FUNCTIONAL AND PHENOTYPIC CHARECTERIZATION OF CAR T CELL POTENCY THROUGH SINGLE CELL AND BULK POPULATION METHODS

Mark Landon[1,2], Carl Dargitz[1], Uma Lakshmipathy[1]
[1]Thermo Fisher Scientific, Carlsbad CA, [2]California Polytechnic State University, San Luis Obispo, San Luis Obispo CA

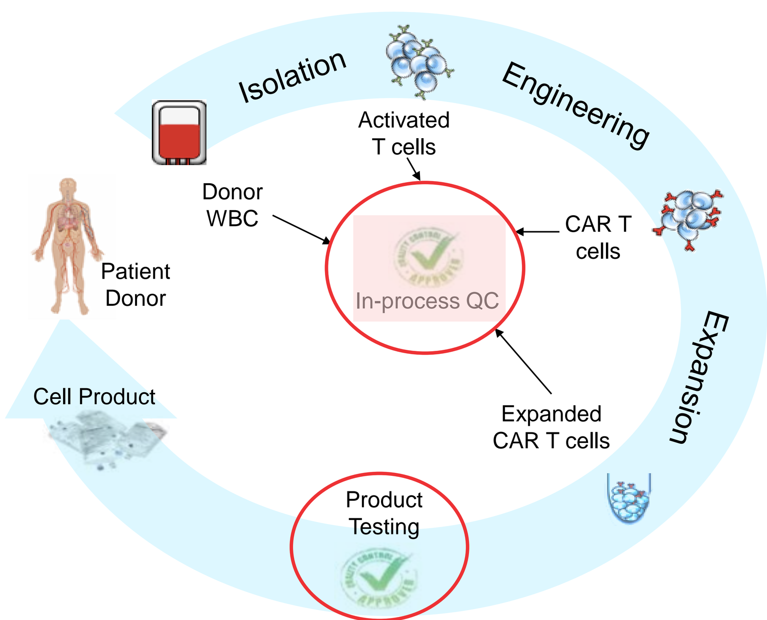
ABSTRACT

Chimeric antigen receptor (CAR) T-cells have proven to be an effective cellular immunotherapy and are approved for the treatment of relapse/refractory hematological malignancies. Current methods to determine their potency rely on assessment of T-cell phenotype, *in vitro* cytotoxicity, and/or a single analyte (IFN-γ) secretion analysis that varies between donors and does not correlate with patient response. Single-cell based technologies have been able to accurately predict CAR T-cell potency by characterizing their polyfunctional strength indices (PSI), which is a measurement of the quantity of cells secreting two or more cytokines and the amount of cytokine secreted by those cells. While able to accurately segregated responders from non-responders, the widespread use of this technology is limited by high cost, long assay duration, and lack of high-throughput capabilities. Multiple methods for determining T-cell potency exist and have high throughput capabilities, but their relation to PSI values is unknown. As the field of cellular immunotherapeutics expands, there exists a need to develop more cost effective, high-throughput, and predictive potency assays.

To address this, T-cells were isolated from healthy donors, expanded *in vitro*, and transduced with a second generation (CD3ζ and 4-1BB) anti-CD19 CAR lentivirus. The anti-CD19 CAR T-cells were characterized by expression of differentiation and exhaustion markers, *in vitro* cytotoxic activity, secretion of cytokines on a bulk population level, and PSI values. Standard measurements of T-cell potency including T-cell phenotype, *in vitro* cytotoxicity, and IFN-γ secretion did not correlate with PSI. Four cytokines (IL-2, IL-5, IL-6, IL-31) were found to be strongly correlated with PSI values. The results from this study identify optimal methods to characterize immune cell potency, and guide development of future potency assays. These can be used to facilitate a simplistic and integrated workflow that is relevant for functional characterization of both autologous and allogeneic CAR T-cell products.

