Correlating CAR-T cell therapy product attributes to clinical outcomes

Irina Gershgorin

Quantitative Sciences and Statistics, Technical Research and Development

Cell and Gene Therapies, Novartis

IABS

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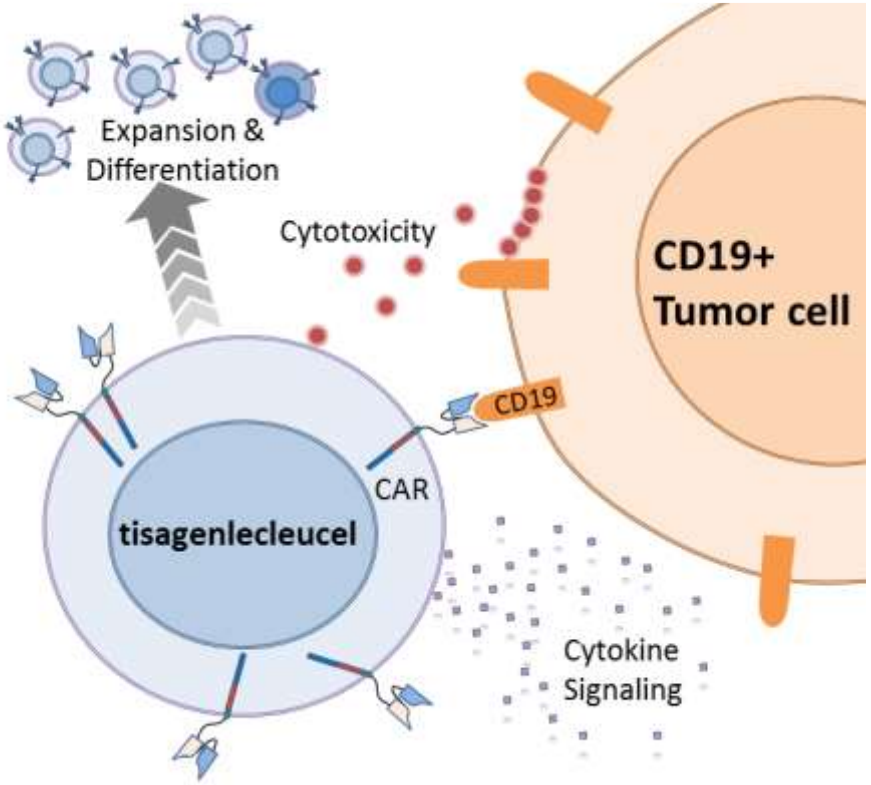
Introduction

- **KYMRIAH – first cell therapy to be commercially approved, 2017**
- **Indications:**
  - Adults: relapsed/refractory Diffuse Large B-cell Lymphoma (**DLBCL**), follicular lymphoma (**FL**)
  - Children and young adults (pediatric): Acute Lymphoblastic Leukemia (**pALL**)
- **How it works:**

Chimeric Antigen Receptor (CAR) T cell therapy product is engineered from a patient's T cells by adding a transgene that codes for a protein that recognizes malignant B cells. Engineered T cells are then re-infused back into the patient.



Personalized therapy:

- 1 batch : 1 patient
- High batch to batch variability
- Small sample sizes
- Highly variable starting material needs to be consistently manufactured into a final product that meets pre-specified release criteria

[download (fda.gov)]

Critical Quality Attributes	
Dose, transduction efficiency, viability, and T-cell function/potency (as measured by the release of IFNγ, a cytokine released by activated T cells)	
Category	**Cell Populations/Markers**
Cell population	• T cells (cytotoxic [CD8+], helper [CD4+]), B cells/blasts, monocytes, NK cells, and dendritic cells • CAR+ and CAR– cells
CD4:CD8 ratio	• CD4:CD8 ratio in CAR+ fraction • CD4:CD8 ratio in CAR– fraction
Differentiation of T cells	• Naive | TSCM CD4/CD8 cells (CD45RA+CCR7+) • Central memory CD4/CD8 cells (CD45RA-CCR7+) • Effector CD4/CD8 cells (CD45RA+CCR7-) • Effector memory CD4/CD8 cells (CD45RA-CCR7-)
Exhaustion (checkpoint inhibitors)	• PD-1, LAG3, TIM3, and 2B4 expression
Senescence	• CD27 and CD28 expression (characterization of early differentiated cells and senescent cells) • Senescent by expression of KLRG1
Activation/T regulatory	• HLA-DR, CD25, and CD38 expression
Clinical baseline characteristics	• Age • High-risk mutations • Time between diagnosis and first relapse • LDH and baseline tumor burden[a]

Correlational analyses of the clinical trials:

- pALL (ELIANA/ENSIGN), N=143 (fewer in cases where immunophenotyping data were not available)
  - **Gershgorin** et al. Impact of tisagenlecleucel product attributes on clinical outcomes in pediatric and young adult patients with relapsed or refractory acute lymphoblastic leukemia (r/r ALL). **AACR, 2021**

- DLBCL (JULIET), N=115 (fewer in cases where immunophenotyping data were not available)
  - Jeschke et al. Impact of Tisagenlecleucel Chimeric Antigen Receptor (CAR)-T Cell Therapy Product. **ASH 2019**

- Clinical endpoints:
  - Efficacy: best overall response (BOR), duration of response (DOR), overall survival (OS), event/progression free survival (EFS, PFS)
  - Safety: cytokine release syndrome (CRS) grade, neurotoxicity (NE) grade

- Predictor variables:
  - Immunophenotypes of final product for 66 attributes via flow cytometry
  - 12 product release attributes examined
  - Percentage and absolute number of each CAR+ T cell population were analyzed

Cross-functional effort

- Initiated by the CMC team to better understand our final product from scientific and manufacturing perspectives
- Advance our understanding of CQAs, manufacturing and control strategy
- Close collaboration with early and late stage development SMEs to understand biological significance
- Partnering with the clinical team to ensure common understanding and alignment on the analyses and their interpretations
- Breaking down silos and building connections across departments to make positive impact

Statistical Approaches applied

- **Univariate analyses**
  - **Wilcoxon/Kruskal-Wallis** tests
  - **Logistic/COX** regression
  - **Kaplan-Meier** graphs and boxplots

- **Multivariate analyses**
  - **LASSO/Elastic net, random forest, decision trees**
    - Exploratory analyses
    - Feature selection – general agreement between the different methods
    - Identify a subset of variables that may distinguish between observed outcomes as a group rather than looking at them one by one
  - **Logistic/COX regression**
    - Run on the subset of predictors picked up by the machine learning methods
    - Incorporate SME input

Challenges & Limitations

Considerations:

a) Small sample sizes
b) Unbalanced groups
c) Missing data
d) p >> n: too many variables for the number of observations (→ risk of overfitting)

***Note*:** All univariate p-values were adjusted for multiple hypothesis testing (Benjamini-Hochberg correction). Boxplots and tables in this presentation show the raw unadjusted p-values.

