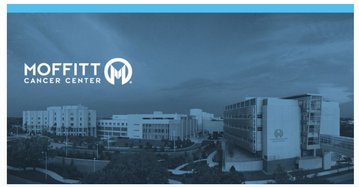

Expansion and identification of neoantigen reactive tumor infiltrating lymphocytes (TIL) from metastatic colorectal cancer (CRC)

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Abstract

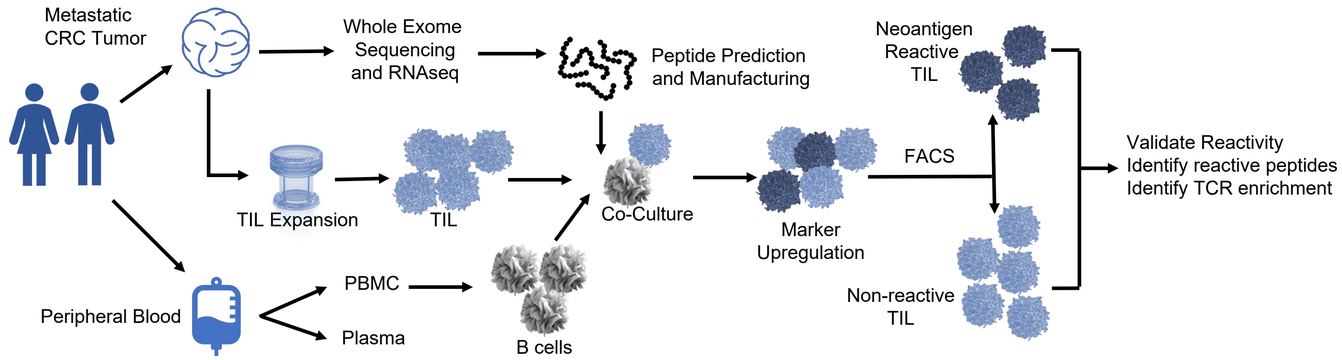
Background
Previously we have shown that neoantigen specific TIL can be enriched from cryopreserved TIL product from melanoma patients. TCGA data for colorectal cancer (CRC) show a median variant count of 111 but with a subset of patients having much higher mutation frequency. Additionally, patients 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 for neoantigen reactive TIL from CRC patients.

Methods
Patient-derived CRC tissue and PBMC were collected at Moffitt Cancer Center under an Ethic's 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.

Background



TIL Expansion

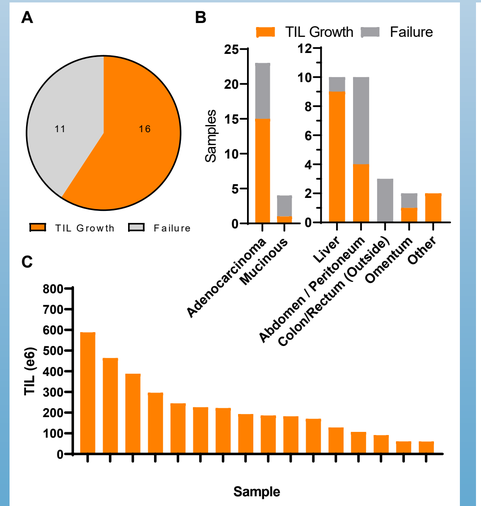


Figure 1: TIL Expansion
Colorectal cancer tumors were enzymatically and mechanically digested, and TIL was expanded in 6000 IU/mL IL-2. A) 16 of 27 CRC samples expanded TIL to levels feasible for downstream enrichment. B) Pathology and tumor location of samples. C) Total TIL expanded following up to 4 weeks of culture.

