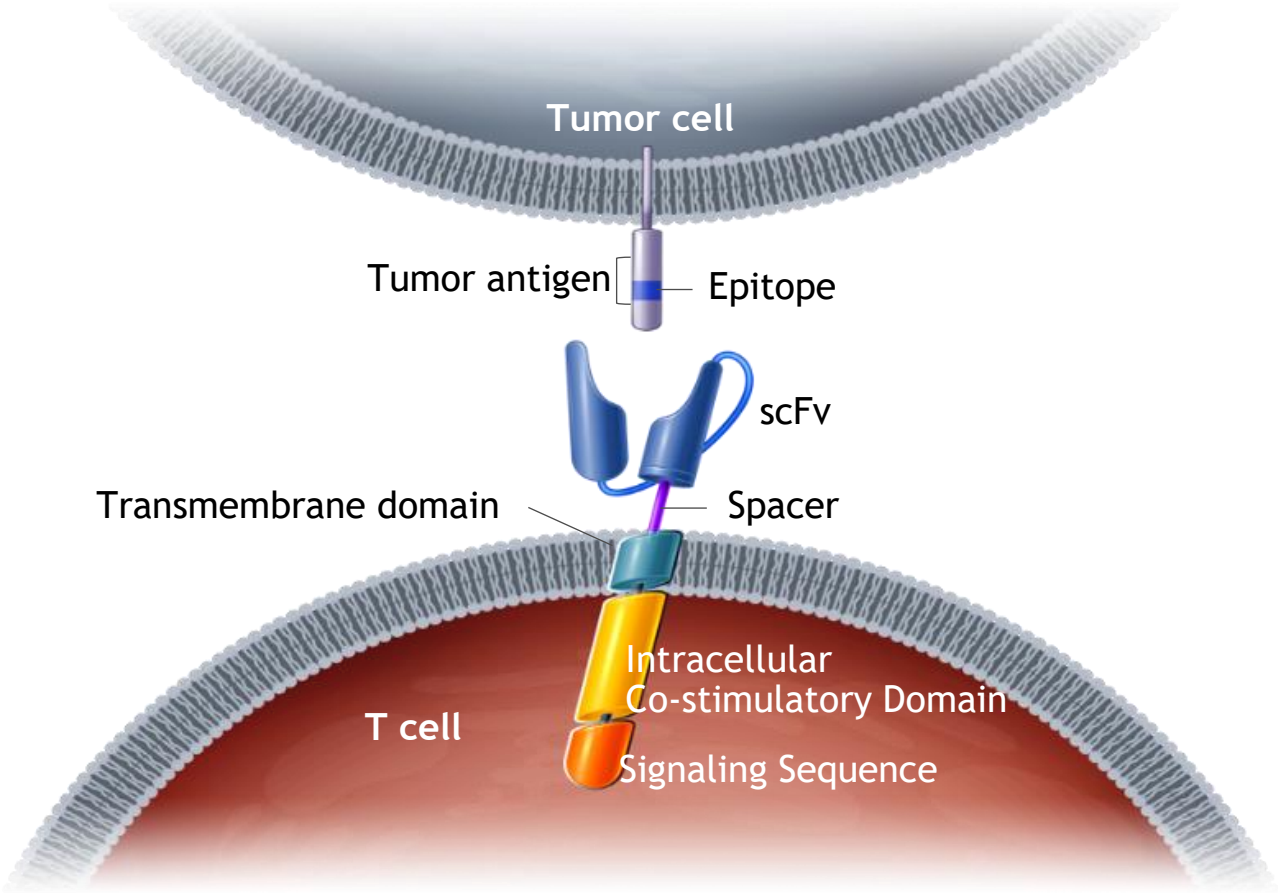
Nolan Polson, Ph.D.
Head of Global Product Quality
Cell Therapy Development and Operations

Bristol Myers Squibb™

Cell therapy specific challenges

Compared to other modalities:

- There are more attributes that can be characterized
- Cell therapy is a relatively new modality, so there is little consensus about which attributes are important to safety and efficacy
- Each lot starts with and is delivered to a single patient with a single clinical outcome, allowing for an assessment of correlation between critical quality attributes and clinical outcomes
- For autologous therapies, the source patient material is by far the most significant contributor to attribute variability, not the manufacturing process



General CAR T- Cell Manufacturing Process Controls:

