Validating CAR-T cell Enumeration by Flow Cytometry Using Novel Synthetic EGFR+ CD3+ Mimic Controls

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Background

Flow cytometry enumeration of transduced CAR T-cells is achieved by detecting a surrogate transgene marker. Quality control labs such as our Fred Hutch Cellular Processing Facility QC lab (CPF QC) use in-house transduced CAR T-cells for validating flow cytometry panels and often as controls for each assay. However, significant lot-to-lot variability and unknown long-term stability of these human control cells limit standardization, reproducibility and comparability of the assay over time.

Study Objective

We evaluated the use of synthetic particles with induced stable CD3 expression (T cell mimics) and expression of the common CAR transduction marker epidermal growth factor receptor (EGFR) to validate our CAR T cell flow cytometry assays and to standardize enumeration of transduced CAR-T cells.

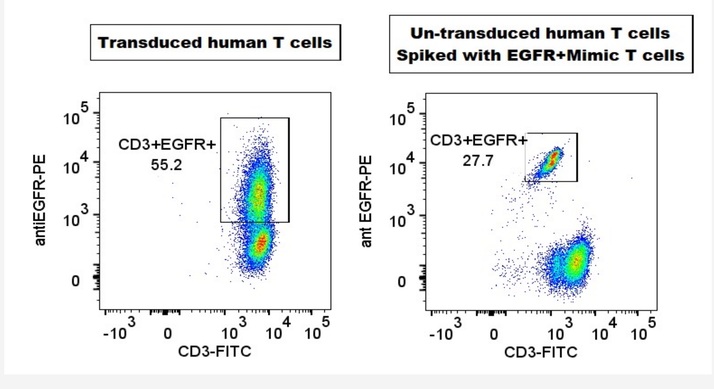
Results

Table 1

Percentage of Recovery [(experimental value/Nominal Value)*100]		
	Cetuximab-AF488	Cetuximab-PE
Mimetic 10% EGFR+	99.5%	100.1%
Mimetic 30% EGFR+	100.4%	99.3%
Mimetic 70% EGFR+	100.5%	100.2%

Custom synthetic T cell mimics with varying percentages EGFR expression: 10% (low), 35% (med), and 70% (high) were custom-ordered from Slingshot Biosciences, Inc. Flow cytometry analysis using the standard CPF QC staining procedure was conducted on the EGFR+ T cell mimics. 100% recovery of the nominal values using PE and AF488 conjugated Cetuximab were achieved.

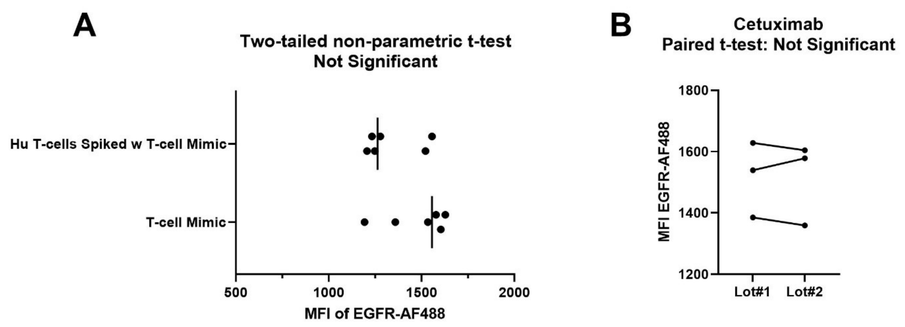
Fig. 1



Homogenous EGFR expression was distinctively detected in human enriched T-cells spiked with EGFR+T-cells Mimic (Right image); no significant difference on CD3 expression was found between human T-cells and transduced CAR-T cells (left vs right image).

Results

Fig. 2



- FIG.2A: Mean Fluorescent Intensity of EGFR on T-cells Mimic were measured with and without spike in human T-cells. No significant difference was found.
- Fig.2B. Two Lots of AF488 conjugated Cetuximab were compared to show consistency between the two lots using EGFR+T-cells Mimic. No significant difference were found.

Conclusion

We showed that Synthetic T-cell mimics expressing a surrogate marker of transduction may serve as a positive control for qualifying new lots of Abs against EGFR, testing binding capacity of Abs conjugated to different fluorochromes, and qualifying CAR T cell enumeration by flow cytometry.

Acknowledgements:
The authors received Assay/Model/Technology Development Funds from FHCC. Special Thanks to SSB.

ISCT NORTH AMERICA REGIONAL MEETING
HOUSTON • TEXAS • USA • SEPT 8–10, 2023

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