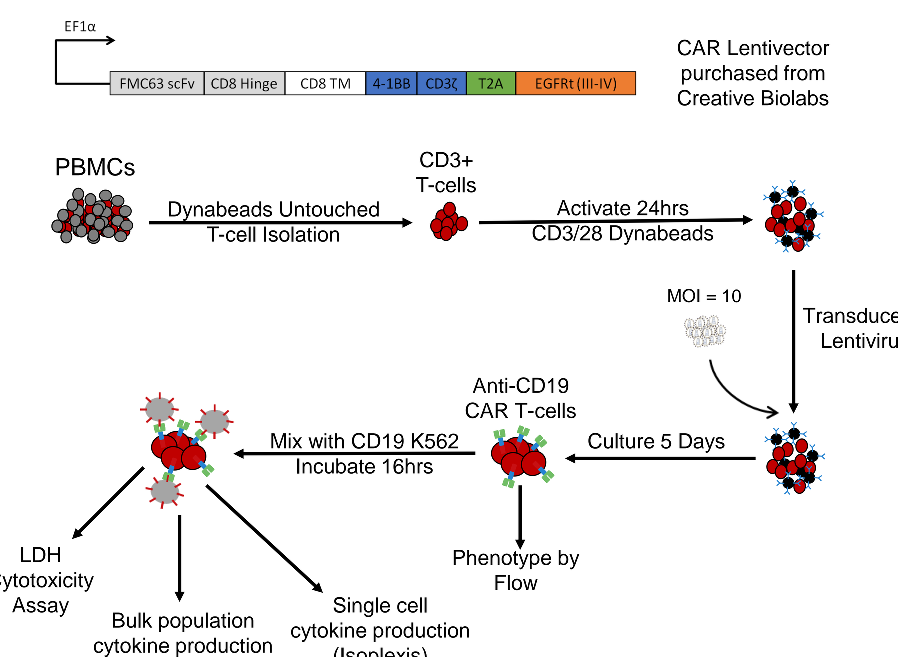
INTRODUCTION

Traditional cancer therapies include surgery, chemotherapy, and/or radiotherapy but are non-specific and inadequate to treat relapse/refractory tumors. Immunotherapy is a promising solution to traditional cancer therapies and harnesses the potency and specificity of the immune system to elicit an anti-tumoral response. CAR T cell therapy is a potent cellular immunotherapy and has seen great success in the treatment of hematological malignancies. CAR T cell manufacturing involves the isolation, activation, engineering, and expansion of patient derived T-cells. Every step of the manufacturing process can effect the CAR T cell potency and requires in-process quality control (QC) to ensure that the product is meeting pre-designed specifications.