Summary of pALL findings

- Some T cell immunophenotypes showed association with efficacy
- No strong associations with safety

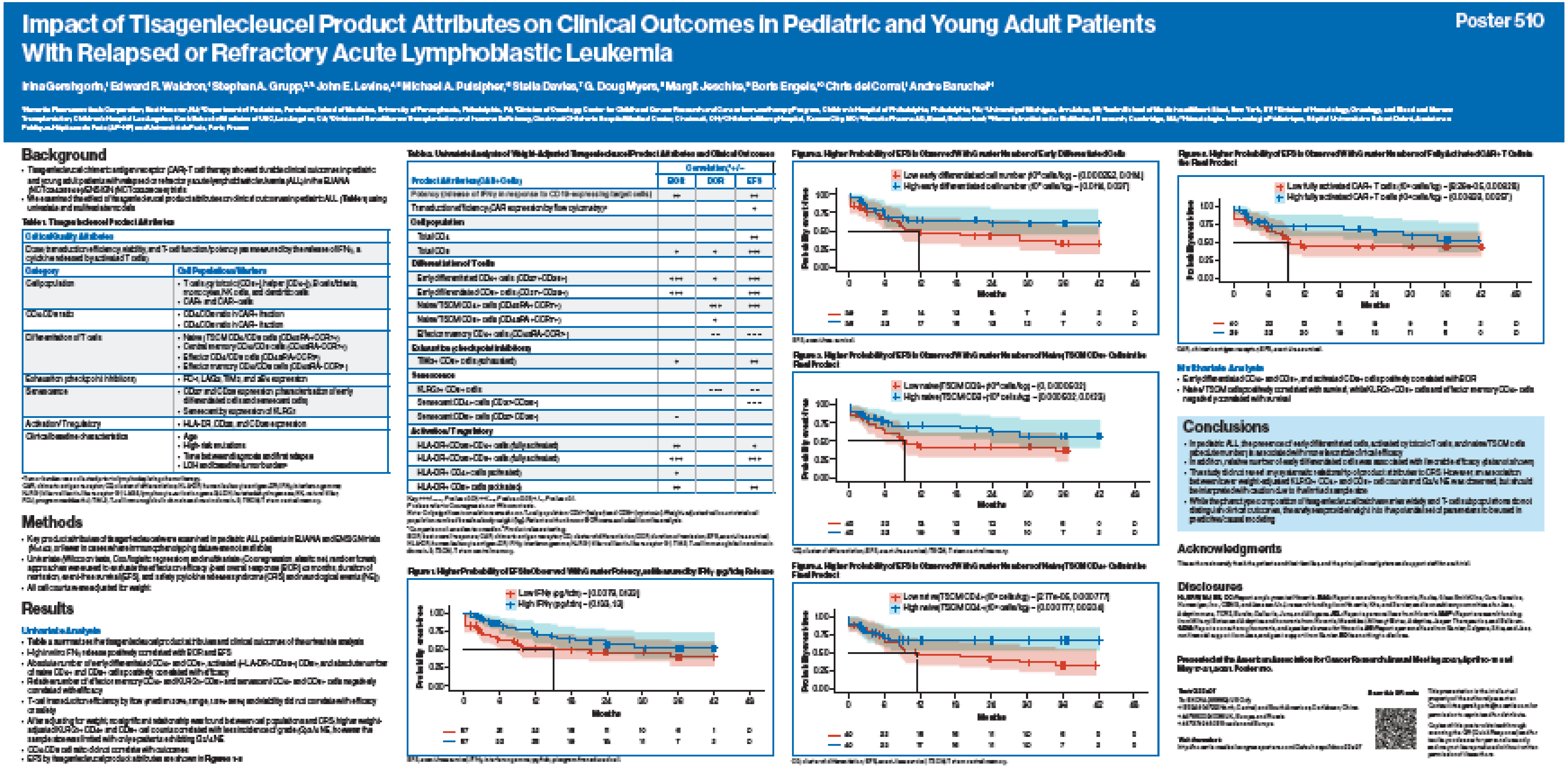


Table 2. Univariate Analysis of Weight-Adjusted Tisagenlecleucel Product Attributes and Clinical Outcomes

Product Attributes (CAR+ Cells)	Correlation,[a] +/–		
	BOR	DOR	EFS
Potency (release of IFNγ in response to CD19-expressing target cells)	++		++
Transduction efficiency (CAR expression by flow cytometry)[b]			+
Cell population			
Total CD4			++
Total CD8	+	+	+++
Differentiation of T cells			
Early differentiated CD4+ cells (CD27+CD28+)	+++	+	+++
Early differentiated CD8+ cells (CD27+CD28+)	+++		+++
Naive/TSCM CD4+ cells (CD45RA+CCR7+)		+++	+++
Naive/TSCM CD8+ cells (CD45RA+CCR7+)		+	
Effector memory CD4+ cells (CD45RA-CCR7-)		– –	– – –
Exhaustion (checkpoint inhibitors)			
TIM3+ CD8+ cells (exhausted)	+		++
Senescence			
KLRG1+ CD8+ cells		– – –	– –
Senescent CD4+ cells (CD27-CD28-)			– – –
Senescent CD8+ cells (CD27-CD28-)	–		
Activation/T regulatory			
HLA-DR+CD38+CD4+ cells (fully activated)	++		+
HLA-DR+CD38+CD8+ cells (fully activated)	+++		+++
HLA-DR+ CD4+ cells (activated)	+		
HLA-DR+ CD8+ cells (activated)	++		++

Key: +++/–––, *P* value <0.01; ++/––, *P* value <0.05; +/–, *P* value <0.1.
P values refer to Cox regression or Wilcoxon tests.