★ GMP / Procedural Controls
★ Raw Material Controls

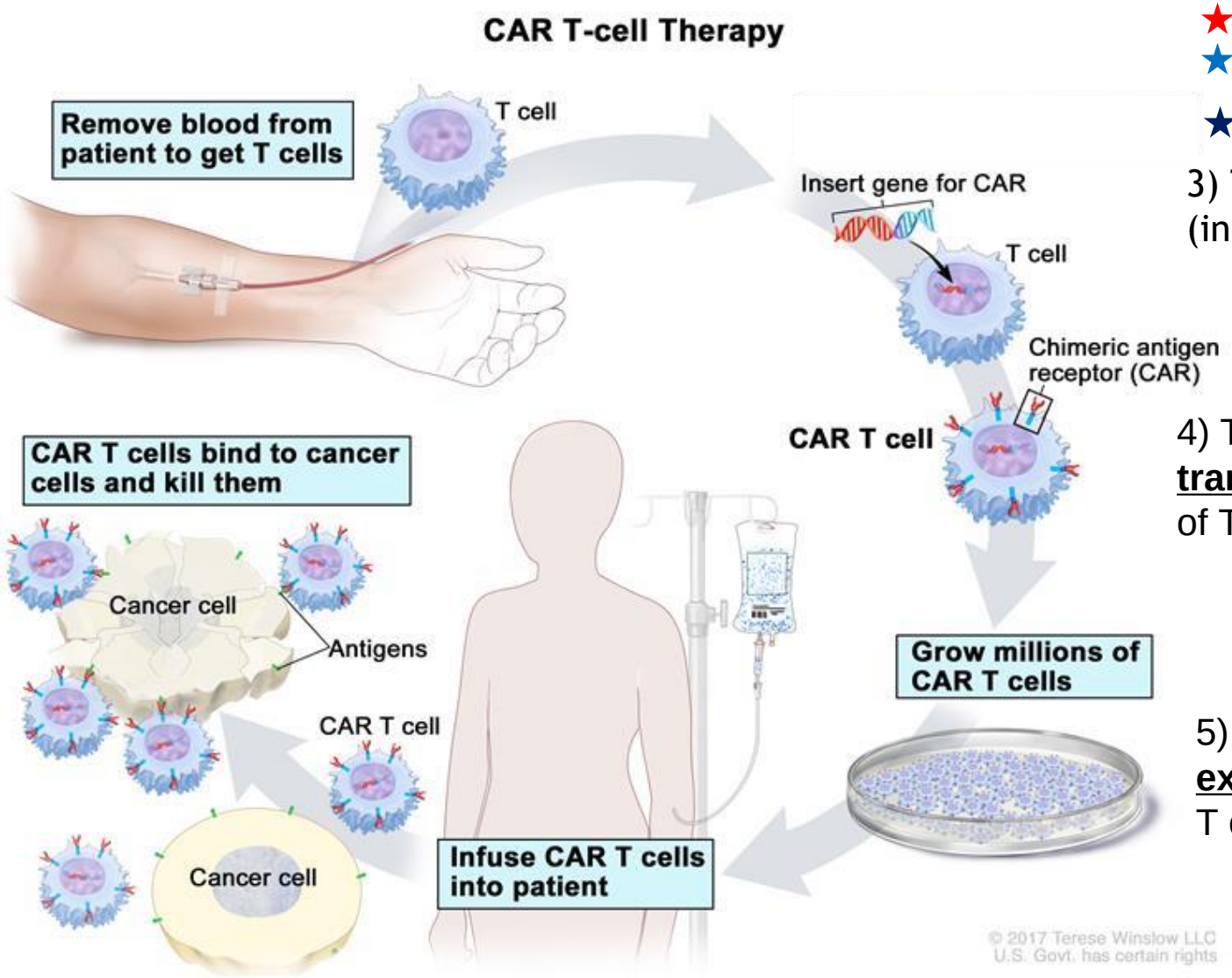
1) Patient's T cells are collected via **leukapheresis** (starting material)

★ Apheresis Specification
★ Characterization Studies

★ Periodic Controls
(PPQ, CPV, Comparability, APR, Stability)

6) **Infusion** back to patient following lymphodepletion

★ Characterization Studies



2) T cell **selection / purification**

★ DP Intermediate Specification
★ In-Process Controls, Critical Process Parameters, etc.
★ Characterization Studies

3) T cell specific **activation** (induce T-cells to proliferate)

★ In-Process Controls, Critical Process Parameters, etc.
★ Characterization Studies

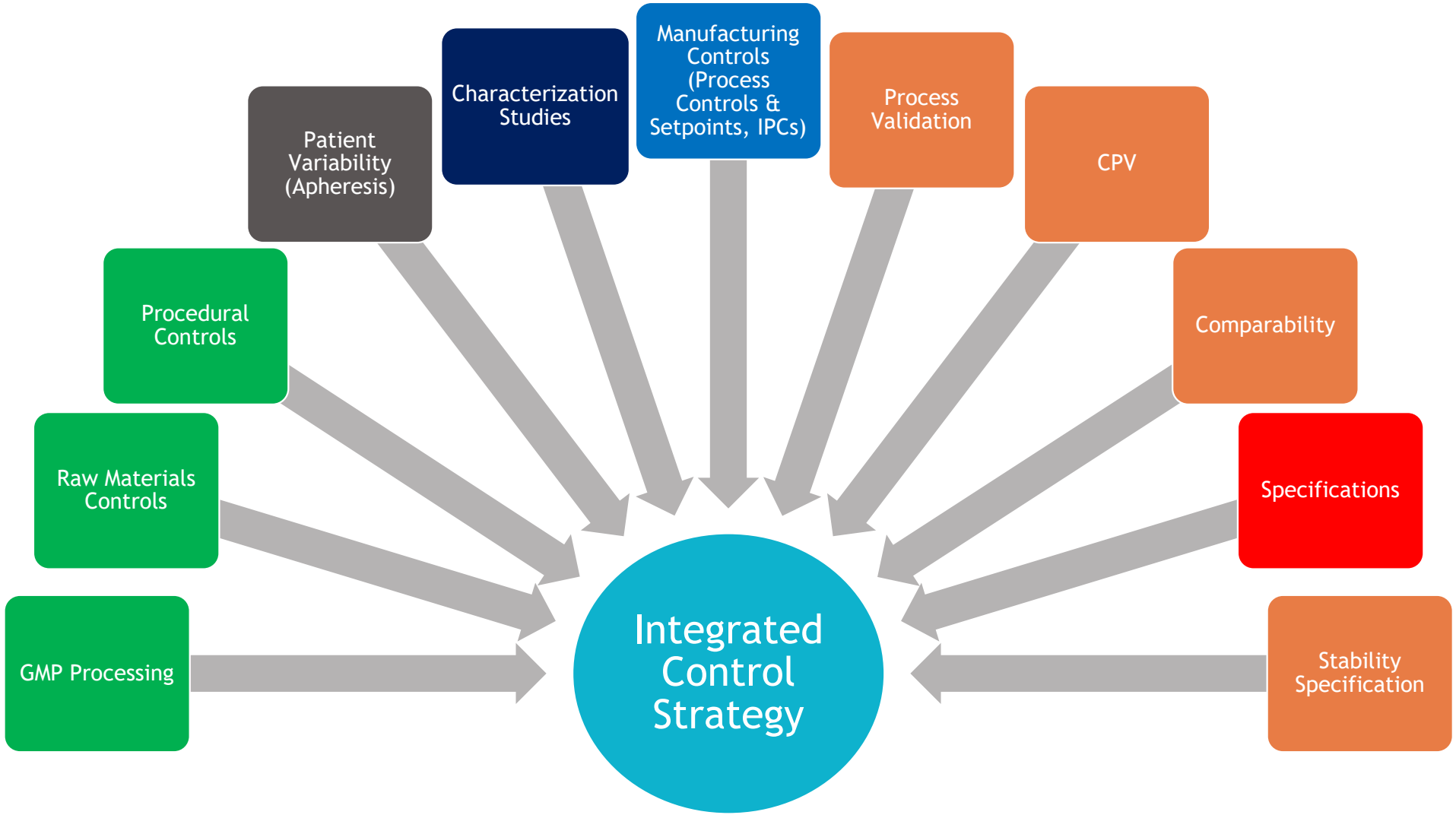
4) Transfer gene to T cells via **transduction** to provide specificity of T cell to target antigen

