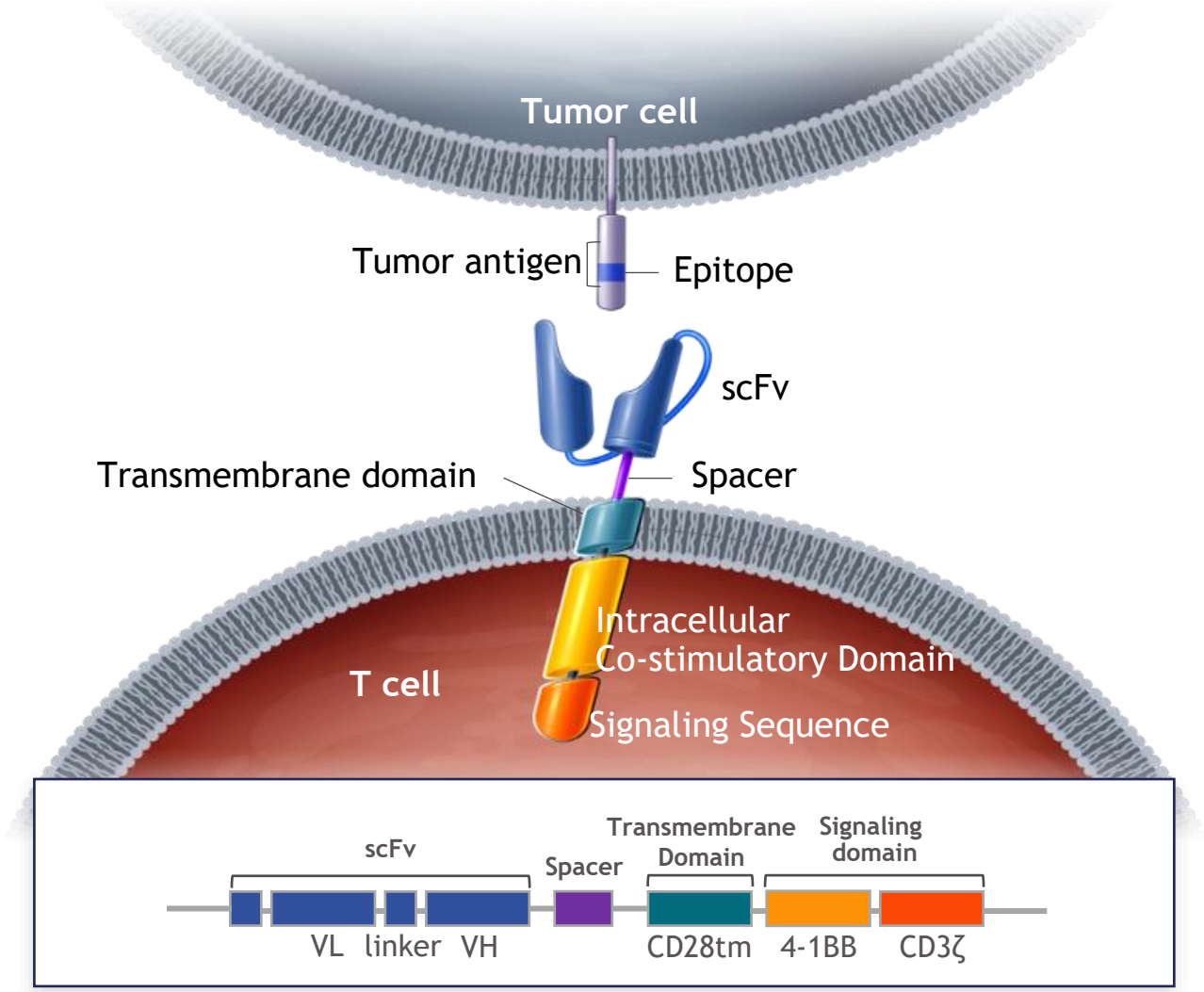
# Patient-centric Specifications for Cell & Gene Therapy Products: Maximizing Patient Access to Safe & Effective Quality Product

