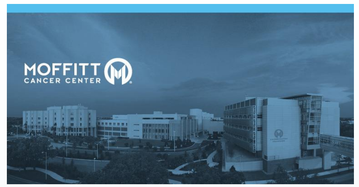


# Expansion and identification of neoantigen reactive tumor infiltrating lymphocytes (TIL) from metastatic colorectal (CRC) and GI cancers.

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## Abstract

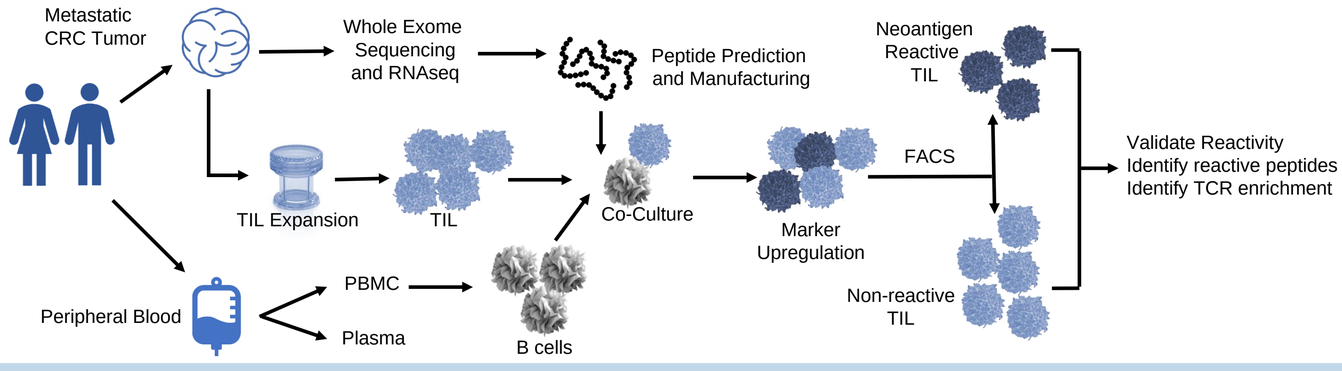
**Background**
Adoptive cell therapy (ACT) with TIL has emerged as a potential treatment of various types of solid tumors. Previously we have shown that neoantigen specific TIL can be enriched from cryopreserved TIL product from melanoma patients. As the TCGA database shows high mutational burdens for colorectal cancer (CRC) and Upper Gastric Cancers (Esophageal and Stomach), these cancers may also be good candidates for enrichment of neoantigen specific TIL. The purpose of this study is to expand, identify, and enrich for neoantigen-reactive TIL from CRC and Gastric cancer patients.

**Methods**
Patient-derived CRC and Gastric Cancer tissue and PBMC were collected at Moffitt Cancer Center under an Ethic's Board approved study (Advarra Pro00043972). Tumor samples were digested to single cell suspensions and cultured for TIL expansion for up to 4 weeks. From DNA and RNA extracted from tumor tissue and autologous PBMC, whole exome sequencing and RNA sequencing were performed. Data was utilized to identify protein-modifying mutations and up to 200 predicted 25mer peptides were synthesized. Neoantigen peptides were pulsed onto patient-derived B-cells and subsequently co-cultured with autologous TIL. TIL were sorted by FACS by upregulation of OX40 and 4-1BB and expanded through the rapid expansion protocol (REP). Neoantigen enriched TIL were analyzed for neoantigen peptide reactivity by flow cytometry for 4-1BB/OX40 upregulation and cytokine release and degranulation via the ELLA platform.

**Results**
TIL expansion was achieved in 64% of CRC samples and 43% of gastric cancer samples. Of those samples, preREP TIL from 6 CRC and 3 gastric cancers were sequenced, co-cultured, and sorted for neoantigen reactive TIL. Upregulation of OX40/4-1BB was seen in 85% (5/6) of CRC and 66% (2/3) GI samples. A subset of these samples showed additional upregulation of Granzyme B, IFNγ, and or TNFα expression. Following sorting of OX40/4-1BB positive TIL and REP, reactivity against pooled neoantigen peptide was validated in 3 of 6 CRC and 1 of 3 GI. Individual peptide screening identified multiple neoantigen peptides driving reactivity in these validated TIL samples.

**Conclusions**
TIL from metastatic colorectal cancer and gastric cancer patient samples were 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 to TIL that did not upregulate OX40 / 4-1BB. These data support further investigation into the use of neoantigen-enriched TIL products to expand the utility of ACT.