★ Viral Vector Specification
★ In-Process Controls, Critical Process Parameters, etc.
★ Characterization Studies

5) Controlled and consistent **expansion** to produce consistent T cell phenotype (dose)

★ Cryopreserved DP Specification
★ In-Process Controls, Critical Process Parameters, etc.
★ Characterization Studies

Total Integrated Control Strategy Focuses on Holistic Control of CQAs



Tools to Support Phase Appropriate Control Strategies

Tool	Purpose	Comments
Critical Quality Attribute (CQA) Assessment	• Establish critical quality attributes that directly impact product quality and/or patient safety • Aligned with clinical outcome through correlative analysis • Must consider attributes on the Quality Target Product Profile	• Initial CQA analysis pre-FIH (pre-clinical characterization, platform knowledge) • CQA assessment refined with additional product / process characterization • CQA confirmation via clinical correlative analysis
Integrated Control Elements Matrix (ICEM)	• Captures impact of process elements on product quality attributes • Compilation of control elements and where they are applied in the process • Defines the control strategy	• The ICEM/PQRA is commonly used in biologics (2009 A-Mab Case Study) and can be adapted for use in cell and gene therapy • Applies the principles of Quality Risk Management (Q9) to the integrated control strategy (ICS) • The ICS in the ICEM is *integrated* in that it accounts for (1) attribute criticality, (2) process understanding and (3) testing controls • The Product Quality Risk Assessment evaluates the integrated control strategy for residual risk to patients
Product Quality Risk Assessment (PQRA)	• Assess the overall risk of CQAs to patient given process capabilities and controls in place (detection mechanisms) • Tool for developing and justifying control strategies to minimize overall risk to patients	

The ICEM/PQRA is Attribute Based

Knowledge of the biological impact of each attribute (specification and characterization) is used to score each attribute for severity along a continuum of potential harm to the patient. Harm can arise through an adverse effect on the patient's health or reduced product efficacy.

The Integrated Control Elements Matrix (ICEM) is used to score occurrence and detection:

Process knowledge is used determine the likelihood (occurrence) that the attribute will be present in sufficient quantity in the drug product to cause the effect considered when assigning the severity score.

The testing program's capability for detection and control of the attribute is considered

Quality Attribute	Experience with Commercial Process	Process Knowledge	Raw Material Testing	DP Release Testing	IPC Action Limit/ Acceptance Criteria	Process Validation	Extended Characterization	Stability
Attribute 1	X	X		X				X
Attribute 2	X	X	X			X		
Attribute 3	X	X					X	
Attribute 4				X				X

Final residual risk to the patient is determined by considering severity, occurrence and detection

The Severity score and the Occurrence score are multiplied to arrive at a Preliminary Hazard level. This reflects the risk to patient without consideration of the testing program.

The Preliminary Hazard Level is multiplied by the detection score to determine the final residual risk to patients from each attribute

Product Quality Risk Assessment

Quality Attribute	Severity Score	Occurrence Score	Preliminary Hazard Level	Detection	Final Residual Risk
Attribute 1	3	5	15	1	15
Attribute 2	2	7	14	3	42
Attribute 3	1	1	1	2	2
Attribute 4	6	7	42	1	42

PQRA tool can be used throughout development to minimize risk to patients as additional product / process knowledge is gained

Using QbD Principles to develop Integrated Control Strategy

CQA assessments, Process Characterization, Integrative Correlative Analysis

Preclinical

- Hypothesis driven QTPP based on **platform CQAs**
- Develop product characterization strategy

Clinical

Pre-Pivotal

- Control Phase appropriate **CQAs** and pCQAs through risk-based approach
- Execute product / process characterization studies
- Perform preliminary **Correlative Analysis** to confirm **CQAs** based on clinical outcomes (safety, efficacy)
- **Initial ICEM / PQRA** to assess process capability, initial control strategy, and preliminary risk to patients

