Conversion of Cellular Kinetic Data for Chimeric Antigen Receptor T-cell Therapy (CAR-T) into Interpretable Units

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Introduction

- The cellular kinetics of CAR-T therapy has been described using both quantitative polymerase chain reaction (qPCR) and flow cytometry assays[1].
- The cellular kinetic measurements for CAR-T therapy by qPCR has been widely reported in units of copies of CAR transgene per µg of genomic DNA, while the flow cytometry assay quantifies the percentage of CAR-T cells as a function of either T cells or white blood cells (WBC) based on their surface expression. Neither measurement accounts for the fact that the WBCs may increase significantly following CAR-T infusion as the patient's hematological counts recover from prior lymphodepletion.
- This warrants the need for converting the measurements into units that are physiologically interpretable, not only for fully appreciating the CAR-T expansion and correlating with outcomes, but also to use in future mechanistic models for CAR-T therapy.
- We propose a formula to convert the qPCR measurements (from copies/µg), and flow cytometry (from percentage of cells) to the more physiologically interpretable cellular concentration in the unit of cells/µl units.

Methods

- Data from 79 relapsed/refractory pediatric acute lymphoblastic leukemia (ALL) patients in the ELIANA (CCTL019B2202) trial were included in this analysis. For converting qPCR measurements from copies/µg to cells/µl, the below equation is proposed. Parameters M, N, F are unknown.

$$\text{Equation 1} \quad \frac{\text{CAR}^+\text{ cells}}{\mu\text{l blood}} = \frac{\text{WBC}}{\mu\text{l blood}} \times \frac{\text{CAR DNA copies}}{\mu\text{g DNA}} \times \frac{M\ \mu\text{g DNA}}{\text{WBC}} \times \frac{1\text{ CAR cell}}{\text{N CAR DNA copies}} \times F$$

M is the number of µg of DNA per white blood cell; N is the number of copies of CAR DNA per CAR-T cell; F is the fraction of cells with CAR DNA that express the CAR.

- For converting flow cytometry measurements from percent of CAR$^+$ cells to cells/µl, the below equation was proposed.

$$\text{Equation 2} \quad \frac{\text{CD3}^+\text{ CAR}^+\text{ cells}}{\mu\text{l blood}} = \frac{\text{WBCs}}{\mu\text{l blood}} \times \frac{\text{CD3}^+\text{ CAR}^+\text{ cells}}{\text{WBC}}$$

- Upon conversion of data to cellular concentration, the exposure-response relationships have been revisited to assess any benefit in response/safety prediction using the exposure in converted units.

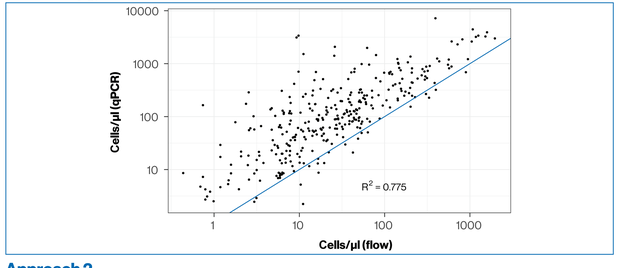
Results

- Three different approaches with varying complexity were taken to estimate (M*F)/N in order to perform the conversion from native units to cellular concentration.

Approach 1

- Assume values for the three parameters, M, N, F in Equation (1). We assume M = 6.6e-6 µg DNA per cell, N = 1 copy/CAR cell, F = 1 (100% of T cells with CAR DNA express the CAR).
- Plotting values for CAR$^+$ T cells measured by flow cytometry and CAR content based upon qPCR estimates using M=6.6e-6 µg, N=1, F=1 yielded DNA-based values for CAR-T consistently higher than flow cytometry-based values for CAR-T (**Figure 1**). We adjusted the assumptions for M, N, and F in Approach 2.

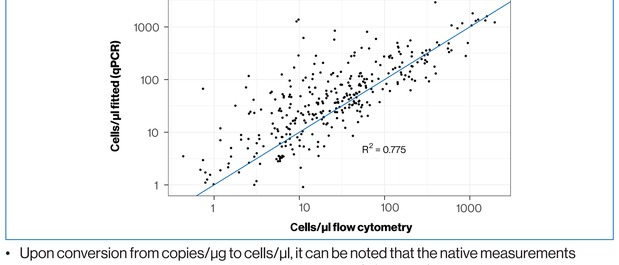
Figure 1. Comparing cells/µl derived from qPCR and flow cytometry assay assuming M=6.6e-6 µg, N=1, F=1



Approach 2

- We perform a regression between WBC x CAR copies/µg from qPCR and cells/µl from the flow cytometry assay to estimate the lumped value of (M*F)/N = 2.68e-6 that gives the best agreement between the flow and PCR estimates.
- Assuming that M = 6.6e-6 µg DNA per cell is correct, there are different possible values for N and F. At one extreme, N = 1 copy of CAR DNA per CAR-T cell, and F = 0.4, or only 40% of the cells with CAR DNA are expressing the CAR.
- At the other extreme, N = 2.46 copies of CAR DNA per CAR-T cell and F = 1, or 100% of CAR-T cells express the CAR (**Figure 2**).