Summary of findings for LASSO

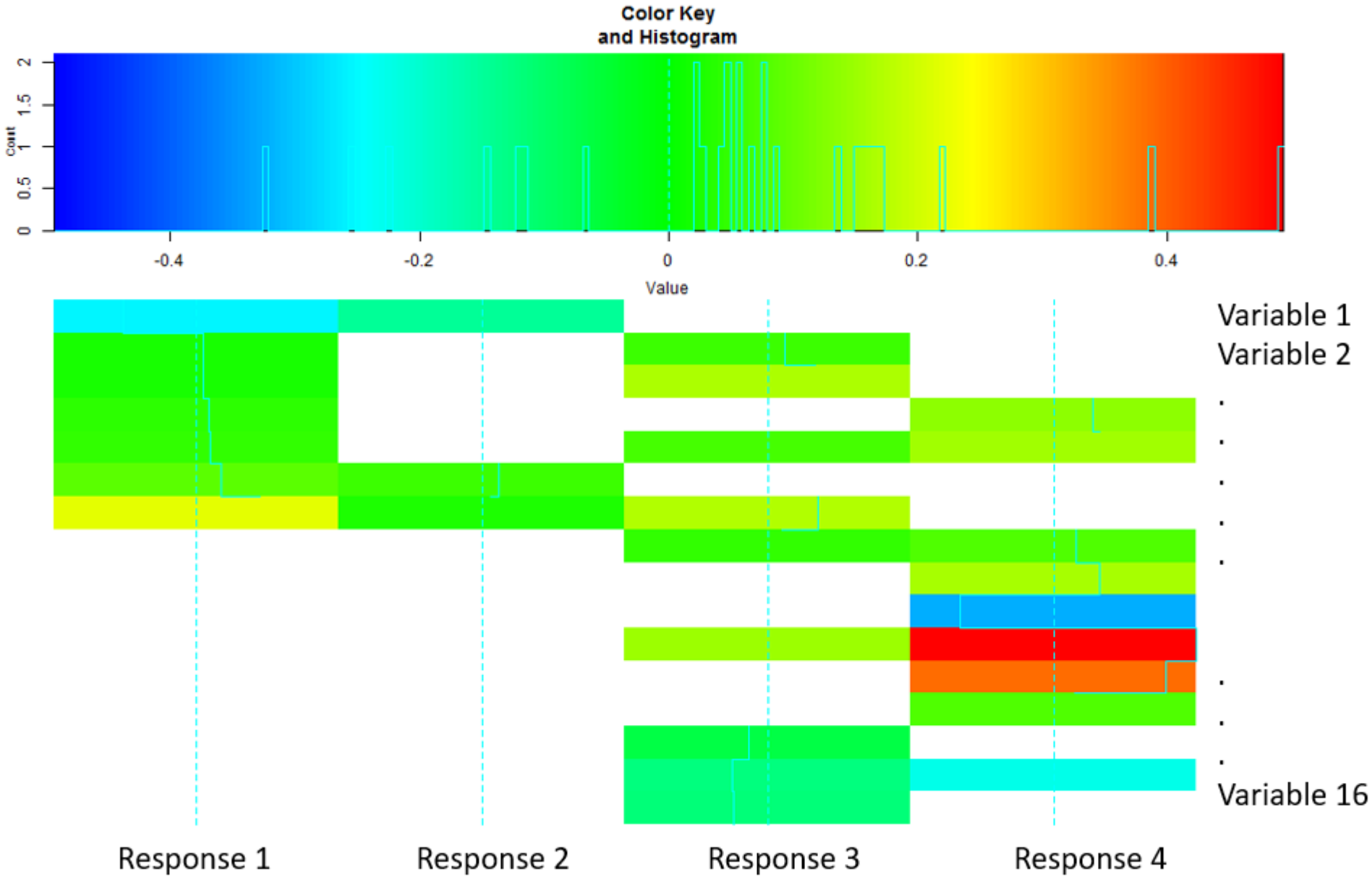
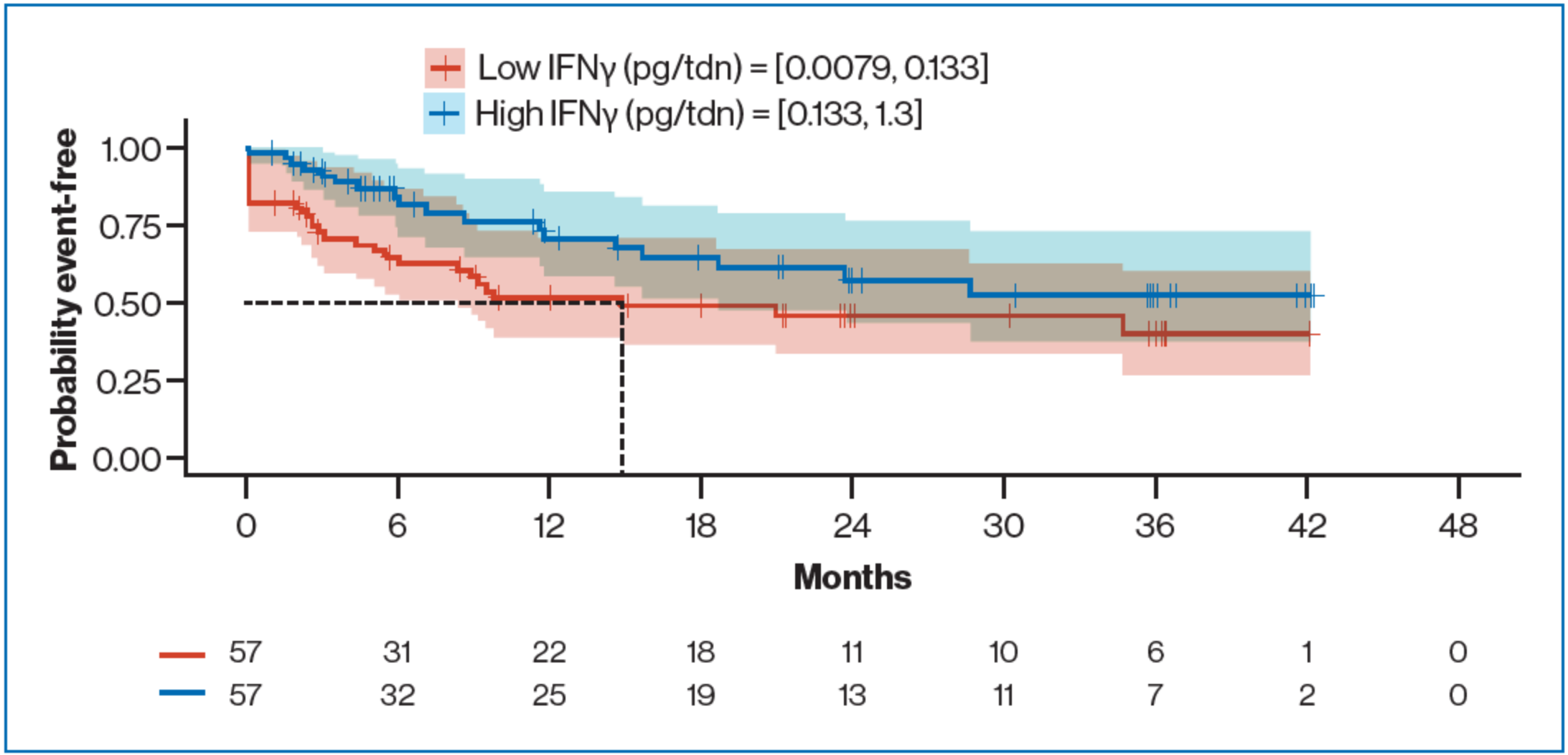
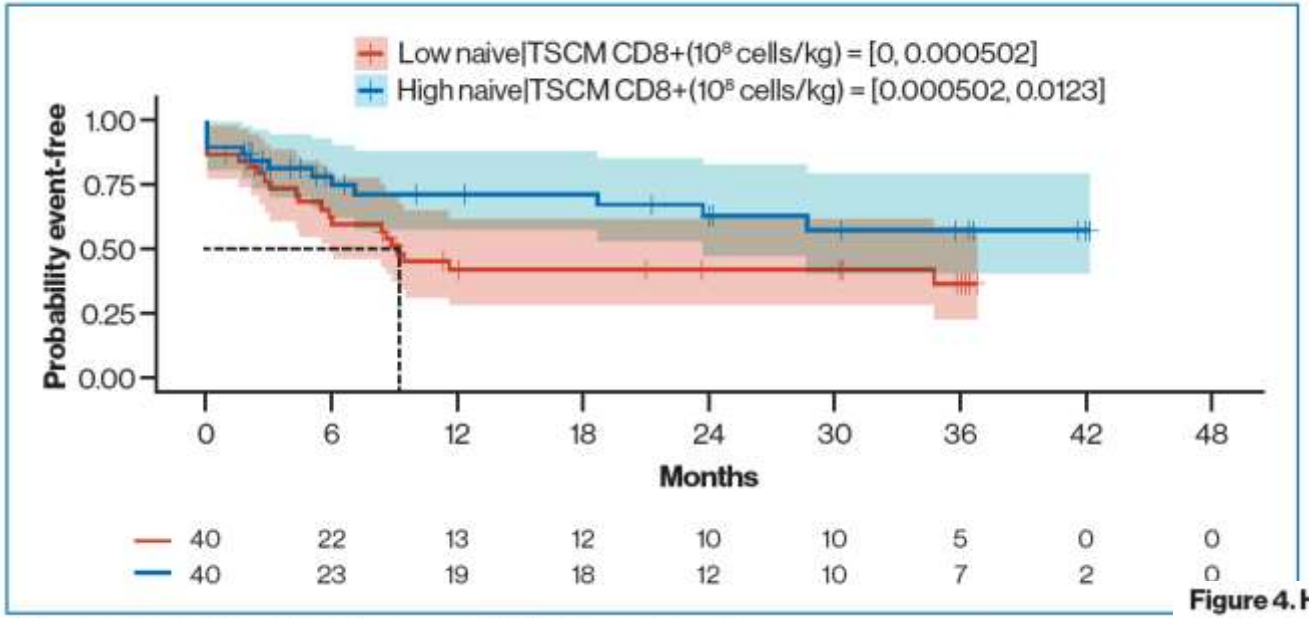


Figure 1. Higher Probability of EFS Is Observed With Greater Potency, as Measured by IFNγ (pg/tdn) Release