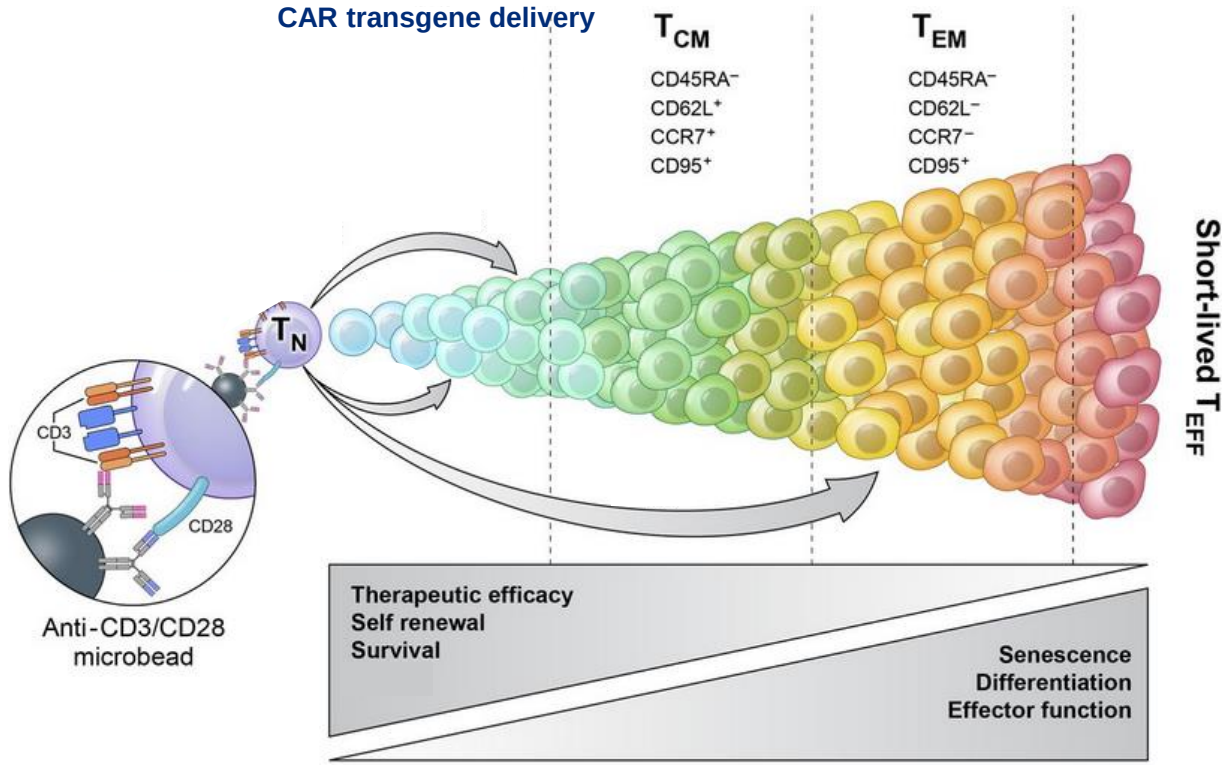
Pivotal

- Develop commercial enabling **Integrated Control Strategy**
- Perform final **Correlative Analysis** to confirm correlation of **CQAs** to clinical outcomes
- Confirm total **Integrated Control Strategy (post PPQ)** to support commercial product
- **Final ICEM / PQRA** to assess process capability, commercial control strategy, and final risk to patients

★ Commercial Filing

Autologous gene engineered T cell products are complicated, with a vast number of attributes that could be characterized by available analytical techniques

Cell Health	• Metabolic State[1] • Apoptosis marker[1] • Cell Viability
Phenotypic Composition	• CD3+ T cell purity[2] • CD4 and CD8 lineage[3] • Memory T cell subsets[4]
Antigen Specific Function	• Cytokine secretion[5] • Polyfunctionality[5] • Proliferative Capacity



Adapted from Gattinoni L, Restifo NP. *Blood*. 2013;121(4):567-568.

Clinical and pre-clinical evidence supports a role for early memory T cells in CAR T cell mediated efficacy

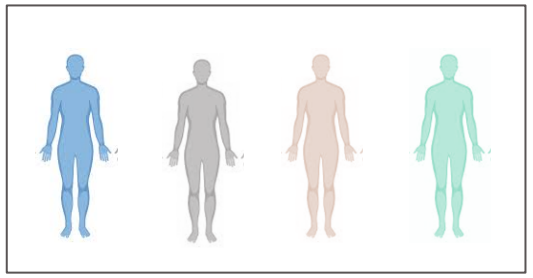
Multiple Myeloma[6], CLL[4], NHL[7], Preclinical mouse model[8,9]

1. Tschumi BO, et al. *J Immunother Cancer.* 2018;6(1):71.
2. Wang X, et al. *Mol Ther Oncolytics.* 2016;3:16015.
3. Golubovskaya V, et al. *Cancers (Basel).* 2016;8(3). pii: E36.
4. Fraietta JA, et al. *Nat Med.* 2018;24(5):563-571.
5. Rossi J, et al. *Blood*. 2018;132(8):804-814.
6. Cohen AD, et al. ASH 2018: Abstract 1974.
7. Larson RP, et al. AACR 2018: Abstract 960.
8. Ghassemi S, et al. *Cancer Immunol Res*. 2018;6(9):1100-1109.
9. Sabatino M, et al. *Blood*. 2016;128(4):519-28.

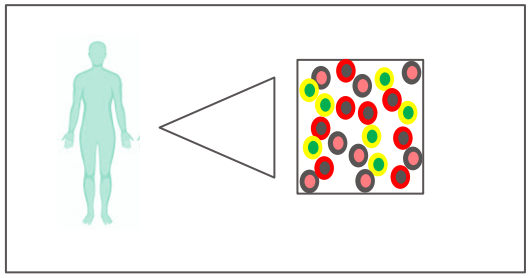
Patient Heterogeneity is the Primary Source of Variability in BMS Cell Therapy Products

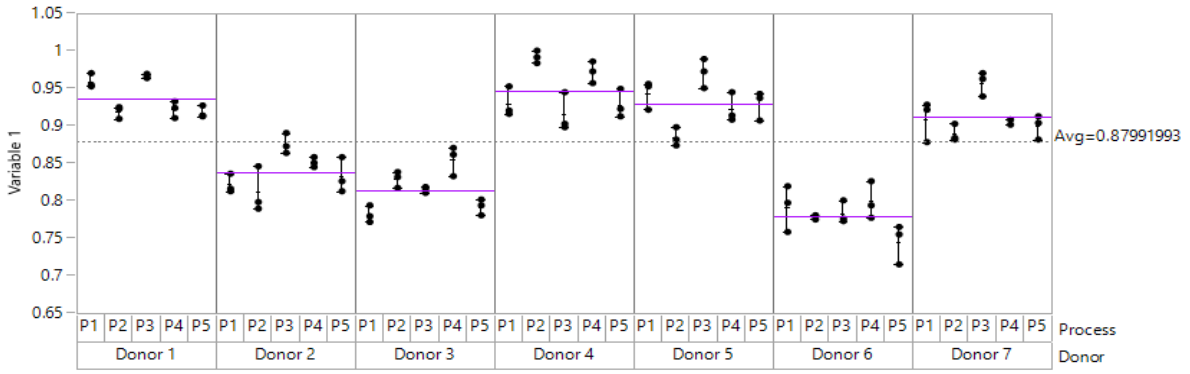
- Patient heterogeneity is represented in the autologous leukapheresis material
- Everyone's leukapheresis cellular composition and T cell subtype composition is different