Ken Riker, Global Product Quality
Neil Haig, Analytical Development

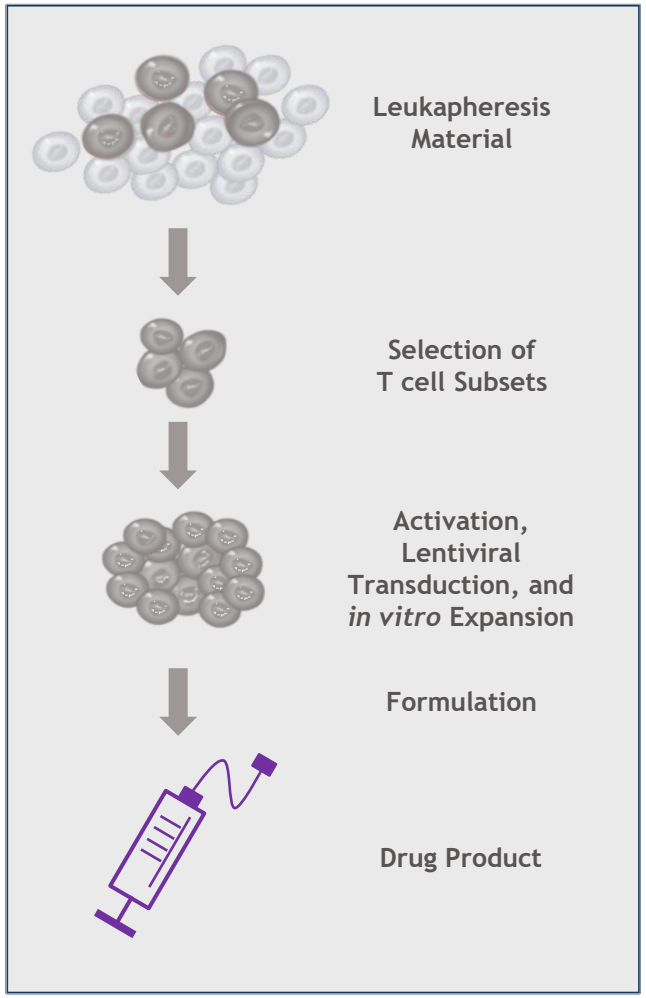
# Overview of Autologous CAR T cell Therapy




LTR, long terminal repeat; scFv, single-chain variable fragment; VH, variable heavy chain; VL, variable light chain

- Autologous Cell Therapy
  - Patient cells are collected, gene is delivered by vector, and the cells expanded *ex vivo* before reintroduction into the donor
- Chimeric Antigen Receptor (CAR) T cell Therapy
  - Binding domain confers specificity
  - Spacer/hinge domain designed
  - Transmembrane domain optimized to minimize tonic signaling
  - Intracellular signaling domain delivers a CD3ζ activation signals and 4-1BB-driven co-stimulation

# Overview of Manufacturing Process




## T Cell Selection

- The composition of T cells are highly variable in patient leukapheresis material. The selection of defined T cell subset(s) reduces one or more layers of this variability, which improves control of product manufacture
  - Non-T cells are depleted in the upstream manufacturing process, not through expansion
  - Enables improved control of dose through a defined composition of T cell subset(s)

## Activation/Gene Delivery

- Controlled activation of patient T cells and gene delivery promote consistent transduction (*i.e.* insertion of CAR into the cellular genome) and expansion
- The risk of transducing a non-T cell is limited by enriching T cells prior to the transduction step

## Expansion

- Process duration and T cell doublings required to achieve target dose are controlled during expansion to control population doublings

# Example Quality Attributes on a CGTP Specification

| | | |
|---|---|---|
| Appearance | • Color<br>• Clarity | • Compendial<br>• Compendial |
| Identity | • Confirmation of Identity | • Flow Cytometry |
| Purity | • Cell Viability<br>• T cell Purity<br>• T cell Subset Purity<br>• Product-related Impurities<br>• Process-related Impurities | • Fluorescent Microscopy & Image Analysis<br>• Flow Cytometry<br>• Flow Cytometry<br>• Flow Cytometry<br>• ELISA |
| Strength | • CAR-positive Viable T cells | • Flow Cytometry |
| Potency | • Antigen-specific Biological Function | • Bioassay |
| Safety | • Transduction Control<br>• Endotoxin<br>• Mycoplasma<br>• Sterility | • qPCR<br>• Compendial<br>• Compendial<br>• Compendial |

# Patient-centric Specifications for Cell & Gene Therapy Products: Maximizing Patient Access to Safe & Effective Quality Product