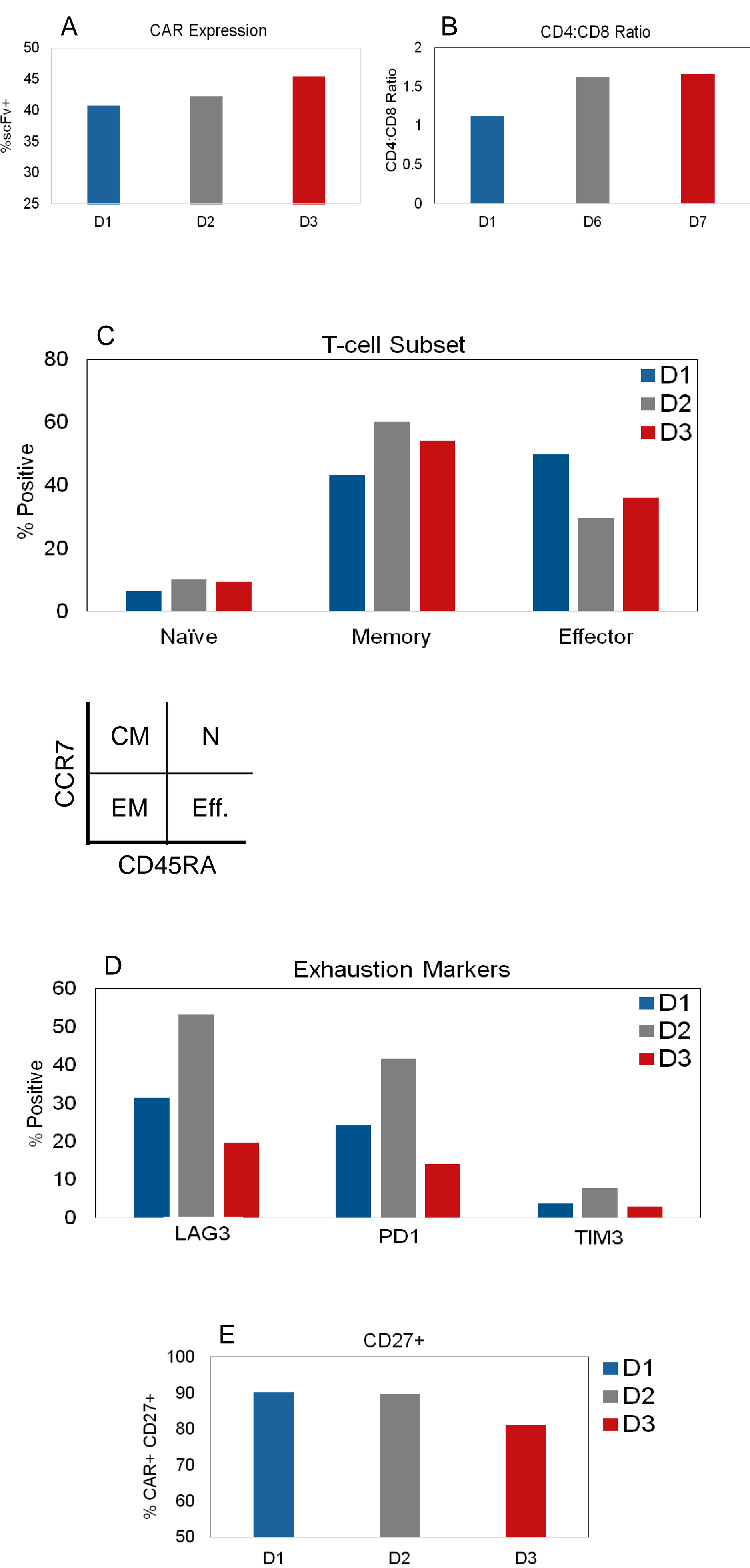
Unfortunately, standard assays for CAR T potency do not correlate to patient response. Single-cell based methods deconvolute T-cell heterogeneity and can better characterize T-cell based products. One such technology assesses cytokine production on a single cell level and has found a positive correlation between patient response and the number of polyfunctional cells present in a product. The objective of this study was to compare multiple potency assays to this technology to identify optimal methods of characterizing CAR T cell potency.