Inter-patient Heterogeneity



Intra-patient Heterogeneity





Donor variability

Process variability

Analytical variability

Total variability

Variance Components

Component	Var Component	% of Total	20 40 60 80	Sqrt(Var Comp)
Donor	0.00439270	82.4		0.06628
Process[Donor]	0.00065038	12.2		0.02550
Within	0.00029072	5.5		0.01705
Total	0.00533380	100.0		0.07303

Donor variance makes up 82.4% of the total variance

Variability as a standard deviation for each of the variance components

$$\sigma^2_{Total} = \sigma^2_{Donor} + \sigma^2_{Process} + \sigma^2_{Analytical} = 0.00439 + 0.00065 + 0.000291 = 0.00533$$

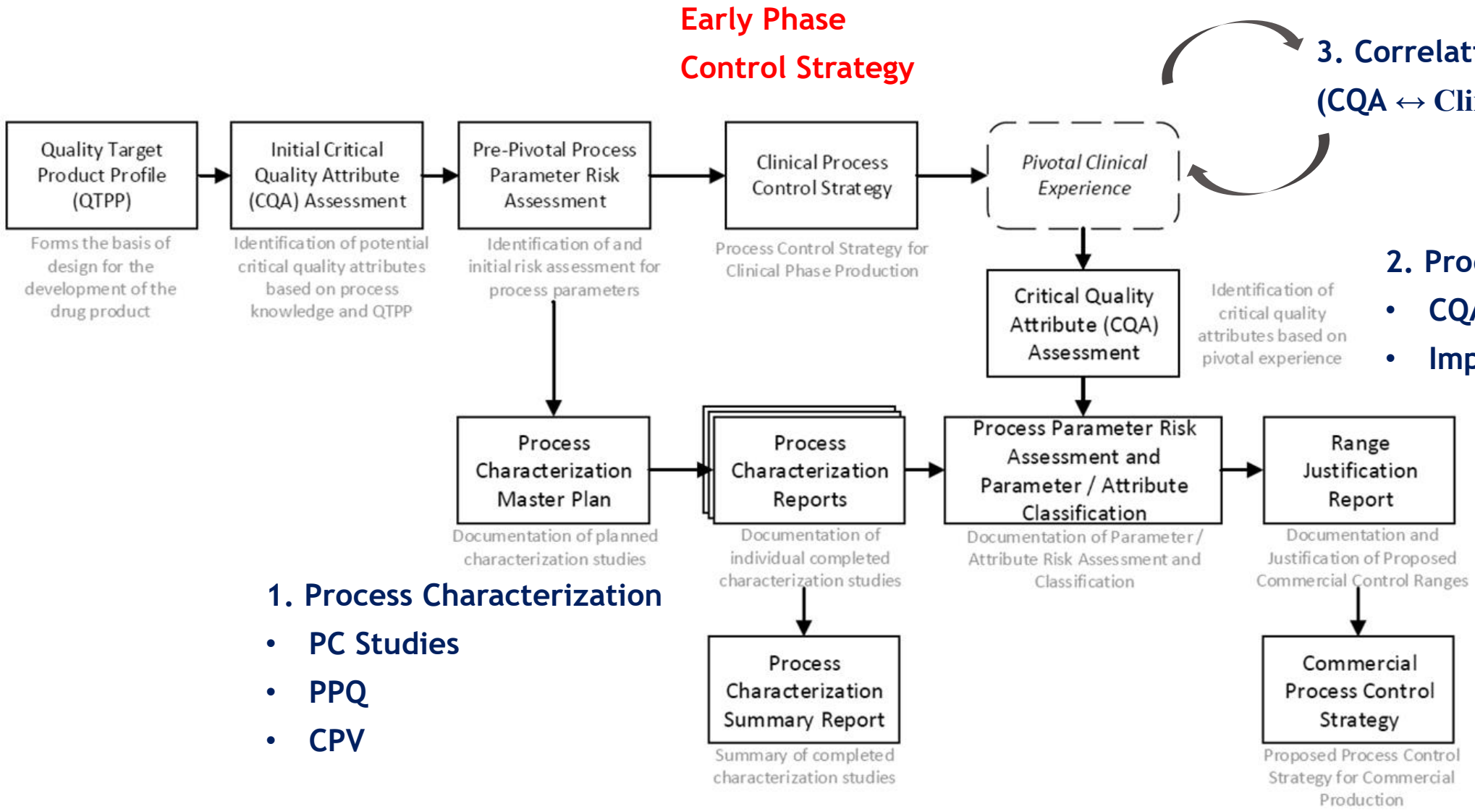
R. Ashton et.al., CASSS CGT Conf. July 2019

- The strongest contributor to variability in autologous drug product quality is patient and starting material heterogeneity
- Understanding the impact of this heterogeneity on product quality and clinical outcomes requires cross-disciplinary integrative correlative analysis

