Background Targeting neoantigens by adoptive cell therapy (ACT) can effectively treat advanced solid tumors. However, the conventional rapid expansion protocol (REP) for T-cell expansion can stimulate bystander cells and cause differentiation and exhaustion of T cells. This can lead to inadequate expansion of neoantigen-reactive T cells and hence ineffective ACT.

Methods We developed an in vitro culture method, termed NeoExpand, where T-cell receptor-engineered T cells (TCR-T) or neoantigen-reactive tumor infiltrating lymphocytes (neoTIL) were selectively expanded by neoantigen-specific stimulation. Briefly, T cells were co-cultured with antigen presenting cells or engineered cell lines loaded with neoantigens for ~2 weeks in the presence of interleukins 2 and 21.

Results When NeoExpand was used to expand TCR-T cells expressing previously identified CD8+ TCRs targeting shared p53 or KRAS neoantigens, selective expansion of TCR-expressing CD8+ T cells were observed when compared to REP (1.6 fold, p<0.001, n=8 (TCRs)). Phenotypically, NeoExpand expanded CD39-CD69- cells, reportedly less differentiated T cells with stem-like features, relative to REP (9.9 fold, p<0.001, n=12).

Next NeoExpand’s ability to facilitate neoantigen-reactive TCR isolation was tested. From 25 TIL samples from tumors expressing p53 or KRAS mutations, the conventional screening identified 14 neoTIL clonotypes (i.e., neoantigen-specific TCRs) (3 CD4; 11 CD8), while NeoExpand enabled identification of 42 clonotypes (14 CD4; 28 CD8), indicating neoTIL’s repertoire expansion during NeoExpand.

Next, we examined the effect of NeoExpand on expansion, phenotypes and functions of neoTIL. When TIL samples from patients with p53-mutated or Ras-mutated gastrointestinal or breast cancer were tested, greater expansion of neoTIL with NeoExpand was noted relative to REP (4.0 fold, p=0.02). Single-cell transcriptome analysis revealed expansion of neoTILs with stem-like memory cell phenotypes uniquely in the NeoExpand conditions. These neoTILs expressed stem and memory markers, including CD62L, IL7R, and TCF1 and lacked exhaustion-associated gene expression, including CD39 and TIM3. Finally, TILs expanded through NeoExpand or REP were functionally compared using xenograft mouse models. Three TIL samples, one containing p53RK175H-reactive TILs and two containing KRASG12V-reactive TILs were expanded through NeoExpand or REP and were adoptively transferred to NSG mice engrafted with p53 RK175H+ TYK-nu human ovarian cancer cells or KRASG12V+ patient-derived xenograft cancer cells. TILs expanded through NeoExpand led to significant tumor regression (p<0.001, n=5 mice/group).

Conclusions Collectively, NeoExpand selectively expands neoantigen-reactive T cells compared to REP and enables sensitive identification of neoantigen-reactive TCRs by expanding neoTIL repertoire. NeoExpand's ability to enhance phenotypes and functions of neoantigen-reactive T cells warrants its evaluation for clinical use.

REFERENCES