MULTIOMIC ANALYSIS OF TIL SUGGESTS THAT CD4+ POLARIZATION REPresses TIL EXPANSION AND CD8+ ACTIVATION AND IS ASSOCIATED WITH PROGRESSION OF DISEASE

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Background Tumor Infiltrating Lymphocyte (TIL) Adoptive Cell Transfer (ACT) is effective in treating malignant melanoma and other solid tumors. The success of TIL ACT relies on the adequate expansion of TIL, with previous studies showing a positive association between number of TIL infused and patient response. To identify characteristics important for TIL expansion, we performed a multiomic analysis of patients’ TIL product.

Methods Expanded TIL products from metastatic melanoma patients enrolled in clinical trials at Moffitt Cancer Center were collected before infusion. CD4+ and CD8+ were isolated and analyzed by RNA-seq (n=13) and pan-acetyl histone 3 ChIP-seq (n=20). The number of TIL, percentage of CD4/CD8 infused, progression free survival (PFS), and overall survival (OS) were recorded. To more evenly divide both RNA-Seq and ChIP-seq samples between groups, patients were categorized into “TIL high” vs “TIL low” based on division at the geometric mean of the number of TIL infused (geometric mean= 4.6e10, range= 9.05e+09 – 1.13e+11). Log2 fold changes of ±0.5 and q-values of <0.1 were considered significant.

Results Individuals in the “TIL high” group had longer (PFS) (median=92 vs 4, p< 0.0001) and OS (OS median=92 vs 10 months , p<0.0001) than those in the “TIL low” group, and the percentage of CD4+ infused was negatively correlated with the number of TIL infused (R²=-0.72, p=7.7e-05). RNA-seq revealed 30 differentially expressed genes (DEGs) in CD4+ between groups. The upregulated genes in the “TIL low” group included TNFRSF4, PTGDR2, IL5, and CCL4, which are associated with Th2 and Treg phenotypes. Pathway analysis of the RNA-seq data further suggested increased Th2 and Th17 polarization in the “TIL low” group. ChIP-seq showed 18 differentially acetylated genes (DAGs) between “TIL high” and “TIL low” CD4+, including increased acetylation at known inducers of Tregs CD200R1, IL12RB2. RNA-seq revealed 3 DEGs between CD8+ in the “TIL high” and “TIL low” groups. ChIP-seq revealed 18 DAGs between “TIL high” and “TIL low” CD8+, with increased acetylation at genes known to be upregulated during CD8+ activation (e.g., SLC4A10, TIGIT and P2RY1).

Conclusions Our results and those of other groups have shown that increased number of TIL infused are associated with positive patient outcomes. Our results further indicate that CD4+ Th2, Th17 and Treg phenotypes in the TIL product are associated with decreased numbers of TIL achieved during expansion. Consequently, we hypothesize that approaches to directing T-cell polarization during TIL expansion may increase patient response rates.

Ethics Approval IRB exempt