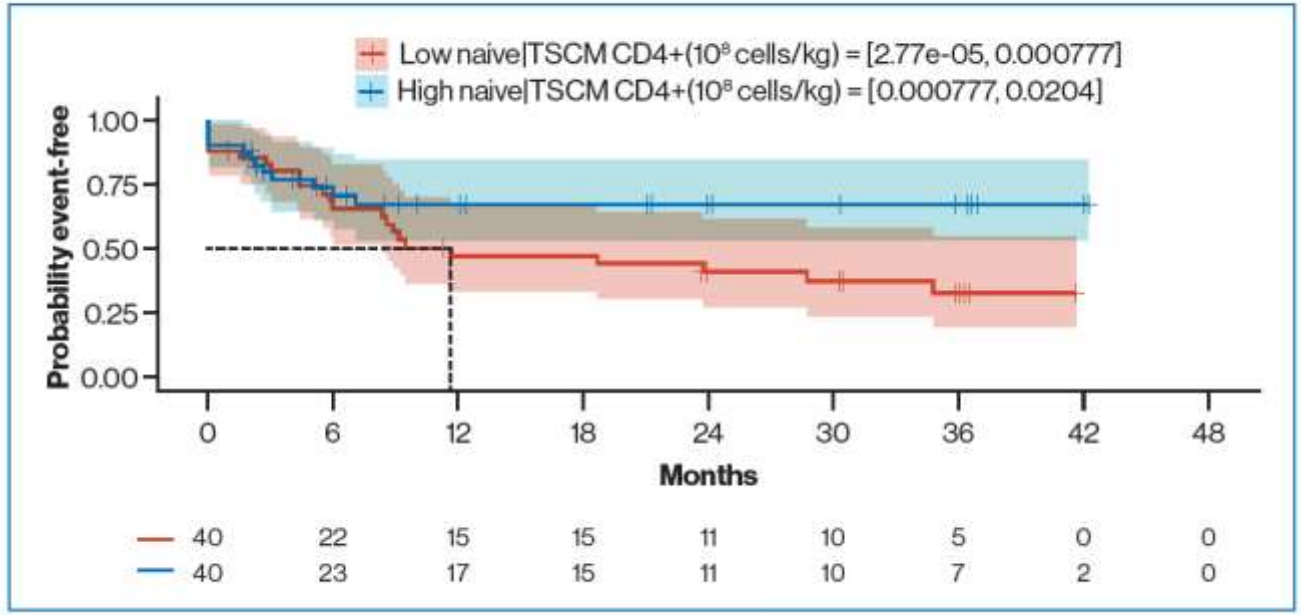
EFS, event-free survival; IFNγ, interferon gamma; pg/tdn, picogram/transduced cell.

Figure 3. Higher Probability of EFS Is Observed With Greater Numbers of Naive|TSCM CD8+ Cells in the Final Product



CD, cluster of differentiation; EFS, event-free survival; TSCM, T stem central memory.

Figure 4. Higher Probability of EFS Is Observed With Greater Numbers of Naive|TSCM CD4+ Cells in the Final Product



CD, cluster of differentiation; EFS, event-free survival; TSCM, T stem central memory.

Multivariate Analysis

- Early differentiated CD4+ and CD8+, and activated CD8+ cells positively correlated with BOR
- Naive/TSCM cells positively correlated with survival, while KLRG1+CD8+ cells and effector memory CD4+ cells negatively correlated with survival

Conclusions

- In pediatric ALL, the presence of early differentiated cells, activated cytotoxic T cells, and naive/TSCM cells (absolute number) is associated with more favorable clinical efficacy
- In addition, relative number of early differentiated cells was associated with favorable efficacy (data not shown)
- The study did not reveal any systematic relationship of product attributes to CRS. However, an association between lower weight-adjusted KLRG1+ CD4+ and CD8+ cell counts and G3/4 NE was observed, but should be interpreted with caution due to the limited sample size
- While the phenotype composition of tisagenlecleucel batches varies widely and T-cell subpopulations do not distinguish clinical outcomes, the analyses provide insight into the potential set of parameters to be used in predictive/causal modeling

Summary of DLBCL findings

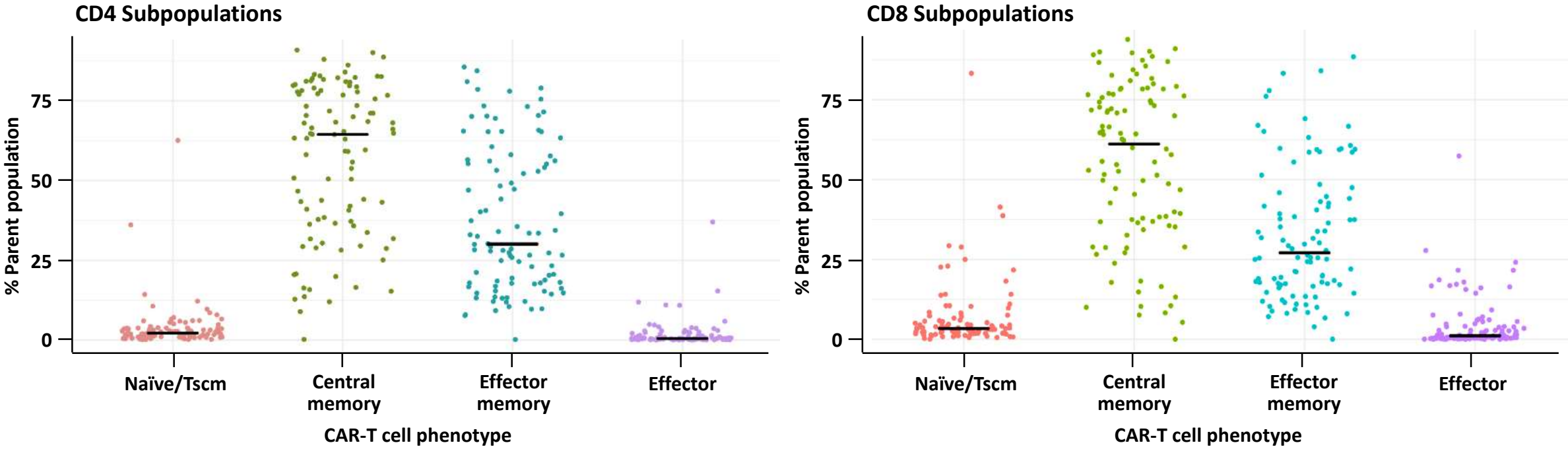
- Did not find specific FP immunophenotypes to correlate with efficacy
- Activated FP T cells showed a positive association with higher CRS grade
- **Machine learning methods were very useful to select a smaller subgroup of key parameters**

Impact of Tisagenlecleucel Chimeric Antigen Receptor (CAR)-T Cell Therapy Product Attributes on Clinical Outcomes in Adults with Relapsed or Refractory Diffuse Large B-Cell Lymphoma (r/r DLBCL)

Veronika Bachanova, Constantine S. Tam, Peter Borchmann, Ulrich Jaeger, Joseph P. McGuirk, Harald Holte, Edmund K. Waller, Samantha Jaglowski, Michael R. Bishop, Charalambos Andreadis, Stephen Ronan Foley, Jason R. Westin, Isabelle Fleury, P. Joy Ho, Stephan Mielke, Takanori Teshima, Gilles Salles, Stephen J. Schuster, Richard T. Maziarz, Koen Van Besien, Koji Izutsu, Marie Jose Kersten, John M. Magenau, Nina D. Wagner-Johnston, Koji Kato, Paolo Corradini, Jufen Chu, **Irina Gershgorin**, Therese Choquette, Lida Bubuteishvili Pacaud, Margit Jeschke