- CGTP Case Study: Patient-centric Specification to Ensure Product Quality and Consistency
  - Autologous CAR T cell therapies are personalized medicine; each patient receives a unique lot that is manufactured using their own T cells
  - The DP has 8 attributes on the Specification with quantitative AC
  - The predominant source of product variance is the patient starting material (*e.g.* apheresis)

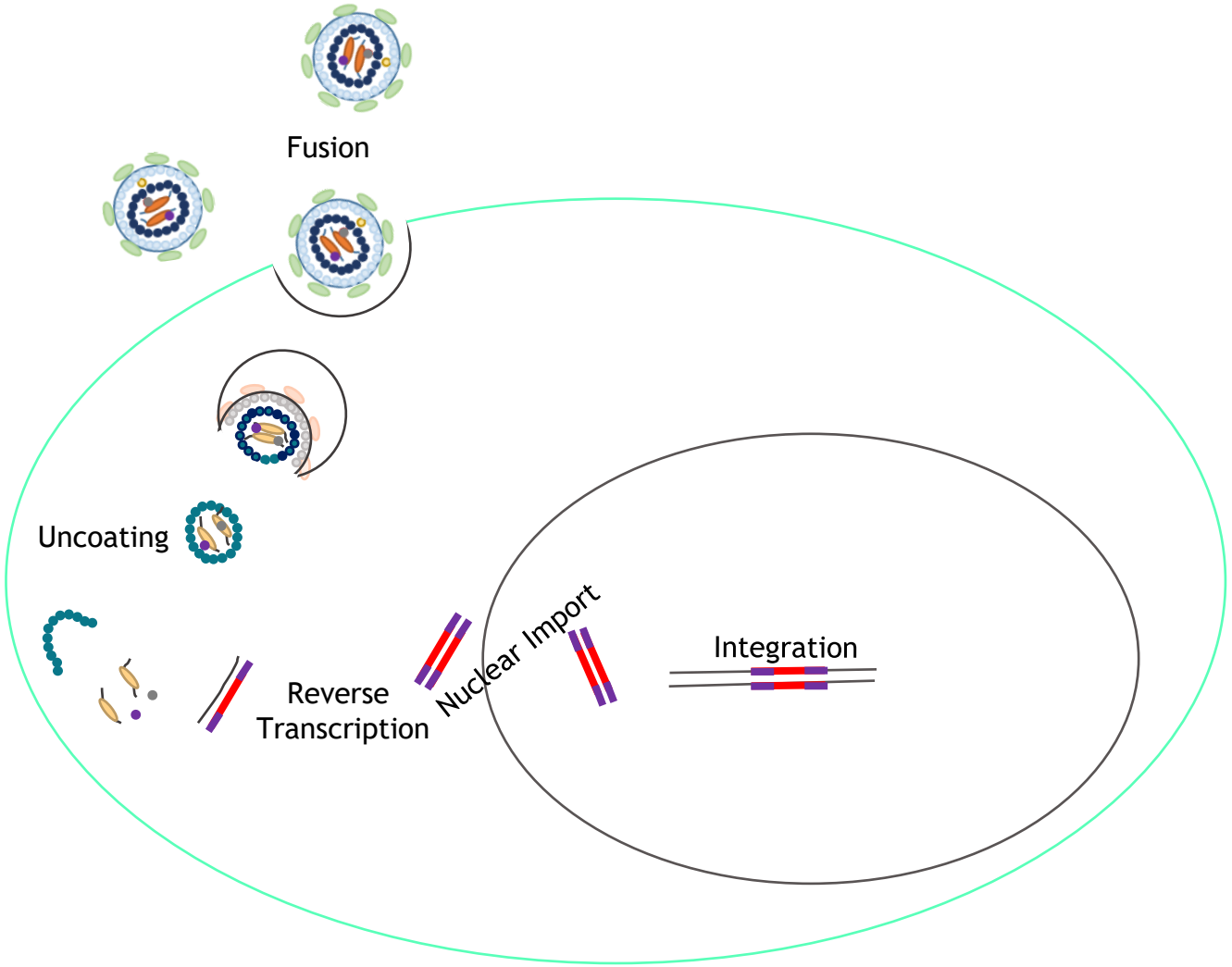
- In concordance with ICH Q6B, ICH Q8(R2), ICH Q9, ICH Q10, and ICH Q11, the proposed commercial Specification considered:
  1. Clinical manufacturing batches used for evaluating clinical safety and efficacy
  2. T cell biology and the quality attribute-clinical outcome relationship established by correlative analyses
  3. Data obtained from manufacturing process development and characterization studies
  4. Capabilities of the analytical procedures
  5. Pharmacopeia specifications

