145 COMPARISON OF CAR-T CELL MANUFACTURING PLATFORMS REVEALS DISTINCT PHENOTYPIC AND TRANSCRIPTIONAL PROFILES

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Background With the clinical success of chimeric antigen receptor (CAR)-T cells against hematological malignancies, investigators are looking to expand CAR-T therapies to new tumor targets and patient populations. To support translation to the clinic, a variety of cell manufacturing platforms have been developed to scale manufacturing capacity while using closed and/or automated systems. Such platforms are particularly useful for solid tumor targets, which typically require higher CAR-T cell doses that can number in the billions. Although T cell phenotype and function are key attributes that often correlate with therapeutic efficacy, it is currently unknown whether the manufacturing platform itself significantly influences the output T cell phenotype and function.

Methods Static bag culture was compared with 3 widely-used commercial CAR-T manufacturing platforms (Miltenyi CliniMACS Prodigy, Cytiva Xuri W25 rocking platform, and Wilson-Wolf G-Rex gas-permeable bioreactor) to generate CAR-T cells against FGFR4, a promising target for pediatric sarcoma. Selected CD4+CD8+ cells were stimulated with Miltenyi TransAct, transduced with lentiviral vector, and cultured out to 14 days in TexMACS media with serum and IL2.

Results As expected, there were significant differences in overall expansion, with bag cultures yielding the greatest fold-expansion while the Prodigy had the lowest (481-fold vs. 84-fold, respectively; G-Rex=175-fold; Xuri=127-fold; average of N=4 donors). Interestingly, we also observed considerable differences in CAR-T phenotype. The Prodigy had the highest percentage of CD45RA+CCR7+ stem/central memory (Tscm)-like cells at 46%, while the bag and G-Rex cultures had the lowest at 16% and 13%, respectively (average N=4 donors). In contrast, the bag, G-Rex, and Xuri cultures were enriched for CD45RO+CCR7- effector memory cells and also had higher expression of exhaustion markers PD1 and LAG3. Gene clustering analysis using a CAR-T panel of 780 genes revealed clusters of genes enriched in Prodigy/de-enriched in bag, and vice versa. We are currently in the process of evaluating T cell function.

Conclusions This is the first study to our knowledge to benchmark these widely-used bioreactor systems in terms of cellular output, demonstrating that variables inherent to each platform (such as such as nutrient availability, gas exchange, and shear force) significantly influence the final CAR-T cell product. Whether enrichment of Tscm-like cells in the final infusion product correlates with response rate, as has been demonstrated in the setting of CD19 CAR-Ts, remains to be seen and may differ for FGFR4 CAR-Ts and other solid tumors. Overall, our study outlines methods to identify the optimal manufacturing process for future CAR-T cell therapies.

http://dx.doi.org/10.1136/jitc-2021-SITC2021.145