398 UTILIZING A TH17 POLARIZATION STRATEGY FOR GENERATING HIGH QUALITY HUMAN TIL PRODUCTS FOR PATIENTS WITH ADVANCED SOLID TUMORS

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Background Tumor infiltrating lymphocyte (TIL) therapy, a form of adoptive cell therapy (ACT), is based on the notion that T cells in the tumor are antigen-specific, and that by culturing them *ex vivo*, they can be expanded to large numbers and reinfused into the patient to potentially generate a strong antitumor response. TIL therapy has only been moderately successful. Efforts to improve efficacy have focused on isolating and expanding neoantigen-reactive TIL, which often involves time-consuming and costly isolation and sorting strategies.[1 2] Previous work has demonstrated\ enhanced efficacy of Th17 polarized cells in murine tumor models.[3 4] We hypothesized that a Th17 polarization strategy for generating TIL from surgical samples of patients with metastatic melanoma would result in a superior cellular product for ACT.

Methods Tumor resected from deidentified melanoma patients were obtained and cut into ~3mm diameter pieces. A single piece per well was placed into culture in a 24 well plate with media containing agonist αCD3 and either the standard, high-dose IL-2 (6000 IU ml^{-1}), or Th17 polarizing cytokines (IL-1β, IL-6, TGFβ1, IL-21, αIFNγ, and αIL-4, as well as low-dose IL-2 [200 IU ml^{-1}]). Remaining pieces were digested into single cell suspensions. TIL cultures were maintained for 2–3 weeks, after which cells were phenotyped by flow cytometry. Pre- and post-expansion TIL were FACS sorted for $CD3^+$ cells and whole transcriptome single cell sequencing was performed in conjunction with TCR sequencing.

Results Th17 polarization robustly enhanced yield of TIL ($p = 0.0068$) compared to commonly used traditional high-dose IL-2 expansion protocols. Additionally, Th17 polarized TIL contained significantly less Tregs ($p = 0.0086$) and vastly more $CD39^+$ $CD103^+$ $CD8^+$ T cells ($p = 0.0021$) when compared with high-dose IL-2 expansion. Subtraction experiments, wherein a single cytokine was individually omitted from the Th17 polarization cocktail, identified that TGFβ1 was the most critical factor for inducing the observed phenotype, though loss of any of the other cytokines resulted in a partial diminishment in yield. Single cell sequencing revealed alterations in the transcriptomic state of pre- vs. post-expansion TIL with the two different expansion strategies.

Conclusions We found that applying a simple Th17 polarization strategy for the generation of TIL products is feasible and results in a potentially superior TIL product. Further testing of TIL reactivity and functionality is ongoing.

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Ethics Approval Deidentified patient tumor specimens were obtained in accordance with an IRB-approved protocol at the Winship Cancer Institute of Emory University.

http://dx.doi.org/10.1136/jitc-2023-SITC2023.0398