Background
Adoptive cell therapy (ACT) targeting neoantigens can achieve durable clinical responses in patients with advanced solid cancer.1-3 However, many T cells among the tumor infiltrating lymphocytes (TIL) are not specific for tumor antigens.4-6 Additionally, ex vivo expansion of TILs often results in further reduction and differentiation of TILs specific for neoantigens.7 The low frequencies and/or differentiated phenotype of TILs can contribute to ineffective ACT.

Methods
We developed a protocol to selectively expand neoantigen-specific TILs by in vitro stimulation of target neoantigens, termed NeoExpand. As a proof-of-concept we performed NeoExpand on p53 neoantigen-specific TILs. TILs were incubated with antigen presenting cells either electroporated with tandem minigene RNAs encoding multiple p53 neoantigens in 25mers or pulsed with mutant peptides (25mers or minimal epitopes) and cultured in media containing 300-1000 IU interleukin (IL) 2 and 30 ng/mL IL-2 for 14 days.

Results
We retrospectively determined the frequencies of neoantigen-reactive TILs in 10 patient infusion products by CDR3B deep sequencing. 62% (41/66) of neoantigen-reactive clones were <1% in the infusion products. TILs from patient 4196 initially containing ~2% of TILs recognizing p53R175H, one of the “hotspot” p53 mutations, were subjected to NeoExpand and the rapid expansion protocol (REP) with feeder cells, OKT3 and IL-2. Following REP, 0.4% of 4196 T cells were reactive with p53R175H, indicating that REP decreased the frequency of the p53R175H-reactive clones. In contrast, the 4196 TILs following NeoExpand contained 8% TILs reactive with p53R175H, which was a 4-fold increase in the p53R175H-reactive cells relative to the starting population and 20-fold higher than that of REP. While conventional TIL screening identified 3 T-cell receptors (TCRs) R4, 10 NeoExpand enabled identification of 7 TCRs including the 3 TCRs identified by conventional screening. In vivo, 4196 TILs expanded through REP was ineffective in treating established TYK-nu human ovarian cancer cells that naturally expressed p53R175H7 while the 4196 NeoExpand TILs effectively regressed the tumor in NSG mice. Single cell transcriptome analysis demonstrated that NeoExpand led to expansion of central memory/stem-like populations among the mutant p53-reactive clones in the 4196 TILs, while REP led to the depletion of the central memory/stem-like, population. In total, we identified 18 TCRs recognizing various p53 neoantigens, including 7 uniquely identified by NeoExpand from 6 patient samples.

Conclusions
In conclusion, these data indicate that TIL specificity for neoantigens can be preferentially expanded by NeoExpand, enabling effective TCR isolation and treatment of p53-mutant cancer in mice.

Trial Registration
Patient samples and healthy donor peripheral lymphocytes were obtained through the tissue procurement protocol NCT00068003.

REFERENCES