TIL Selection

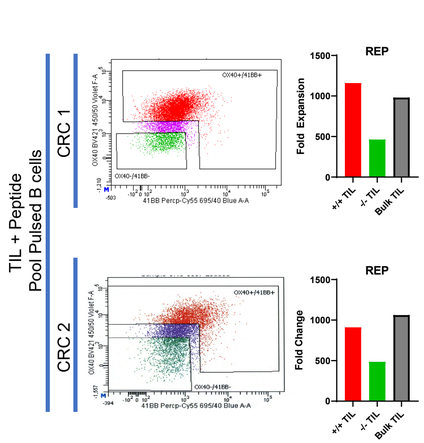


Figure 2: TIL Selection
CRC sample TIL from 2 specimens was co-cultured with autologous B cells pulsed with neoantigen specific 25-mer peptides. TIL was sorted for 4-1BB+ and OX40+ upregulated TIL (+/+). Bulk starting TIL, neoantigen reactive (+/+), and non-reactive (-/-) TIL was expanded by rapid expansion protocol (REP).

TIL Reactivity

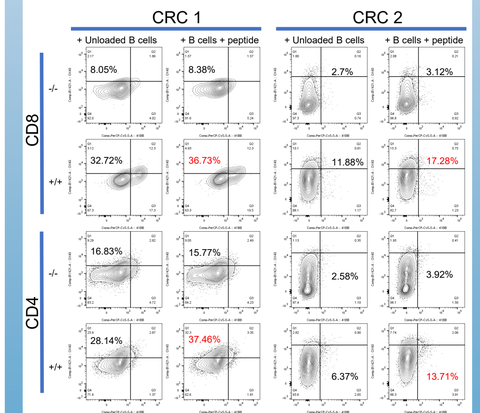


Figure 3: TIL Reactivity
Neoantigen reactive (+/+) and non-reactive (-/-) TIL following REP were cocultured with autologous B cells pulsed with a pool of neoantigen specific 25-mer peptides to verify reactivity of sorted samples. Reactivity was measured by 4-1BB and OX40 upregulation by flow analysis.

TIL Reactivity

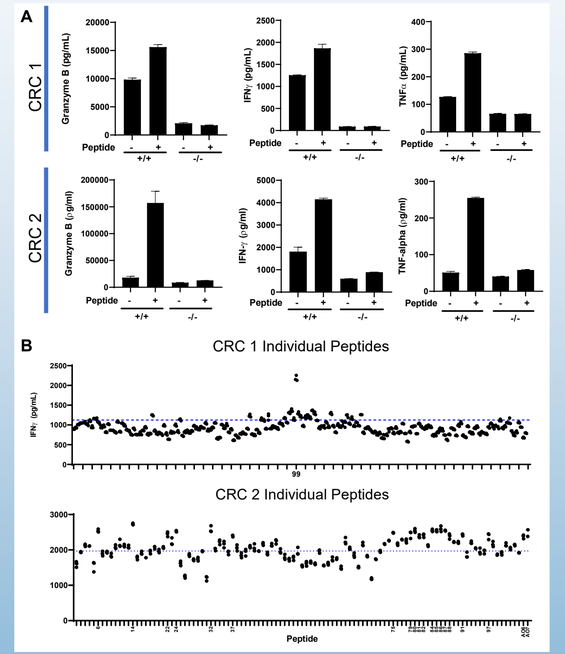


Figure 4: TIL Reactivity
Neoantigen reactive (+/+) and non-reactive (-/-) TIL following REP were cocultured with autologous B cells pulsed with a pool of neoantigen specific 25-mer peptides to verify reactivity of sorted samples. A) Reactivity was measured by upregulation of Granzyme B, IFNγ, and TNFα secretion in supernatant as measured by an ELLA assay. B) Peptides were further screened for individual reactivity by upregulation of IFNγ secretion.

Conclusions

- TIL were successfully expanded from 16 of 27 digested CRC samples.
- Neoantigen reactive TIL can be enriched and expanded from CRC expanded TIL
- Neoantigen reactive TIL maintains reactivity following expansion.

Acknowledgements

Specimens were collected under IRB approved protocols from consented patients.
Funding: This research was supported by a sponsored research agreement with Turnstone Biologics and supported in part by the Flow Cytometry and Genomics Core Facilities at the Moffitt Cancer Center, an NCI designated Comprehensive Cancer Center (P30-CA076292).