EXPANSION AND IDENTIFICATION OF NEOANTIGEN REACTIVE TUMOR INFILTRATING LYMPHOCYTES (TIL) FROM METASTATIC COLORECTAL CANCER (CRC)

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Background Previously we have shown that neoantigen specific TIL can be enriched from cryopreserved TIL product from melanoma patients. TCGA data for colorectal cancer (CRC) show a median variant count of 111 but with a subset of patients having much higher mutation frequency. Additionally, patients with higher tumor mutation burden (TMB) have been shown to have improved response to immune checkpoint therapy compared to patients with low TMB. Thus, CRC samples with higher mutational frequency may be an ideal candidate for enrichment of neoantigen specific TIL. The purpose of this study is to expand, identify, and enrich neoantigen reactive TIL from CRC patients.

Methods Patient-derived CRC tissue and PBMC were collected at Moffitt Cancer Center under an Ethics Board approved study (Advarra Pro00043972). TIL was expanded from digested tumor tissue. Whole exome sequencing and RNA sequencing were performed on DNA and RNA extracted from tumor tissue and autologous PBMC. Sequencing and expression data were utilized to identify protein-modifying mutations. Peptides were predicted for their ability to be presented on MHC molecules, prioritized, and synthesized. Neoantigen peptides were loaded onto patient-derived B-cells and co-cultured with autologous TIL. These TIL were then sorted by FACS by upregulation of 4-1BB and OX40 and expanded through the rapid expansion protocol (REP). Enriched TIL were screened for neoantigen reactivity and analyzed by flow cytometry for 4-1BB/OX40 upregulation and cytokine release and degranulation via the ELLA platform.

Results TIL expansion was successfully achieved in 9 of 10 liver metastasis (90%) while only 4 of 10 (40%) samples from the peritoneal cavity expanded TIL. Of the CRC samples that expanded TIL, one patient showed a high mutation frequency with 1710 mutations identified. Restimulation of enriched neoantigen-specific TIL resulted in upregulation of 4-1BB/OX40 from the positive sorted TIL but minimal upregulation from the negative control sorted TIL. This coincided with increased granzyme B, IFNγ, and TNFα in response when compared to their non-reactive TIL counterpart. Of the 196 peptides screened, one peptide corresponding to a known mutation in HDHD3 stimulated 4-1BB/OX40 enriched TIL.

Conclusions TIL from metastatic colorectal cancer patient samples were successfully expanded from multiple disease sites. TIL from these samples can be screened for neoantigens and enriched for neoantigen-reactive TIL. These enriched TIL 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.

Ethics Approval The study was approved by Moffitt Cancer Center's Institutional Review Board, approval number 00043972. Patients gave informed consent before taking part in this protocol.