Figure 2. Comparing cells/µl derived from qPCR and flow cytometry assay by estimating a population average (M*F)/N

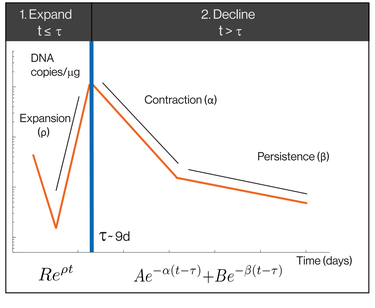


- Upon conversion from copies/µg to cells/µl, it can be noted that the native measurements and cellular concentration values are highly correlated (R^2 = 0.752). Thus, a difference in the exposure-response relationship between the two units is not expected.

Approach 3

- The population cellular kinetic (popCK) model proposed by Stein et al[2] was fit to both the qPCR and flow cytometry data together with an estimate for the (M*F)/N ratio both for the population and for each individual, in addition to estimating the other parameters that capture the CAR-T kinetics (e.g., CAR-T expansion) (**Figure 3**).

Figure 3. Schematic showing the mathematical model describing Tisagenlecleucel expansion



Fold expansion = $e^{\rho\tau}$

- The population estimate for the (M*F)/N ratio is 1.58e-6. A comparison of the parameters using the various data sources (unconverted copies/µg vs cells/µl) are shown in **Table 1**.

Table 1. Comparison of crucial popCK model parameters using the native qPCR units vs cells/µl

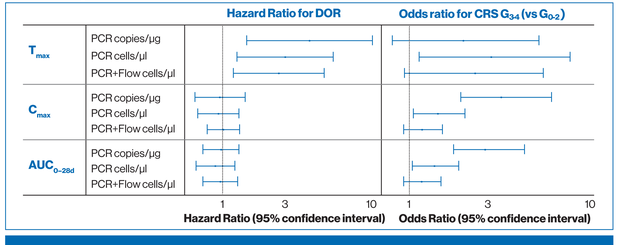
	Copies/µg fit	Cells/µl pooled fit (Approach 3)
Fold expansion	3366	39044
T_{max}	8.87 days	9.43 days
α	0.17/day	0.31/day
β	0.0026/day	0.0024/day

- A 12x higher expansion of CAR-T cells can be observed based on the parameter estimates using copies/µg as compared to cells/µl. In this trial, this could be attributed to the simultaneous increase in WBCs as well as CAR$^+$ cells that lead to a higher expansion when considering cellular concentration. The units of copies/µg gives the CAR expression normalized by the total WBC DNA, thus the expansion quantified in copies/µg units does not capture the increase in the total number of CAR$^+$ cells *in vivo*.

Exposure-Response

- For assessing the exposure-response relationship, cellular kinetic parameters (C_{max}, T_{max}, AUC_{0-28d}) that were derived from the popCK model were used to fit a regression model with a response variable.
- The time-to-event response variables explored were event free survival (EFS), duration of remission (DOR), and B-cell recovery time. For time-to-event response variables, a Cox proportionality regression model was fit.
- Logistic regression models were fit to two categorical variables: 28-day response, grade ≥3 cytokine release syndrome (CRS). The odds ratio provides the propensity of a categorical response upon doubling the exposure (**Figure 4**).

Figure 4. Forest plot showing the hazard/odds ratio describing the exposure-response relationship of time-to-event and categorical variables



Conclusions

- We have proposed equations to convert cellular kinetic measurements from qPCR and flow cytometry assays to physiologically meaningful units of cells/µl.
- The CAR-T fold-expansion in cells/µl is observed to be ~12x higher as compared to copies/µg. This can be attributed to the simultaneous expansion in WBCs along with the CAR$^+$ T cells.
- When assessing the exposure-response relationship, using units of cells/µl provides a similar result compared to the native copies/µg units, likely due to a high correlation between exposure measurements by either set of units.

References

1. Thudium et al, Blood (2017) 130 (21): 2317–2325. **2.** Stein et al, CPT: Pharmacometrics and Systems Pharmacology (2019), 8 (5): 285-295.

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