IDENTIFICATION AND ENRICHMENT OF NEOANTIGEN-REACTIVE T CELLS TO OPTIMIZE ADOPTIVE CELL TRANSFER WITH TUMOR-INFILTRATING LYMPHOCYTES (TIL)

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Background Adoptive cell transfer (ACT) using tumor-infiltrating lymphocytes (TIL) has achieved an overall response rate of 39% in metastatic melanoma patients at Moffitt Cancer Center. In these trials, a substantial fraction of patients were non-responders by RECIST, but demonstrated a mixed response to therapy. These results suggest that the infused TIL product contained tumor-reactive T cells with therapeutic potential, which could be further optimized to improve ACT with TIL. We hypothesized that outcomes might be improved by identifying and enriching neoantigen-reactive TIL within bulk products. The purpose of this study is to define approaches to optimize ACT with TIL, by identifying, enriching, and analyzing neoantigen reactive TIL from the ACT infusion product of previously treated metastatic melanoma patients.

Methods Patient-derived cryopreserved tumor tissue, PBMC, and TIL from completed metastatic melanoma TIL trials were used for this study. Whole exome and RNA sequencing were performed on DNA and RNA extracted from tumor tissue and compared to DNA from autologous PBMC. Genetic sequencing and gene expression data were utilized to determine protein-modifying somatic mutations. Peptides were then predicted for their ability to be presented on MHC molecules, prioritized, and up to 192 custom 25-mers were synthesized per patient sample. Neoantigen peptides were loaded onto patient-derived dendritic cells (DC) and co-cultured with autologous TIL. These TIL were then sorted by FACS on their ability to upregulate 41BB and OX40 and expanded through the rapid expansion protocol (REP). Enriched TIL were subsequently screened for neoantigen reactivity by 41BB/OX40 upregulation, cytokine release, and degranulation.

Results Protein-altering somatic mutations from metastatic melanoma tissues ranged from 49 to 1631 mutations (median = 389). On average, 16.2% of TIL were sorted for upregulation of 41BB/OX40 upon co-culture with DC pulsed with the neoantigen peptide pool (range: 2.7–31.1%). CD4+ TIL displayed a 3.75-fold upregulation of 41BB/OX40, while CD8+ TIL saw a 1.88-fold increase (n=6). This coincided with substantial production of IFN γ , TNF α , and granzyme B (n=6). Neoantigen-reactive (41BB+/OX40+) and non-reactive (41BB-/OX40-) TIL expanded to similar degrees in REP (average of 639-fold vs. 611-fold; n=6). Restimulation of enriched neoantigen-specific TIL resulted in superior pro-inflammatory functionality (granzyme B, IFN γ , and TNF α) when compared to non-reactive TIL.

Conclusions TIL from metastatic melanoma patient samples were successfully enriched for neoantigen-reactive TIL, which maintained increased reactivity against these predicted peptides upon restimulation when compared non-reactive TIL. These data support further investigation into the use of neoantigen-enriched TIL products to enhance efficacy of ACT.

Trial Registration NCT01005745, NCT01659151, NCT01701674

Ethics Approval NCT01005745 was approved by USF IRB approval number Ame5_107905. NCT01659151 was approved

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