## Background




## TIL Expansion

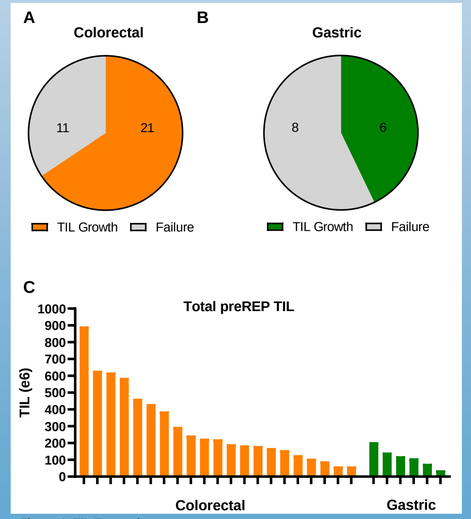
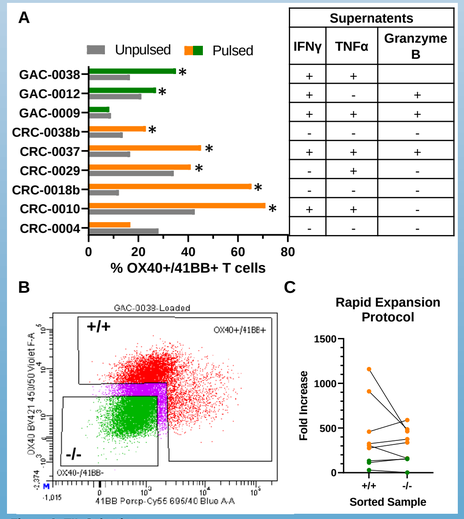



**Figure 1: TIL Expansion**
Colorectal and gastric cancer tumors were enzymatically and mechanically digested, and TIL was expanded in 6000 IU/mL IL-2. (A) 21 of 32 CRC samples and (B) 6 of 14 gastric cancer samples expanded TIL to levels feasible for downstream enrichment. C) Total PreREP TIL expanded following up to 4 weeks of culture ranged from 894e6 to 38e6.

## TIL Selection




| Supernatents | | |
|---|---|---|
| IFNγ | TNFα | Granzyme B |
| + | + | |
| + | - | + |
| + | + | + |
| - | - | - |
| + | + | + |
| - | + | - |
| - | - | - |
| + | + | - |
| - | - | - |

**Figure 2: TIL Selection**
(A) CRC (n=6) and gastric cancer (n=3) TIL were co-cultured with autologous B cells pulsed with neoantigen specific 25-mer peptides. TIL were sorted for 4-1BB+ and OX40+ upregulated TIL (+/+). Supernatants from sorting co-culture were tested for IFNγ, TNFα, and Granzyme B expression (A). Example research-scale gating for sorting of neoantigen peptide reactive (+/+) and non-reactive (-/-) TIL (B). Neoantigen reactive (+/+) and non-reactive (-/-) TIL were expanded by rapid expansion protocol (REP) (C). * samples showing upregulation of OX40 / 4-1BB following co-culture.

## TIL Reactivity

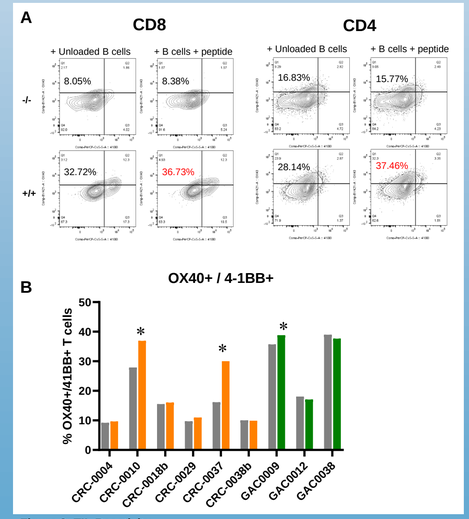



**Figure 3: TIL Reactivity**
Representative sample of neoantigen reactive (+/+) and non-reactive (-/-) TIL following REP that were cocultured with autologous B cells pulsed with a pool of neoantigen specific 25-mer peptides to verify reactivity of sorted samples. Reactivity was measured by 4-1BB and OX40 upregulation by flow analysis (A). Summary of 4-1BB and OX40 upregulation following co-culture against pooled peptides (B). * samples showing upregulation of OX40 / 4-1BB following co-culture.

## TIL Reactivity

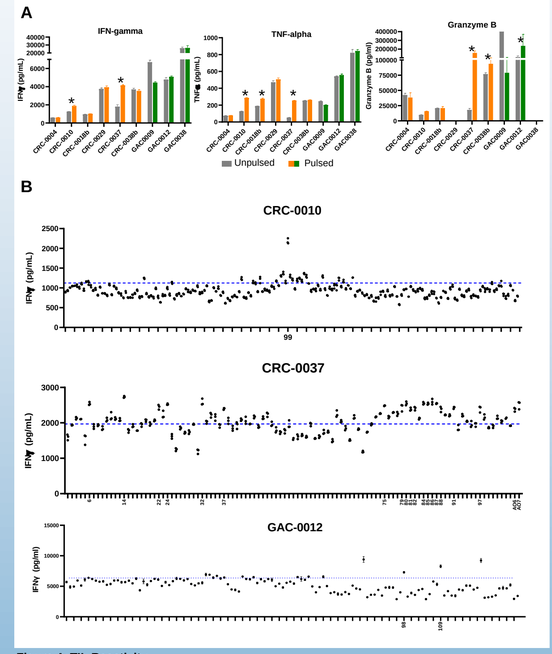



**Figure 4: TIL Reactivity**
Neoantigen reactive (+/+) and non-reactive (-/-) TIL following REP were cocultured with autologous B cells pulsed with a pool of neoantigen specific 25-mer peptides to verify reactivity of sorted samples. Reactivity was measured by upregulation of IFNγ, TNFα and Granzyme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

## Summary

- TIL were successfully expanded from 21 of 33 digested CRC samples and 6 of 8 gastric cancer 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.
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