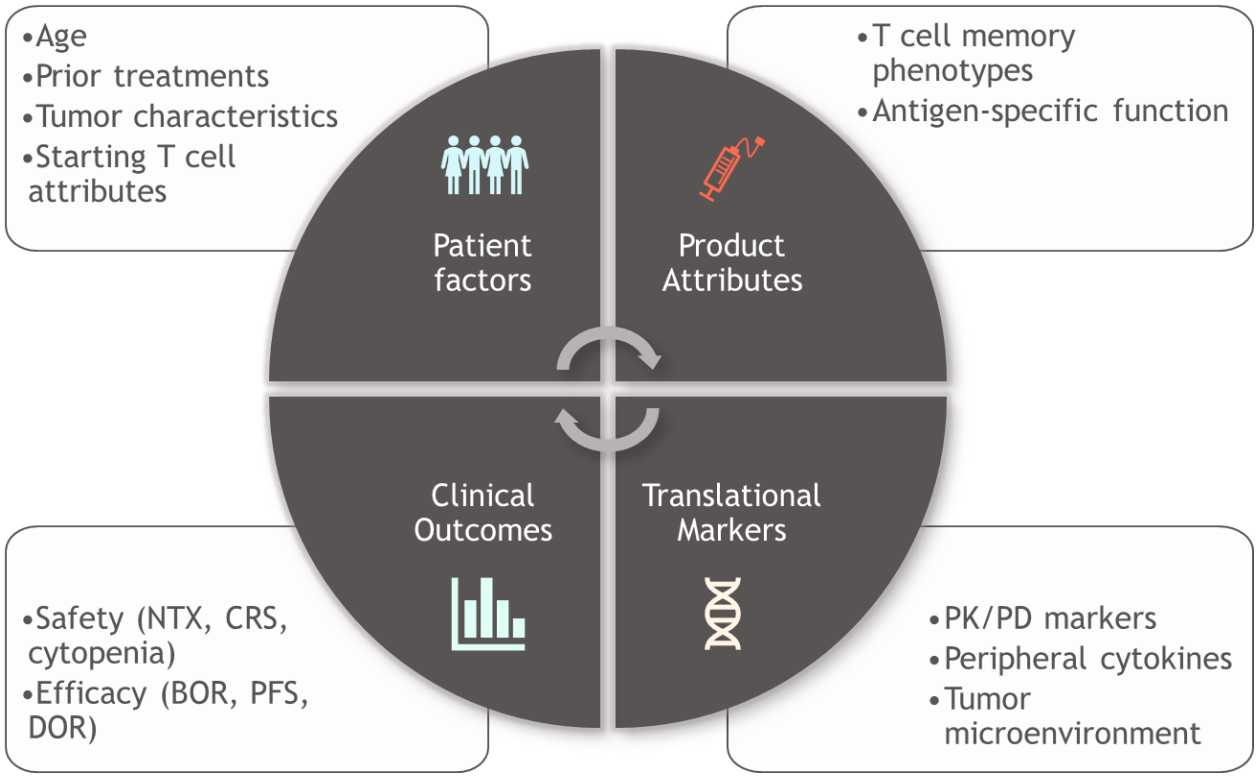
Initial Early Phase Autologous T Cell Therapy Specification

Quality Attribute	Parameter	Methodology	Specification
Appearance	Color	Compendial	Description of color
	Clarity	Compendial	Description of turbidity
Identity	Confirmation of ID	Flow Cytometry	Anti-XXX CAR+ cells detected (ID confirmed)
Purity	Cell Viability	Fluorescent Microscopy & Image Analysis	Initial Specifications based on Platform Knowledge (Product, Process), Patient Population, Health Authority Guidance, and Risk-Based Approach
	T cell Purity	Flow Cytometry	
	Product-Related Impurities	Flow Cytometry	
	Process-Related Impurities	ELISA	Initial Specification based on historical process understanding, initial impurity risk assessment or tox assessment
Strength	CAR + Viable T cells	Flow Cytometry	> XX CAR+ cells/mL (Strength in lieu of potency)
Safety	Transduction Controls	qPCR	Initial Specifications based on Platform Knowledge (Product, Process), Patient Population, Health Authority Guidance, and Risk-Based Approach. Strength alternative orthogonal control, develop in later phase of development.
	Endotoxin	Compendial	XX EU/mL
	Mycoplasma	Compendial	Not detected
	Sterility	Compendial	No growth

Pathway from Early Phase to Commercial Control Strategy



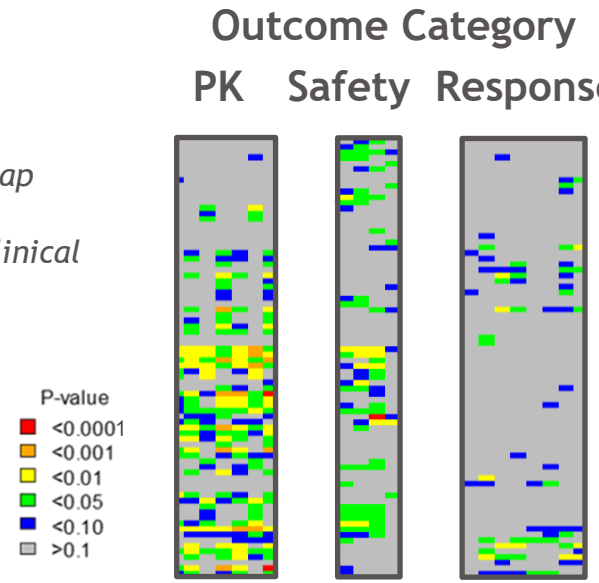
Correlative Analysis: Bridge between clinical outcomes and patient centric specifications throughout development



Correlative analysis: use of statistical methods and SME review to determine potential associations between two or more features

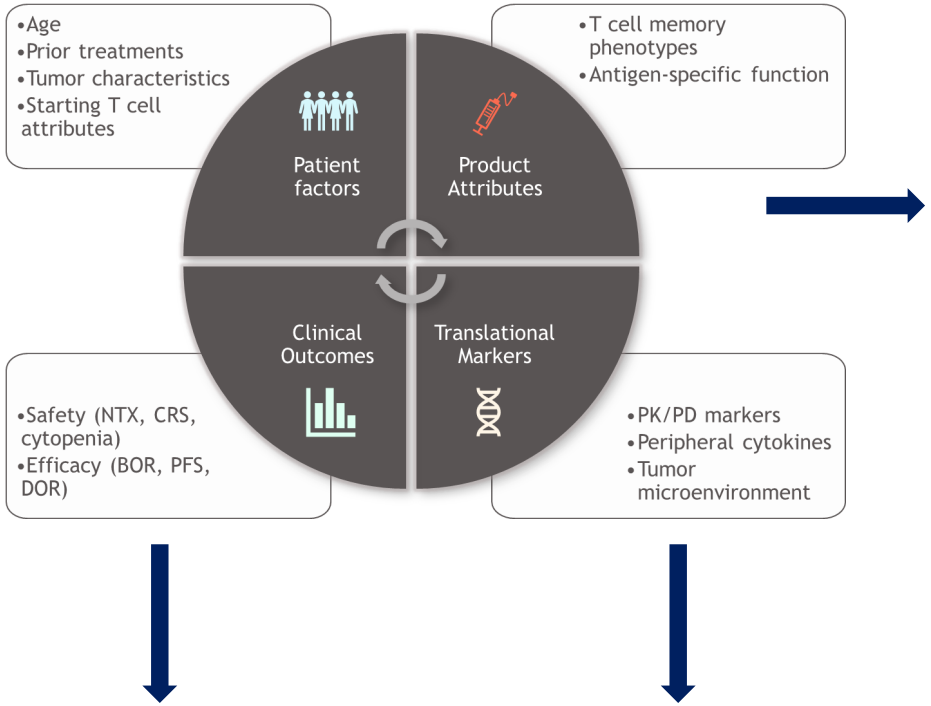
- The type of analysis performed will depend on the CQA and clinical outcome relationship being evaluated
  - Time-to-event (e.g. PFS, DOR)
  - Continuous (e.g. PK)
  - Categorical (e.g. Responder/Non-responder)