# CGTP Case Study: Patient-centric Specification to Ensure Product Quality and Consistency

A. **T cell Transduction Frequency Specification: Attribute, Assay, and Acceptance Criterion**

B. Orthogonal Control of T cell Transduction

# T cell Transduction



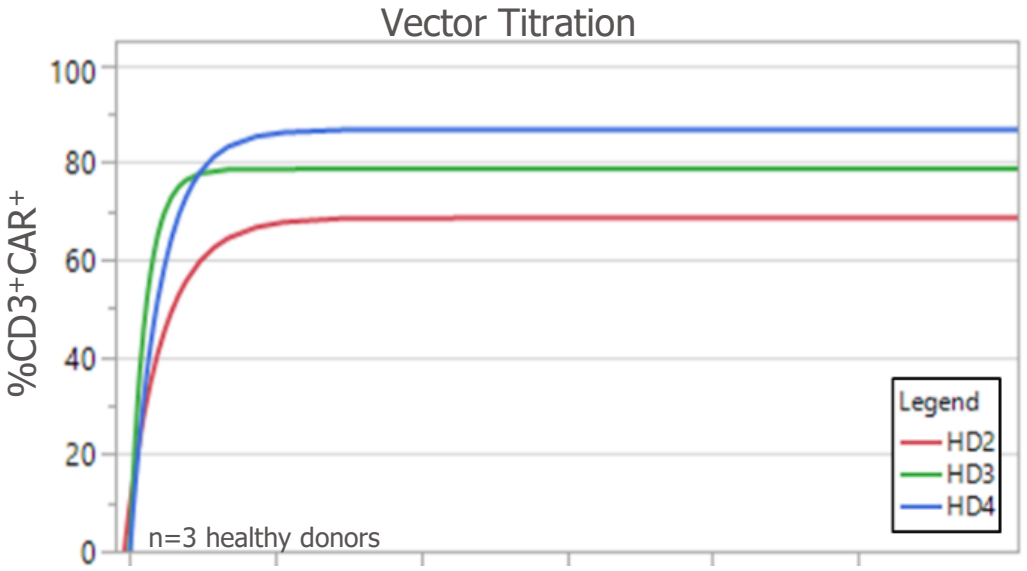

- T cell Transduction
  - Lentiviral vector delivers the CAR for integration into the cellular genome, which is then expressed as a functional protein on the cell surface
  - Successful transduction can be confirmed and quantified through a number of related attributes
- Two Perspectives on T cell Transduction:
  - Transduction frequency (%$CD3^+CAR^+$) is a CQA that should be included in the Potency matrix and have acceptance criteria in the Specification
  - The combination of Identity, Strength (concentration of viable $CD3^+CAR^+$ cells), Potency, and the number of integration events per cell should be justified instead of the transduction frequency

# T cell Transduction Frequency

| T cell Transduction Frequency (%CD3$^{+}$CAR$^{+}$) | | | | | | |
|---|---|---|---|---|---|---|
| Sample Size (n) | Distribution Fit | Range | | Mean | Median | Standard Deviation |
| | | Minimum | Maximum | | | |
| 267 | Johnson Sl | 16 | 92 | 63 | 65 | 15.4 |

- A high-degree of variation in transduction frequency has been observed in the proposed commercial manufacturing process
- The predominant source of this variance is caused by patient variability

