Real time, point-of-need measurement of Blood CD19 CAR-T vector load is concordant with standard ddPCR method

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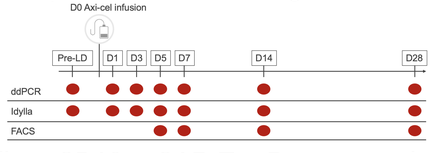
Introduction

- CD19-directed chimeric antigen receptor T cell (CAR-T) therapy has revolutionized treatment of non-Hodgkin lymphoma; however, significant toxicities have been associated with CAR-T therapies.
- Peripheral blood quantification of CAR-T vector load may have prognostic value and can aid in toxicity management for large B cell lymphoma (LBCL) patients undergoing axicabtagene ciloleucel (axi-cel) treatment.
- We assessed the concordance of the Idylla™ platform, a point-of-need (PON), cartridge-based system for real-time assessment of CAR-T vector load from peripheral blood, with standard droplet digital polymerase chain reaction (ddPCR) and flow cytometry (FACS) methods.

Methods

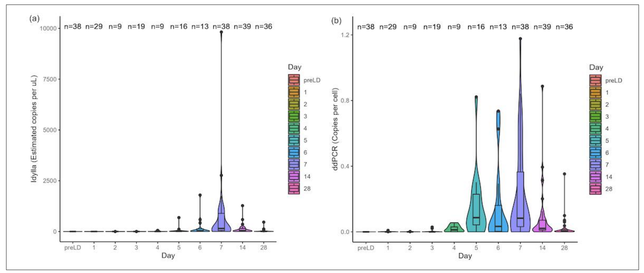
- Thirty-nine consecutively enrolled subjects who received axi-cel CAR-T therapy for LBCL were consented on an IRB-approved protocol. 246 samples were collected across the 39 participants according to the schema below.



- The Idylla CD19 CAR-T Prototype is a fully automated, qPCR based-assay that quantitatively detects CD19 CAR transgene starting from 500µL EDTA peripheral blood.
- ddPCR was used to detect and quantify T cells carrying the anti-CD19 CAR transgene from PBMCs.
- CD19 CAR-T expansion was measured by FACS with anti-idiotype-FMC63 conjugated to Dylight 65013.
- The concordance between Idylla, ddPCR, and FACS analysis was evaluated using Robust Z-score standardization and Bland-Altman analysis.

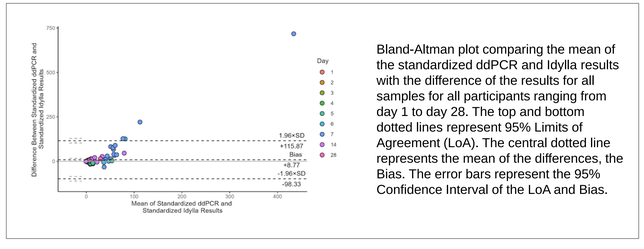
Results

Distribution of CAR-T Vector Load



CAR-T vector load was measured by Idylla (estimated copies per uL) and ddPCR (copies per cell) over the first 4 weeks after axi-cel infusion for each participant.

Concordance Between Idylla and ddPCR Measurements

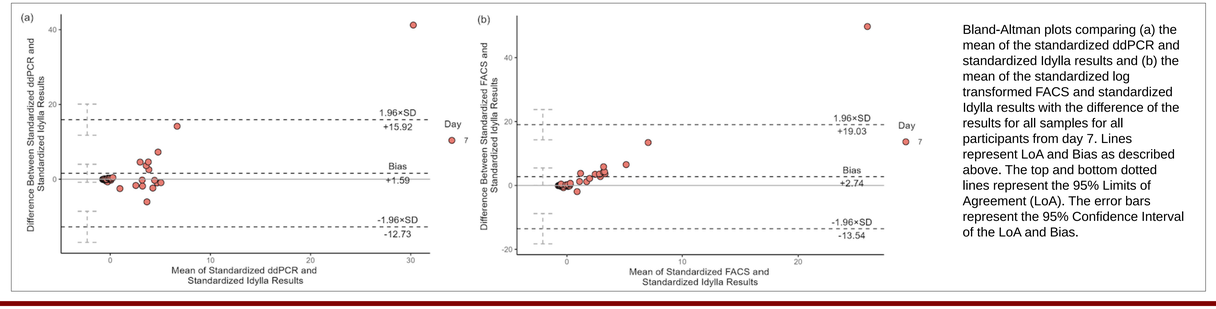


Bland-Altman plot comparing the mean of the standardized ddPCR and Idylla results with the difference of the results for all samples for all participants ranging from day 1 to day 28. The top and bottom dotted lines represent 95% Limits of Agreement (LoA). The central dotted line represents the mean of the differences, the Bias. The error bars represent the 95% Confidence Interval of the LoA and Bias.

A Bland-Altman analysis was performed on the standardized CD19 transgene values of all samples collected between days 1-28. The concordance between Idylla and ddPCR measurements was within the acceptable range, with 95% of all samples within ± 2 SD of the mean difference.

Concordance Between Idylla, FACS, and ddPCR Measurements at Peak CAR-T Expansion (Day 7)

Restricting analysis to day 7, Bland-Altman analysis also found concordance between standardized Idylla and log transformed ddPCR (a) and FACS values (b) for samples collected at the time of peak CAR-T expansion.



Bland-Altman plots comparing (a) the mean of the standardized ddPCR and standardized Idylla results and (b) the mean of the standardized log transformed FACS and standardized Idylla results with the difference of the results for all samples for all participants from day 7. Lines represent LoA and Bias as described above. The top and bottom dotted lines represent the 95% Limits of Agreement (LoA). The error bars represent the 95% Confidence Interval of the LoA and Bias.

Conclusions

- Previous studies of CAR-T expansion have used PCR-based and/or FACS approaches, which require technical laboratory skills and have multi-day turnaround times.
- The Idylla platform enables fully automated detection of CAR-T vector load, with 2 minutes of hands-on time. Results are reported in ~90 minutes.
- The concordance between Idylla and ddPCR measures was within the acceptable range (95% of all samples within +/- 2 SD of the mean difference), and also concordant with FACS data.
- This PON device may ultimately enable standardization of CAR-T vector load measurements across clinical sites, development of predictive algorithms for toxicity, and management of patients showing primary or secondary treatment failure.
- The peak blood CAR-T vector load and its association to toxicity and efficacy outcomes will be reported in future studies.

References

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Support