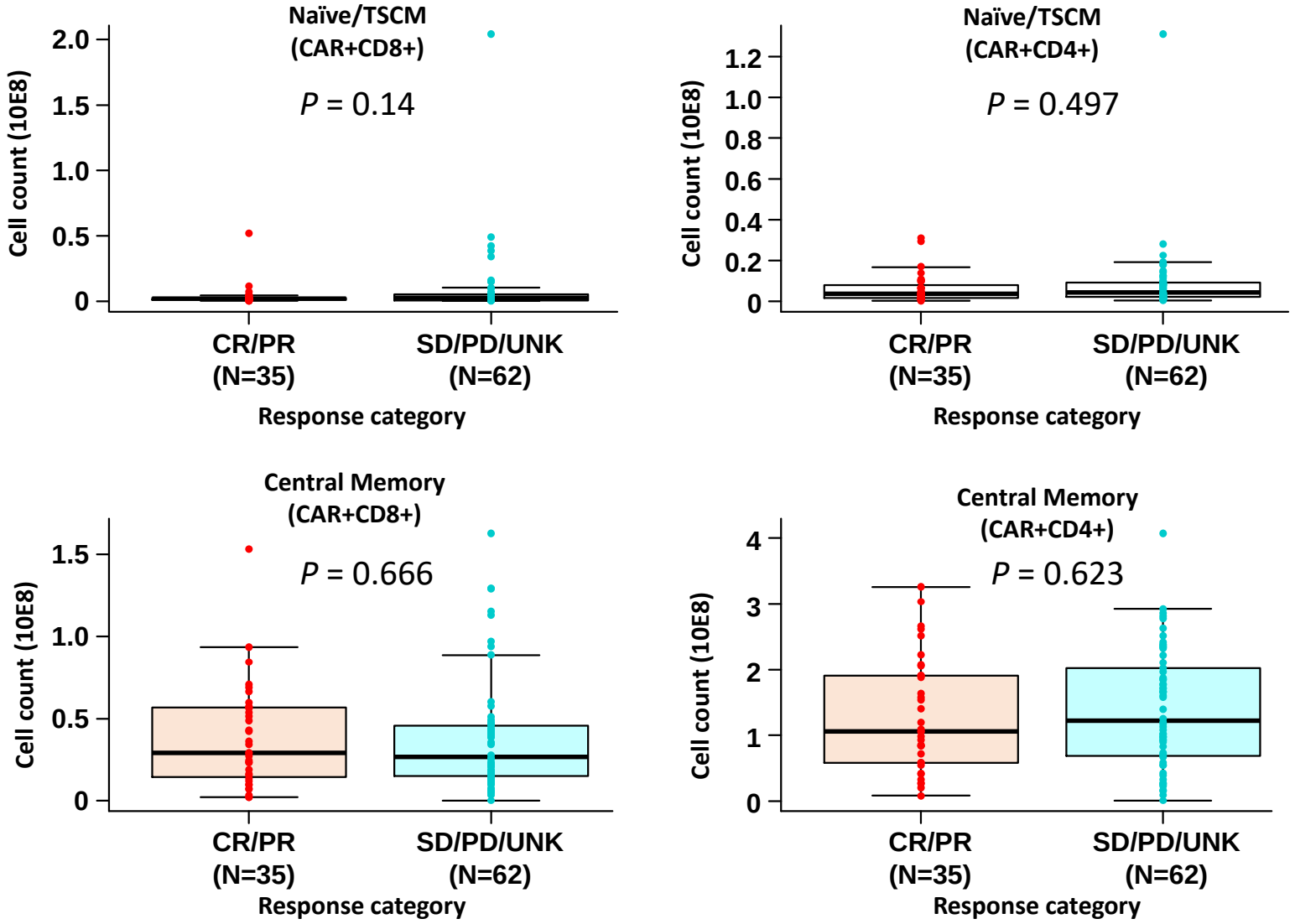
Phenotypic variability of CAR+ T cells

- Central memory cells were the predominant subpopulation of both CD4+ and CD8+ CAR+ T cells



Parent population: viable CAR+ T cells. Immunophenotyping of T cell maturation stages: Naïve/TSCM [CD197+CD45RA+], central memory cells [CD197+CD45RA-], effector memory cells [CD197-CD45RA-], T-effector cells [CD197- CD45RA+].
CAR, chimeric antigen receptor; TSCM, stem central memory T cells.

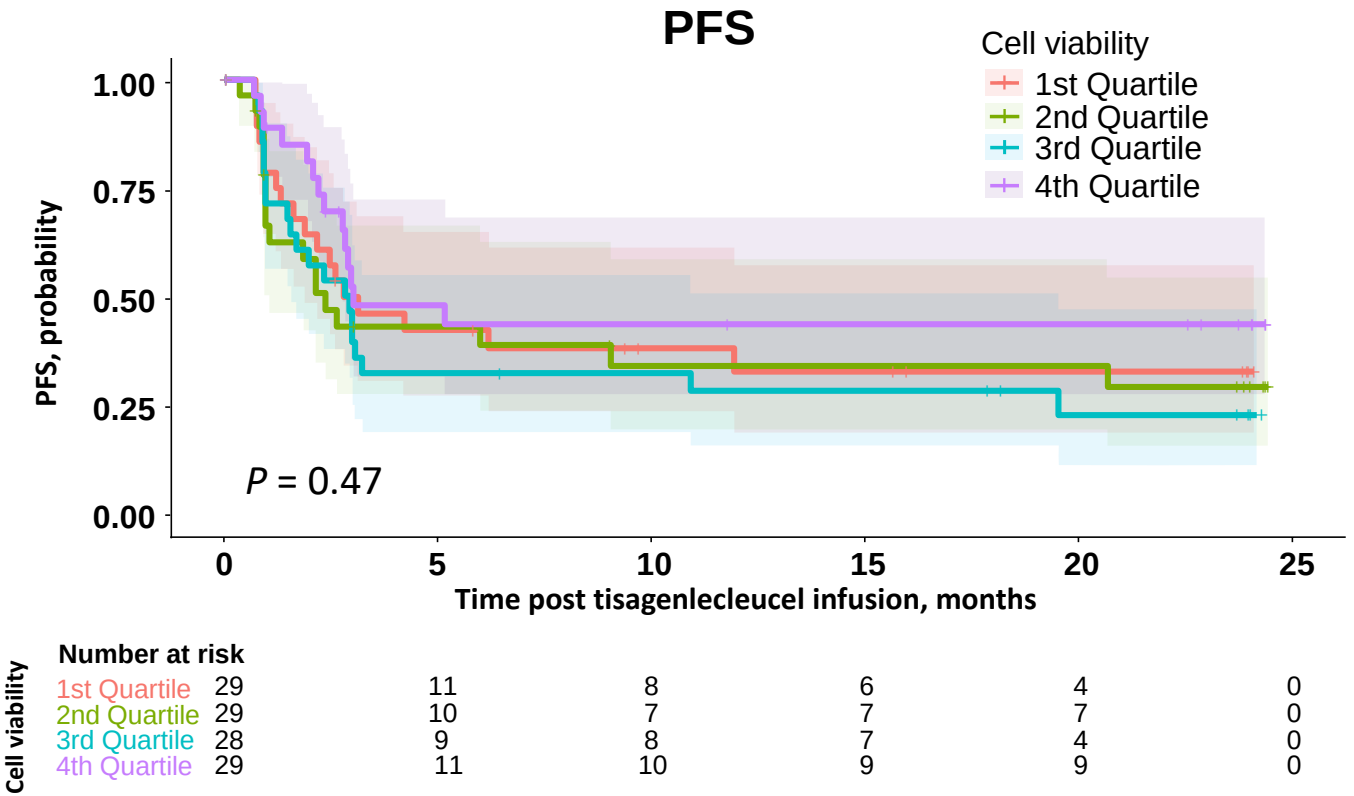
Relationship of less-mature CAR+ T cells in the product with efficacy in JULIET