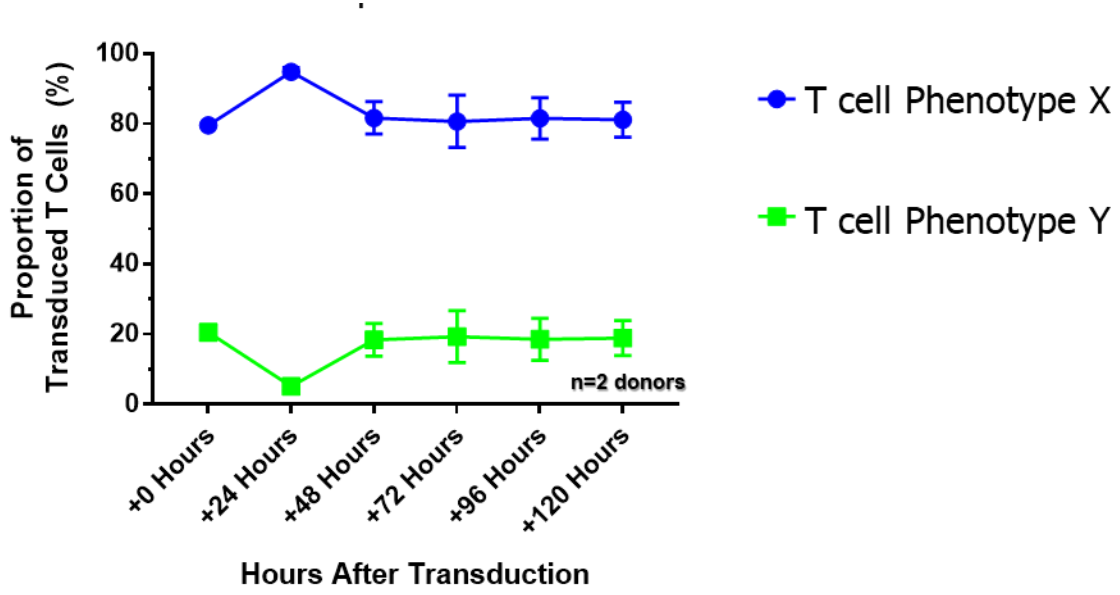
# Variance in the Transducibility of Patient T cells




- The dominant source of variance in T cell transduction frequency is patient T cells
  - Subsets of T cells are not transducable by the lentiviral vector
  - The proportion of transducable T cells varies between donors

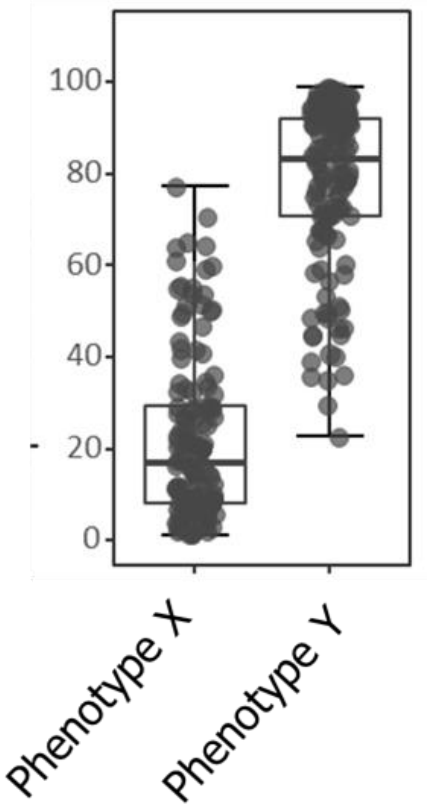
- For example, vector titration experiments demonstrate variation in the maximum transducable frequency of T cells between donors. This cannot be overcome by increasing the titration of vector, demonstrating inherent differences in starting material
  - The data shows the variance for one vector lot. These observations have been confirmed across multiple different lots of vector.

# Transducibility of T cell Subsets




- The transducibility of T cells is correlated with phenotypic properties of the patient cells
  - Phenotype X exhibit greater susceptibility to transduction
  - Phenotype Y exhibit less susceptibility to transduction

# Patient Variance in Transducible T cells




- The relative proportion of these T cell subpopulations in selected patient material are highly variable between patient material at the transduction unit operation (T cell phenotype prior to transduction shown, n=145).
- Consequently, the variability of transduction frequency between lots of DP is primarily the result of differences in the patients' composition of T cell subsets
- In released patient DP, T cell transduction frequency is correlated with the percentage of Phenotype X immediately prior to the transduction step (n=133, Spearman's rank correlation coefficient, p value < 0.0006, Rho 0.302)

# Patient Variance in Transducible T cells

- In a qualified small-down model using healthy donor material, which is representative of the full-scale manufacturing process, approximately 70% of the total variance in T cell transduction frequency was attributable to donor heterogeneity (n=70 experiments using 8 vector lots, 6 donors, and analyzed by mixed model restricted maximum likelihood)