MATERIALS AND METHODS



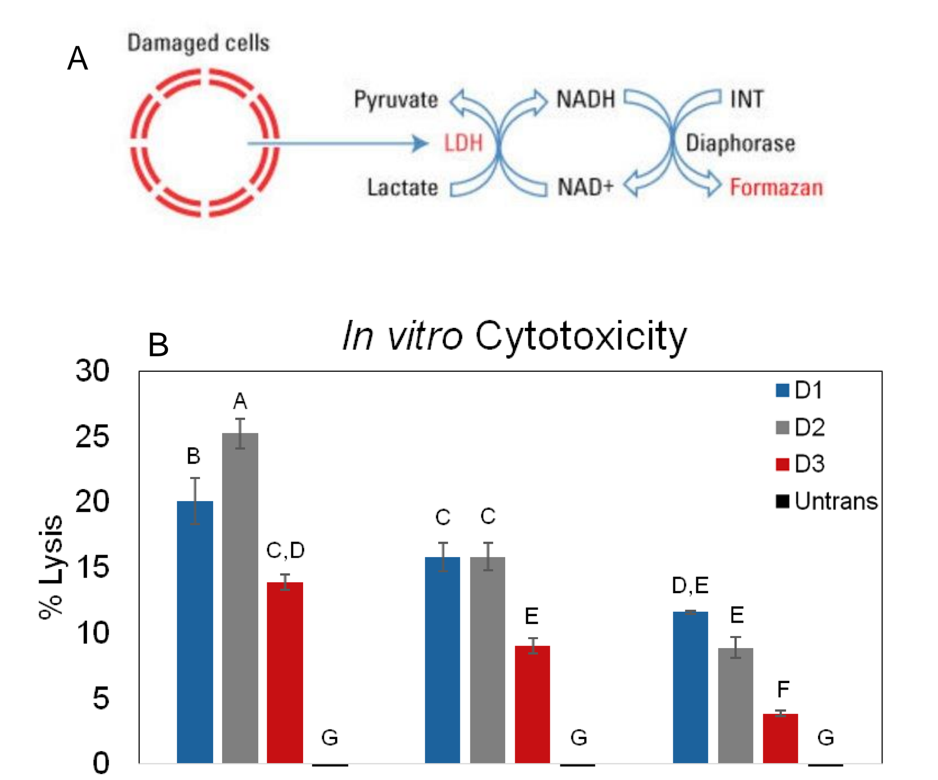
RESULTS

Figure 1. CAR T-cell phenotype



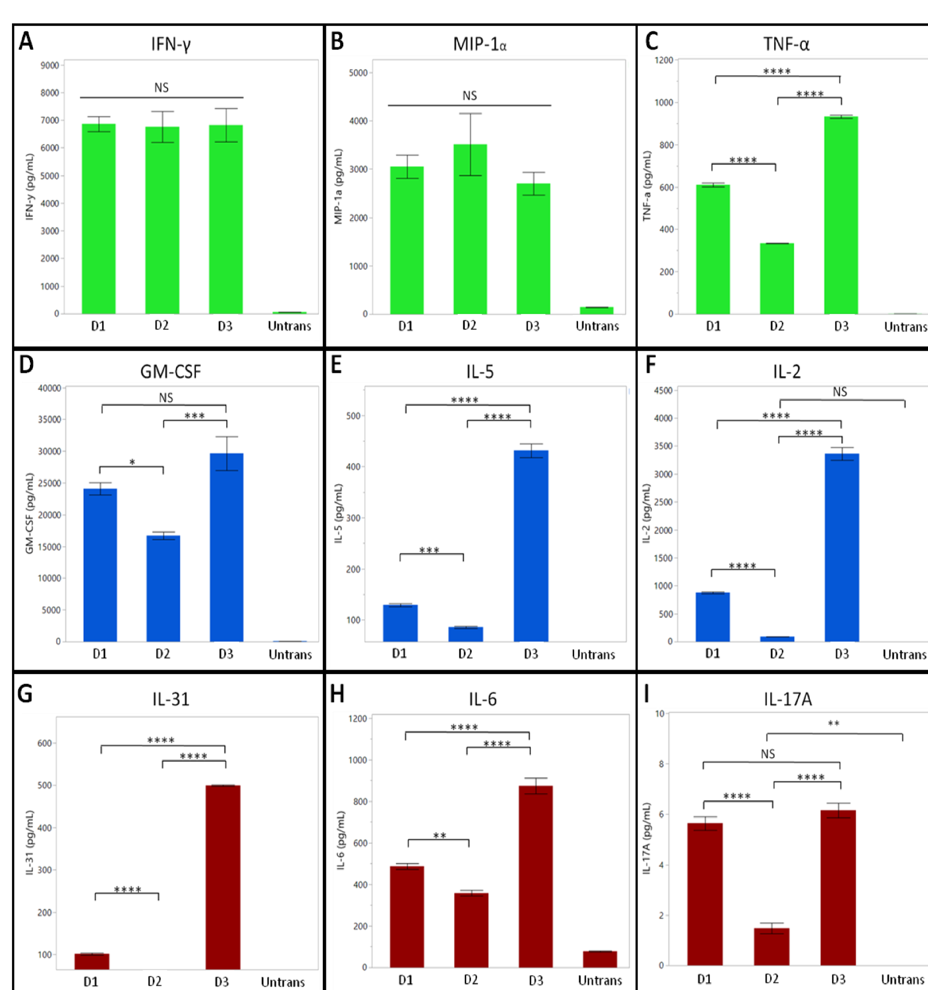
A. Expression of CAR construct detected via goat anti-mouse IgG antibody. Expression is non-variable between donors at the time of co-culture.
B. CD4:CD8 ratio between donors.
C. CAR T cell subset composition is relatively similar between donors. Naïve, memory and effector populations delineated by expression of CCR7 and CD45RA. Abbreviations are as follows: naïve (N), central memory (CM), effector memory (EM), effector (E).
D. Expression of exhaustion markers LAG3, PD1, and TIM3 within CAR+ populations for all donors.
E. Expression of pro-survival marker CD27 within CAR+ populations for all donors.

