**Background** Tumor infiltrating lymphocyte (TIL) therapy has proved to be most effective in melanoma. Additionally, enhancing tumor reactivity by selective expansion of individual TIL subpopulations, screened for neoantigen reactivity, has demonstrated some success in breast cancer.1 However, while other solid tumors such as colorectal cancer (CRC) have been shown to contain neoantigen reactive TIL,2 the ability to selectively enrich these cells has been challenging. Here we demonstrate that the TIDAL-01 process, which utilizes tumor-specific mutation containing peptides to select neoantigen reactive TIL by fluorescence-activated single cell sorting (FACS), can lead to a neoantigen targeted Selected TIL product in CRC.

**Methods** TIL were expanded from a cryopreserved dissociated CRC tumor sample and antigen presenting cells (APCs) were generated from patient-matched blood. Mutations were identified by sequencing and 13-40 amino acid peptides containing the mutation were generated. TIL were selected by FACS from a coculture of TIL and peptide pulsed APCs based on the activation markers CD134 and CD137. Selected TIL were cultured with a rapid expansion protocol (REP) and the expanded TIL were phenotyped and co-cultured with neoantigen pulsed APCs to confirm reactivity. The unselected TIL was expanded by REP as a bulk control for comparison.

**Results** A clear positive population of activated TIL (3.93%) were selected from the co-culture and expanded >1000 fold. The final TIL product was 93.6% CD4 cells and 4.60% CD8 cells and >90% of CD4 and CD8 cells expressed markers of an effector memory phenotype. Single cell sequencing of the T cell receptor repertoire revealed 12 clonotypes that were enriched in sorted TIL vs bulk TIL. In response to co-culture, 31.2% of CD8 cells and 25.6% of CD4 cells in the selected TIL product were positive for IFN-g by intracellular cytokine staining. IFN-g and TNF-a were 53 and 360-fold higher in the co-culture supernatants of selected TIL compared to bulk TIL, respectively. As a measure of killing potential, 20.9% of CD8 cells degranulated in co-culture based on CD107 expression and granzyme B secretion was increased 16.5-fold over bulk TIL. Deconvolution of the peptide pool identified one CD4 and one CD8 antigen driving the neoantigen reactivity.

**Conclusions** These data provide non-clinical proof of concept that neoantigen enriched TIL can be selected by FACS and expanded into a TIL product that contains a marked increase in neoantigen reactive TIL compared to bulk expanded TIL from a CRC tumor.

**REFERENCES**

**Ethics Approval** All human material was obtained from a commercial source, Discovery Life Sciences, which ensures IRB and ethics committee compliance.