- Variance component analyses of the process performance qualification (PPQ), which used a split-run approach (using material from a single donor), determined that >70% of variance in transduction frequency was attributable to donor heterogeneity

- The DP has consistent safety and efficacy across the range of transduction frequency experience gained during clinical trials

- While this variability in T cell transduction frequency exists between patient DP lots, dose is achieved by adjusting the volume of dose based on Strength (Concentration of viable $CD3^{+}CAR^{+}$ cells). This ensures a consistent delivery of viable $CAR^{+}$ T cells across patients.

# CGTP Case Study: Patient-centric Specification to Ensure Product Quality and Consistency

A. T cell Transduction Frequency Specification: Attribute, Assay, and Acceptance Criterion

**B. Orthogonal Control of T cell Transduction**

# CGTP Case Study: Patient-centric Specification to Ensure Product Quality and Consistency

## Our Experience has Shown:

- Acceptance criterion for T cell transduction frequency (%$CD3^+CAR^+$) is not required to ensure control of transduction, potency, or to ensure dose. Control of these attributes are assured through the acceptance criteria for vector copy number/transduced T cell (VCN), antigen-specific cytokine secretion (Potency), and concentration of viable $CD3^+CAR^+$ cells (Strength), respectively.

- %$CD3^+CAR^+$ is not ideal for demonstrating process control owing to the high-degree of variability imparted by patient T cells and is not correlated with safety or efficacy across the wide range of attribute experience gained in clinical trials.

- Accordingly, the volume of the drug product is adjusted based on its Strength to achieve the intended dose.

# Orthogonal Control of Transduction: Transduction Control & Potency

- Transduction Control (VCN)
  - Lentiviral transduction is controlled because it has a theoretical risk of causing insertional oncogenesis
  - The average number of vector insertions per transduced T cell (vector copy number, or VCN) is monitored in every released DP lot
  - To mitigate the theoretical risk of insertional oncogenesis, two-sided statistically-derived acceptance criteria based on the capability of the manufacturing process has been established to ensure transduction remains in the state of control. The upper limit ensures the theoretical risk of insertional oncogenesis is controlled within process capability

- Potency
  - Measured through antigen-specific cytokine secretion by defined number of transduced T cells ($CD3^{+}CAR^{+}$)
  - To ensure released DP remains within the distribution of experience demonstrated to be safe and effective during clinical trials, two-sided statistically-derived acceptance criteria based on the clinical experience gained with the manufacturing process has been established

# Orthogonal Control of Transduction: Identity & Strength

- Identity
  - Evaluated by direct detection of the CAR by a fluorescently-labelled anti-idiotypic antibody
  - Consequently, T cell transduction frequency (%$CD3^+CAR^+$) is measured during release of all DP but acceptance criteria has not been proposed for the reasons discussed in earlier slides

- Strength
  - Determined by multiplying the viable cell concentration (viable cells/mL) by the frequency of transduced T cells (%$CD3^+CAR^+$)
  - Acceptance criteria for concentration of viable $CD3^+CAR^+$ cells (Strength) ensures dose requirements are met for every released DP lot; the manufacturing process has greater than a 99% success rate in conforming to this acceptance criteria.

# Summary of Transduction Controls in Case Study

- Conforming to the acceptance criteria matrix for Identity, Strength, Potency, and VCN ensure:

  A. The Identity of the DP

  B. Strength is sufficient for Dose to be achieved

  C. Potency is within the distribution of clinical experience demonstrated to be safe and efficacious

  D. Transduction remained controlled within process capability

# Case Study Conclusions

- Transduction Frequency (%$CD3^+CAR^+$)
  - Acceptance criteria for Identity, Strength, Potency, and Transduction Control (VCN) provide the necessary assurances of product quality
  - In contrast, the variability in transduction frequency, which is primarily driven by patient heterogeneity, is not ideal for ensuring process control and acceptance criteria would be redundant
  - The DP has consistent safety and efficacy across the range of transduction frequency experience gained during clinical trials

- To maximize patient access to safe and effective quality product, the variance that is contributed by patient heterogeneity and product knowledge of the quality attribute-clinical outcome relationship must be considered to establish a patient-centric Specification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# Thank you

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