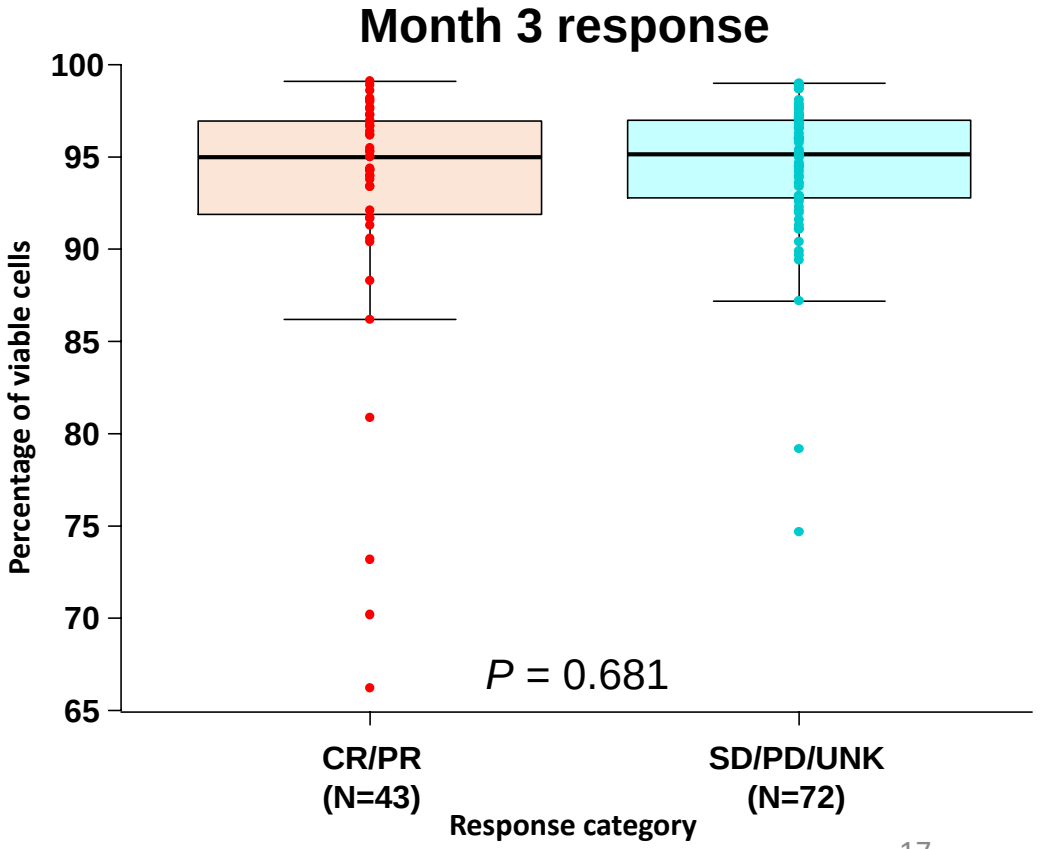


CAR, chimeric antigen receptor; CR, complete response; PD, progressive disease; PR, partial response; SD, stable disease; TSCM, stem central memory T cell; UNK, unknown.

Impact of cell viability on PFS and Month 3 response

- Tisagenlecleucel dose is formulated based on the number of viable CAR+ T cells
  - Viability (post-thaw, %) is not related to efficacy (overlapping distributions)

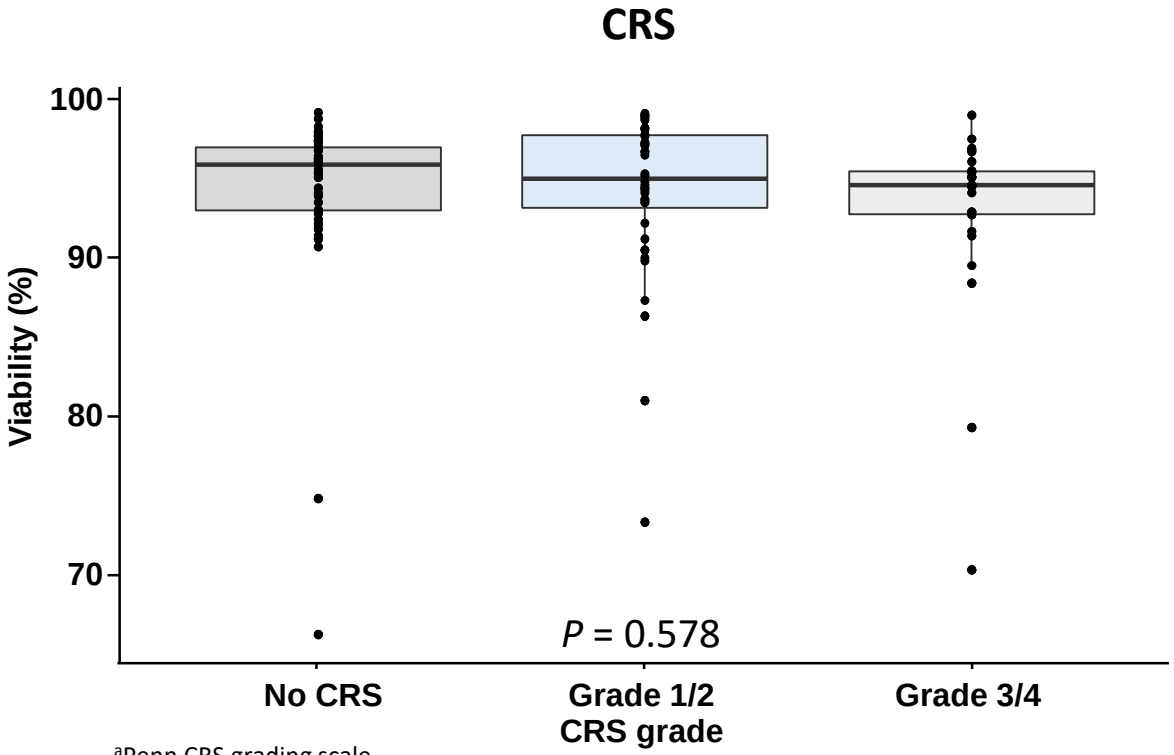


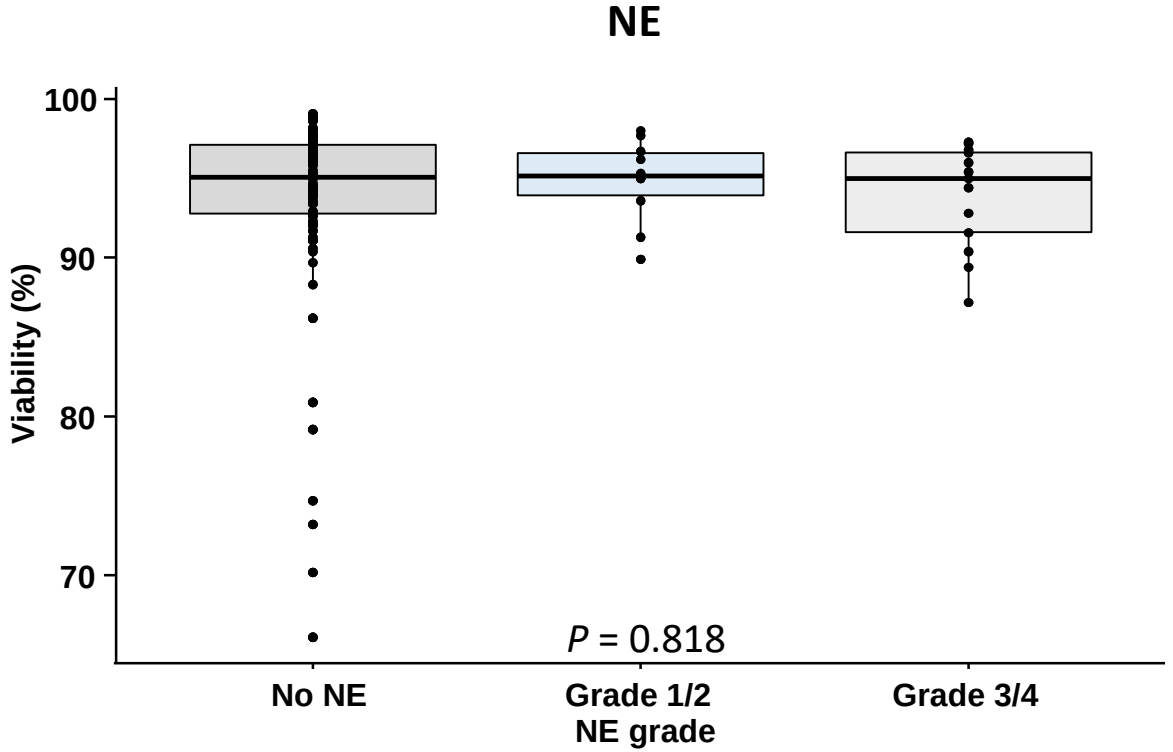


1st quartile = 66.1-92.8; 2nd quartile = 92.8-95; 3rd quartile = 95-97; 4th quartile = 97-99.1.
CR, complete response; PD, progressive disease; PFS, progression-free survival; PR, partial response; SD, stable disease; UNK, unknown.