Figure 2. *In vitro* Cytotoxicity



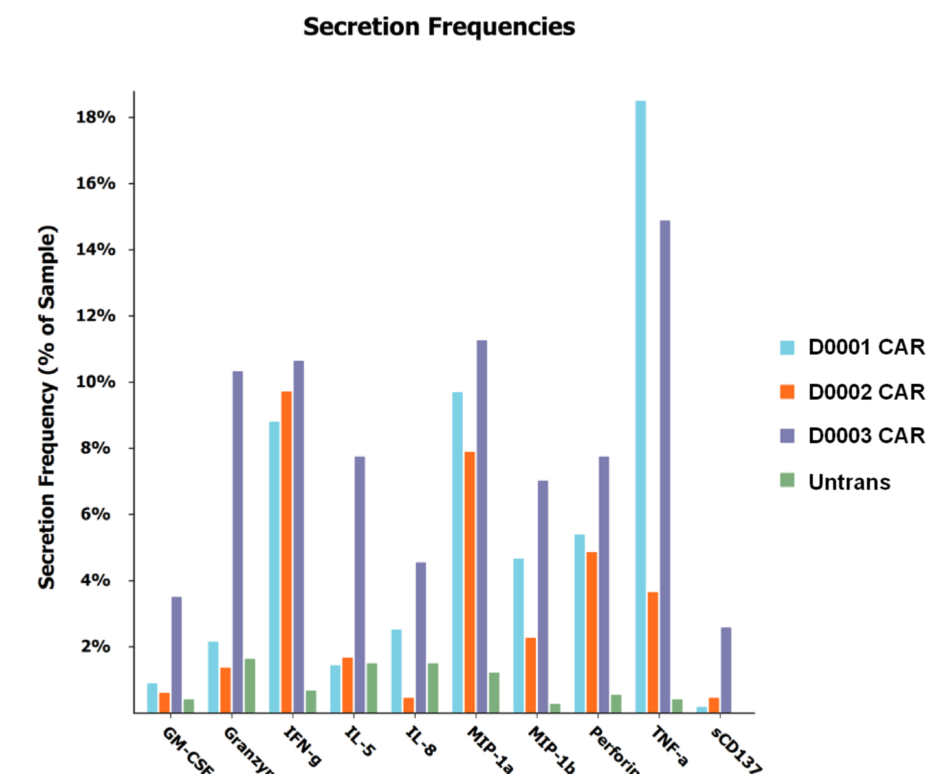
A. *In vitro* cytotoxicity was detected via lactate dehydrogenase (LDH) release. LDH is released in the supernatant by dead/dying cells and catalyzes the reaction of lactate into pyruvate, reducing NAD+ to NADH. NADH is oxidized by diaphorase to convert a tetrazolium salt (INT) into formazan. Absorbance of formazan at 490nm is directly proportional to the amount of LDH, which is proportional to the amount of dead/dying cells present.
B. Percent lysis for all donors after a 16hr co-culture with CD19 K562s. CAR T cells exhibit more lysis compared to untransduced controls (Untrans) at all effector to target (E:T) ratios (n=3 technical replicates). CAR T-cells lytic capability decreased as E:T decreased. Data are presented as means. Error bars denote standard error. Statistical differences performed by comparisons of means with Tukey's post-hoc comparisons. Groups not connected by same letter are statistically different (p≤0.05).