Nominal p-value heat map for univariate Product Quality Attribute and Clinical Outcome Relationships:



R. Larson et.al., AACR 2018

Quality Attributes evaluated in Correlative Analysis includes attributes measured on release / characterization



Quality Parameter	Attribute Name
Purity	Cell viability
Identity & strength	%CD3+CAR+
Strength	Viable cell concentration and transduction frequency
Purity	%CD3+
	%CD8+
	%CD4+
Potency and bioactivities	Antigen-specific function (cytokine secretion, cytolytic activity, proliferations, etc.)
Safety and vector integration	Vector copy number (VCN)
Additional phenotypes	Memory T cell composition
Cell health	Cell health markers

Clinical Category	Clinical Endpoints
Clinical Safety	Cytokine Release Syndrome, Neurotoxicity, Cytopenia
Clinical Efficacy	Overall Response Rate, Complete Response Rate, Progression Free Survival, Duration of Response
PK	Area Under the Curve (AUC), Maximum Concentration (C_{max}), Time at which C_{max} Achieved (T_{max})

Example: Evaluating Correlative Analysis Findings in Support of Specification Acceptance Criteria Setting

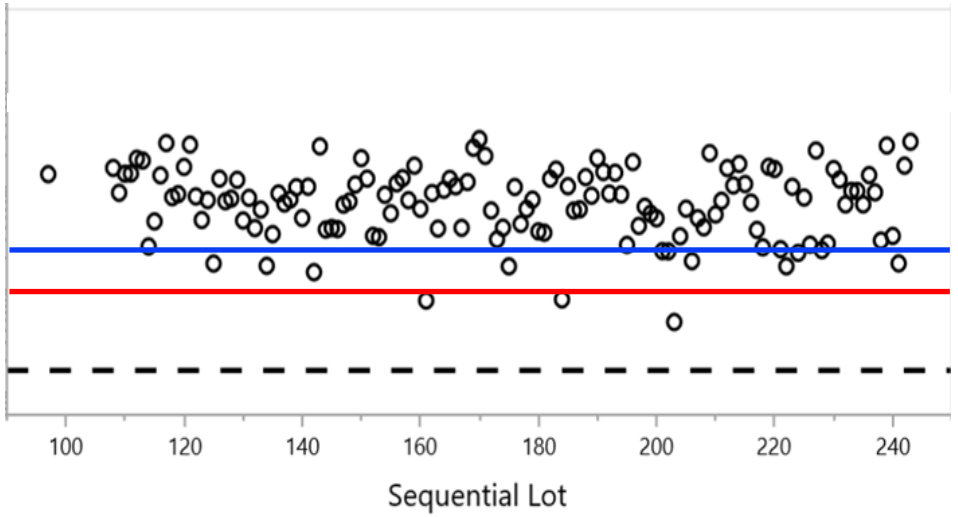
Autologous gene-engineered T cell products exhibit variability in CQAs (Patient, Process, Analytical)

- *Source variability is an important part in justifying specification limits*

Tolerance interval-based approach can be used to establish acceptance criteria based on understanding of process capability (95%95%, 95%/97%, 95%/99%, etc.)

- *Reducing coverage may result in rejection of lots that demonstrate safety and efficacy*

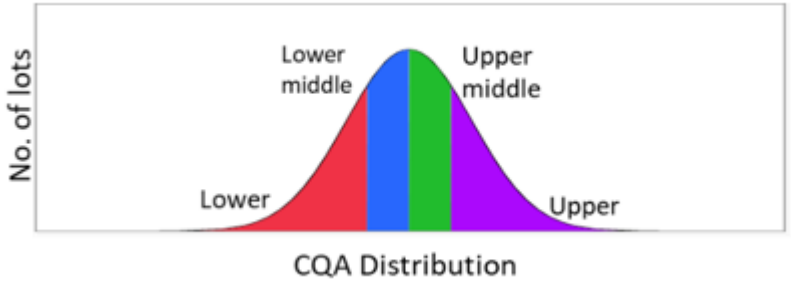
Example CQA Data:



⇐ Are these lots outliers or random patient variability?