Impact of cell viability on CRS and NE

- Tisagenlecleucel dose is formulated based on the number of viable CAR+ T cells
  - Viability (%) is unrelated to safety (overlapping distributions)
  - Total viable cells and dead cells infused are unrelated to safety (not shown)

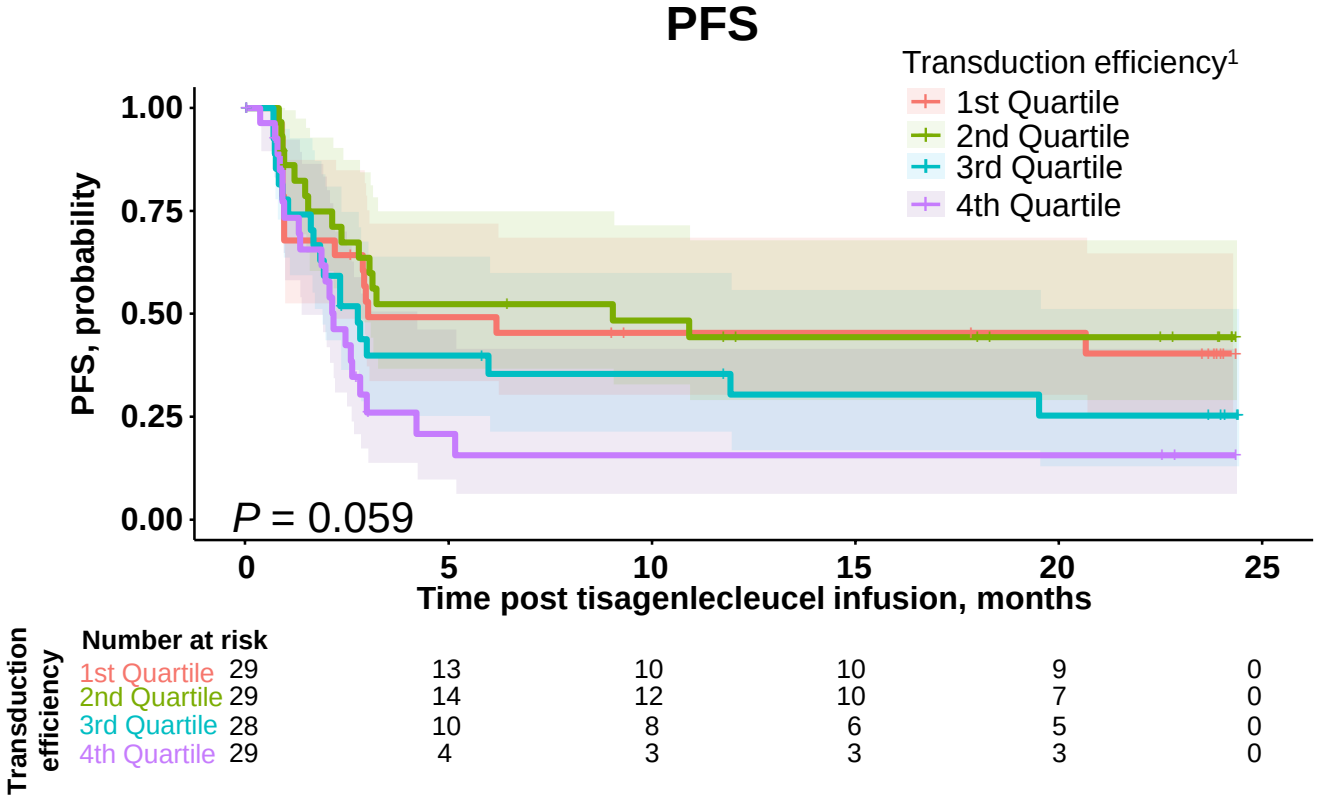


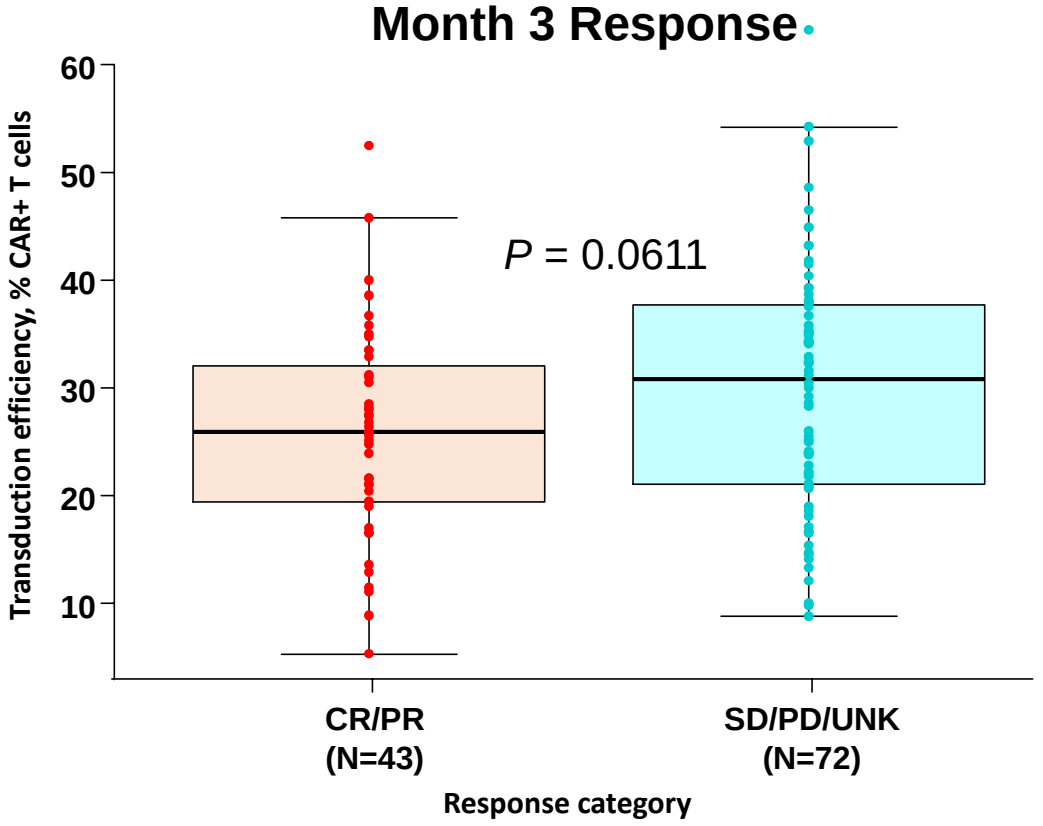


[a]Penn CRS grading scale.
P values = 1-way ANOVA tests.
CAR, chimeric antigen receptor; CRS, cytokine release syndrome; NE, neurological events.

Relationship between transduction efficiency (% CAR+ T cells) and efficacy

- Median percent CAR+ T cells by flow cytometry was 28% (range, 5.3% to 63.2%)
- Univariate analysis (as shown below) suggested a relationship
- No relationship when adjusted for CAR+ T cell dose and other factors in multivariate analysis (shown later)





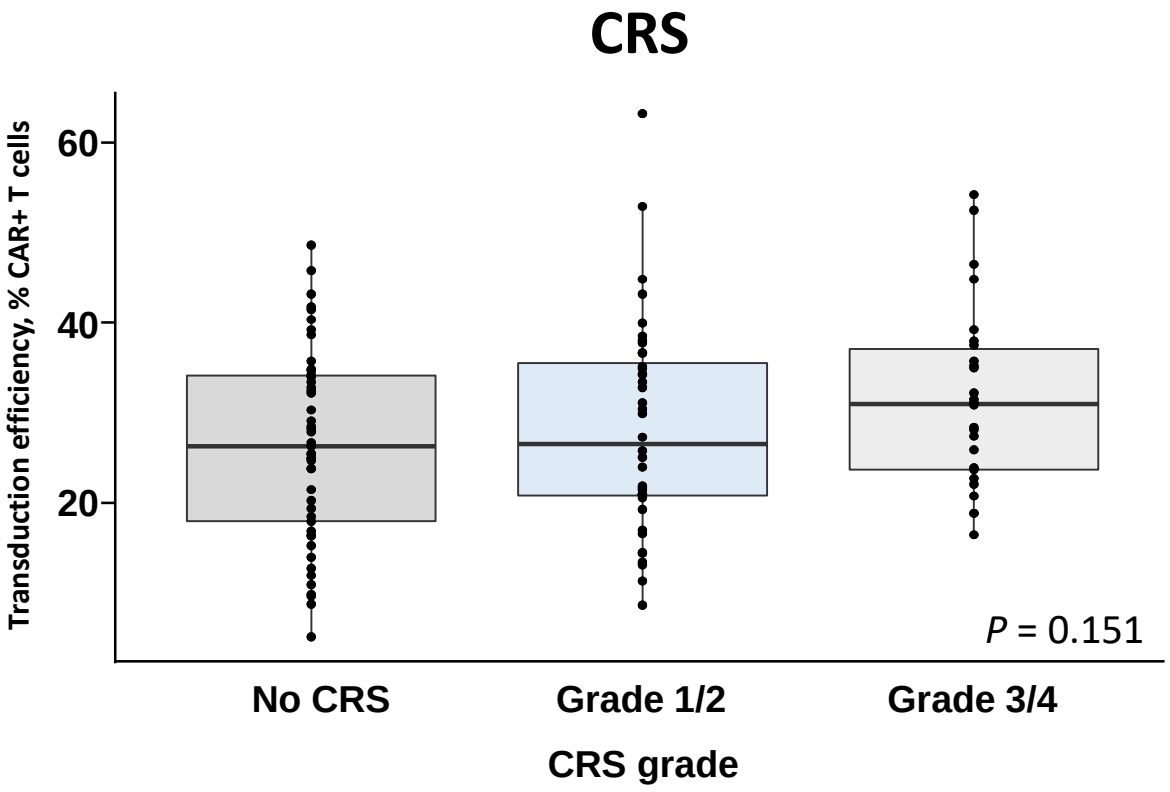
1st quartile = 5.3-20.8; 2nd quartile = 20.8-28; 3rd quartile = 28-35.2; 4th quartile = 35.2-63.2.