Figure 3. Cytokine production on a bulk population level



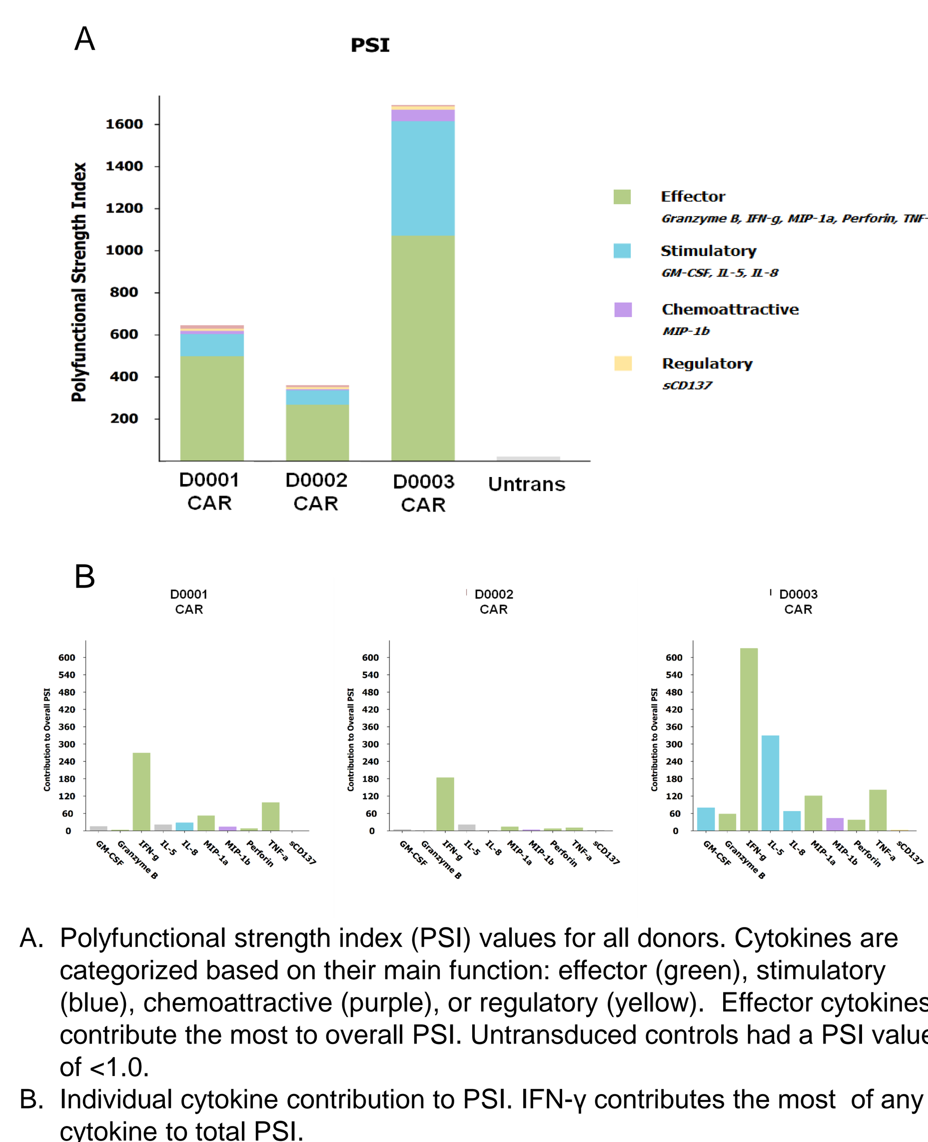
Cytokines produced after overnight stimulation with CD19 expressing K562s were detected on a population level through 34-Plex Human ProcartaPlex™ Panel 1A and categorized based on their main function: effector (A-C), stimulatory (D-F), or inflammatory (G-I) cytokines. All donors produced higher levels of cytokines compared to untransduced (Untrans) controls (p≤0.0001) unless displayed otherwise. Abbreviations are as follows: Interferon gamma (IFN-γ), macrophage inflammatory protein 1 alpha (MIP-1α), granulocyte macrophage colony stimulating factor (GM-CSF). Data presented are means of technical replicates (n=4), error bars denote standard error. Statistical differences performed by comparisons of means with Tukey's post-hoc comparisons (* p≤0.05, ** p≤0.01, *** p≤0.001, **** p≤0.0001).

