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 Ethics 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 65% 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% (3/6) of CRC and 66% (2/3) GI samples. A subset of these samples showed additional upregulation of Granzyme B, IFNg, and or TNFa 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.

Acknowledgements This study was supported by a sponsored research agreement with Turnstone Biologics. This work was supported in part by the Tissue Core Facility at the Moffitt Cancer Center, and in part by the Cancer Center Support Grant P30 CA076292 from the National Cancer Institute.