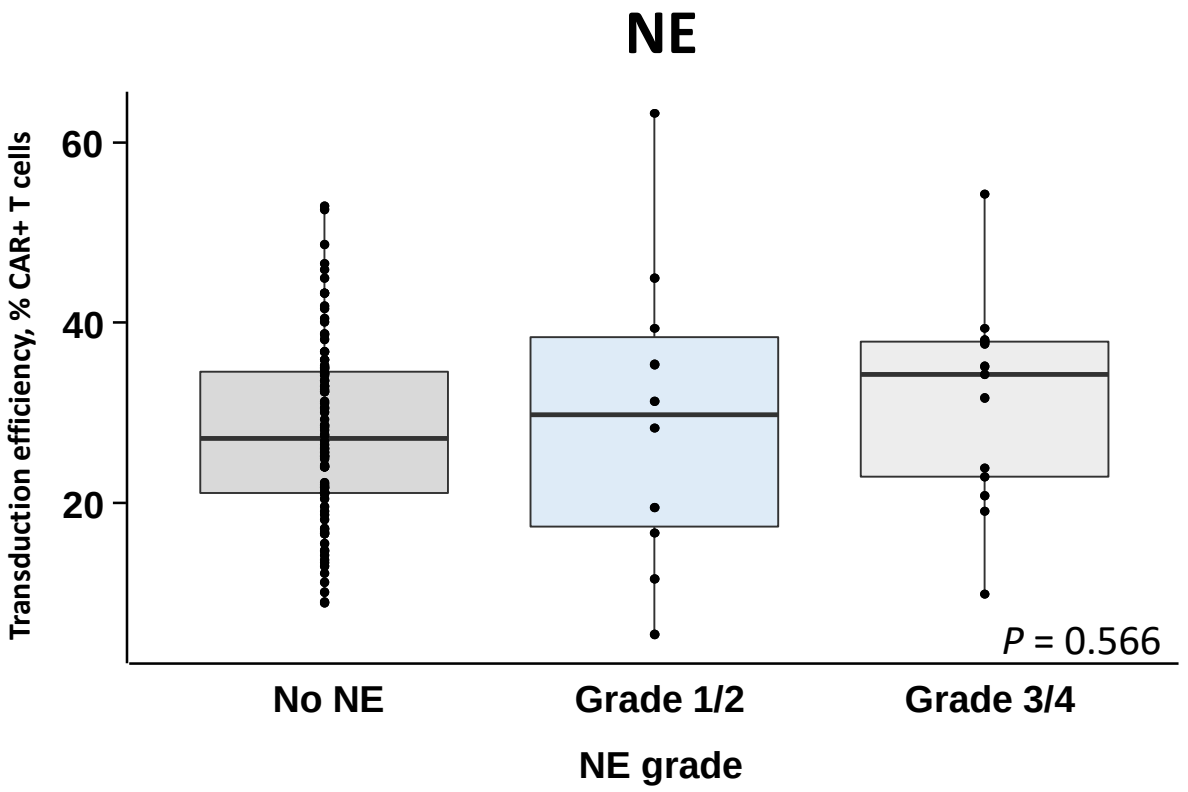
CAR, chimeric antigen receptor; CR, complete response; PD, progressive disease; PFS, progression-free survival; PR, partial response; SD, stable disease; UNK, unknown.

[1] CAR protein expression (=T cell transduction efficiency) is measured by flow cytometry using a CAR19-specific anti-idiotypic antibody conjugated with phycoerythrin (PE).

Relationship between transduction efficiency (% CAR+ T cells) and CRS / NE

- Median transduction efficiency (% CAR+ T cells) by flow cytometry was 28% (range, 5.3% to 63.2%)

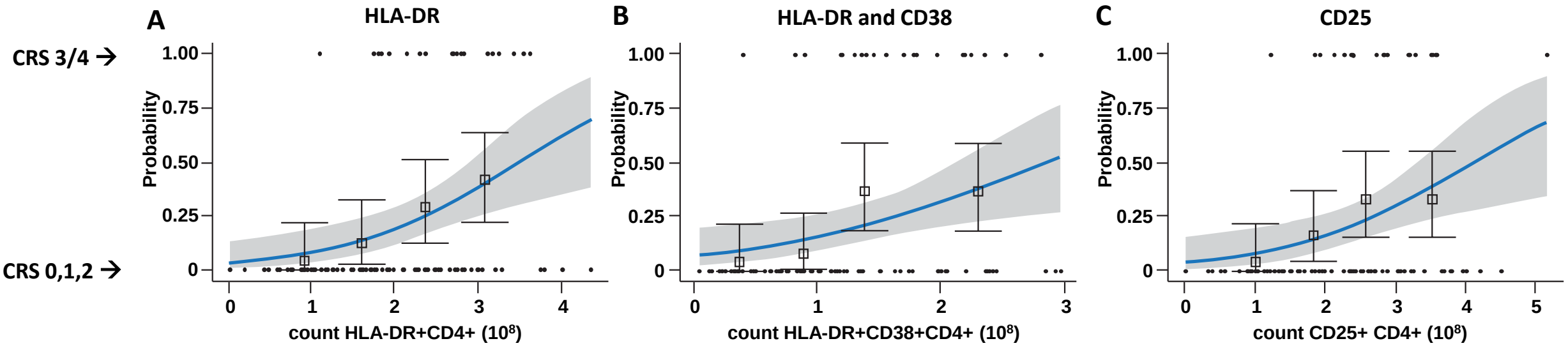




[a]Penn CRS grading scale.
P values = 1-way ANOVA tests.
CAR, chimeric antigen receptor; CRS, cytokine release syndrome; NE, neurological events.

Relationship of CAR+ T cell activation and grade 3/4 CRS

- Total number of activated CD4+ CAR+ T cells was associated with grade 3/4 CRS
  - Activation is defined by expression of the following T cell activation markers: HLA-DR (figure A), HLA-DR and CD38 (=fully activated, figure B), as well as CD25 (figure C)
- HLA-DR+ CD38+CD4+ T cells showed a correlation with grade 3/4 CRS (OR 3.5; 95%CI 1.2-10.7)



- No product attributes were significantly related to grade 3/4 NE

[a]Penn CRS grading scale.

CAR, chimeric antigen receptor; CI, confidence interval; CRS, cytokine release syndrome; HLA-DR, human leukocyte antigen-DR isotype; NE, neurological events.

Key takeaways:

- No single parameter distinguished between the outcomes on its own
- Feature selection and multivariate analyses were instrumental in identifying a set of parameters that may have an explanatory role in the observed outcomes as a group rather than individually
- Correlating product parameters to clinical endpoints helps clarify the criticality of the different quality attributes
- Correlational analyses in cell therapy are in important piece in specification setting and product development strategy

Acknowledgments:

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Thank you!!