Outliers likely due to patient starting material. If clinical safety / efficacy is acceptable for these outlier patients, then wider acceptance criteria may be justified to maximize patient

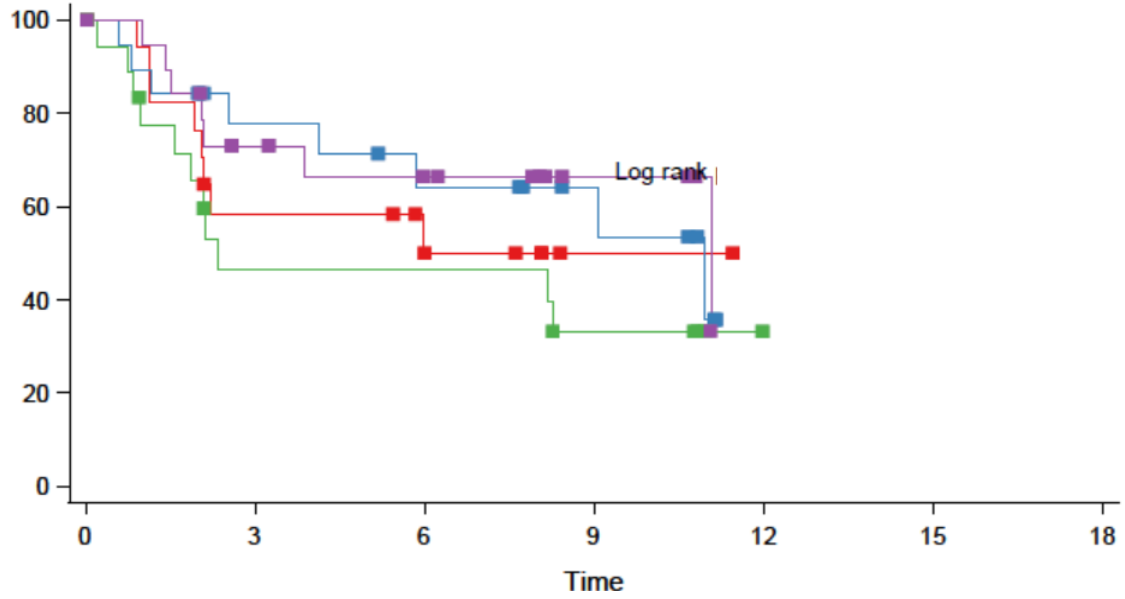
Example: Assessing Clinical Outcomes Across Critical Quality Attribute Range of Experience Through Correlative Analysis



- Clinical outcomes analyzed by CQA range quartiles
- Evaluation should include relevant efficacy and safety readouts
- Interpretation requires close collaboration with Clinical staff (benefit/risk)

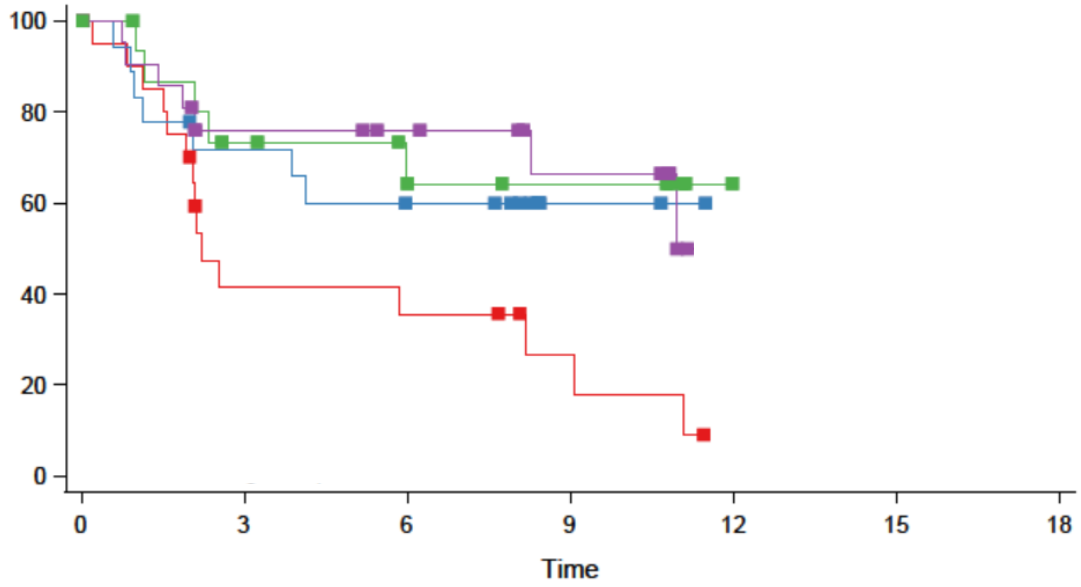
Scenario 1: Durable efficacy is the same across range

→Justifies a Wider Specification Acceptance Criteria



Scenario 2: Trend in reduced durable efficacy at lower end of range

→Justifies a Tighter Specification Acceptance Criteria



*Analysis should also include evaluation of safety across quality attribute range

R. Larson et.al., CASSS CGTP June 2020

Commercial Autologous T Cell Therapy Specification

Quality Attribute	Parameter	Methodology	Specification
Appearance	Color	Compendial	Description of color
	Clarity	Compendial	Description of turbidity
Identity	Confirmation of ID	Flow Cytometry	Anti-XXX CAR+ cells detected (ID confirmed)
Purity	Cell Viability	Fluorescent Microscopy & Image Analysis	Meaningful Specification Established per Clinical Correlative Analysis
	T cell Purity	Flow Cytometry	
	Product-Related Impurities	Flow Cytometry	
	Process-Related Impurities	ELISA	Meaningful Specification Established per Process Characterization / Impurity Risk Assessment
Strength	CAR + Viable T cells	Flow Cytometry	> XX CAR+ cells/mL
Potency	Antigen-specific Function	Bioassay	Product-Specific Acceptance Criteria Established per Clinical Correlative Analysis (potency, transduction controls)
Safety	Transduction Controls	qPCR	
	Endotoxin	Compendial	XX EU/mL
	Mycoplasma	Compendial	Not detected
	Sterility	Compendial	No growth

Control Strategies evolve from Development to Commercialization

- Unique aspects of CAR T requires that industry adapt to develop novel approaches for developing phase appropriate control strategies (leverage industry tools: ICEM, PQRA)
- Integrated control strategy is based on multiple aspects of GMP, characterization, routine control elements (IPCs, release specifications), and periodic control elements (PPQ, CPV, comparability, stability, etc.)

- Phase appropriate control strategies are reflective of historical/initial knowledge of process and product and must adapt over time
- Commercial control strategy is refined based on product / process characterization, characterization of patient variability, and clinical correlative analysis
- Correlative Analysis represents the ultimate “patient centric” specification strategy tied directly to clinical outcomes in the commercial setting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