Figure 4. Cytokine production on a single-cell level



Single cell cytokine production was analyzed via IsoPlexis Single-Cell PF Strength Kit after an overnight incubation with CD19 K562s. Secretion frequencies denote the percent of single cells within the population secreting cytokines above background. Only cytokines secreted above background are displayed.

Figure 5. PSI Values for CAR T cells



A. Polyfunctional strength index (PSI) values for all donors. Cytokines are categorized based on their main function: effector (green), stimulatory (blue), chemoattractive (purple), or regulatory (yellow). Effector cytokines contribute the most to overall PSI. Untransduced controls had a PSI value of <1.0.
B. Individual cytokine contribution to PSI. IFN-γ contributes the most of any cytokine to total PSI.

CONCLUSIONS

- CAR T cell therapy has emerged as a promising solution to the limitations of traditional cancer therapies, but standard potency assays fail to accurately predict efficacy of CAR T cells once infused
- There is a need for a cost-effective, high throughput, and accurate assay to determine CAR T cell potency
- CAR T cells generated in house using clinical relevant methodology and had high, non-variable expression of the CAR construct
- Minimal differences in CAR T cell composition between donors, but increased expression of exhaustion markers was seen in Donor 2
- Potency assays detected signal in all CAR+ samples but not in untransduced controls, suggesting response was antigen specific and from the CAR T-cells specifically
- Dramatic differences in PSI suggest differences in potency exist, but standard assays to detect potency (T-cell phenotype, *in vitro* cytotoxicity, and IFN-γ release) failed to detect these differences
- Comparing assays to PSI via linear regression analysis revealed that:
  - No correlation between CAR expression, CD4:CD8 ratio, T-cell subset composition, or expression of exhaustion markers exists
  - No correlation between *in vitro* cytotoxicity exists. In fact, a negative correlation was found (R^2=0.850)
  - Certain cytokines detected on a bulk population level have a strong correlation: IL-2 (R^2=0.975), IL-5 (R^2=0.993), IL-6 (R^2=0.910), and IL-31 (R^2=0.973)
- Cytokine production on a bulk population level mirrored PSI values with Donor 3 having high, Donor 1 having intermediate, Donor 2 having low, and untransduced controls having almost absent levels of cytokine secretion.
- Correlative cytokines are produced by Th1 and Th2 cells, which usually exist in bipolar states. Their formation is controlled by T-bet and GATA3, respectively. Future studies could investigate the distribution of these transcription factors and assess whether they correlate with PSI
- Correlative cytokines are all activators of STAT3 signaling pathway, which is involved in the formation of memory T-cells. Increased STAT3 signaling within CAR+ cells has been correlated with improved clinical outcome.
- Results from this project can guide future studies and be used to develop products that assess CAR T potency in an accurate, cost-effective, high-throughput manner

ACKNOWLEDGEMENTS

This study was funded by the California Polytechnic State University CIRM Bridges to Stem Cell Research Training Grant.

TRADEMARKS/LICENSING

© 2018 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. For Research Use Only. Not for use in diagnostic procedures.