366 NEOANTIGEN-SPECIFIC STIMULATION OF T CELLS FOR EFFECTIVE CANCER ADOPTIVE CELL THERAPIES

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Background Targeting neoantigens by adoptive cell therapy (ACT) can effectively treat advanced solid tumors.[1–5] However, the conventional rapid expansion protocol (REP) for T-cell expansion can stimulate bystander cells[6–8] and cause differentiation and exhaustion of T cells.[9–11] 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,[4 12] 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,[7] 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[13] identified 14 neoTIL clonotypes (i.e., neoantigen-reactive 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 11 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 $p53^{R175H}$-reactive TILs and two containing $KRAS^{G12V}$-reactive TILs were expanded through NeoExpand or REP and were adoptively transferred to NSG mice engrafted with $p53^{R175H+}$ TYK-nu human ovarian cancer cells or $KRAS^{G12V+}$ 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

1. Tran E, *et al.* T-Cell Transfer Therapy Targeting Mutant KRAS in Cancer. *N Engl J Med* 2016;**375**:2255–2262. https://doi.org:10.1056/NEJMoa1609279
2. Tran E, *et al.* Cancer immunotherapy based on mutation-specific CD4+ T cells in a patient with epithelial cancer. *Science* 2014;**344**:641–645. https://doi.org:10.1126/science.1251102
3. Zacharakis N, *et al.* Immune recognition of somatic mutations leading to complete durable regression in metastatic breast cancer. *Nat Med* 2018;**24**:724–730. https://doi.org:10.1038/s41591–018-0040–8
4. Kim SP, *et al.* Adoptive Cellular Therapy with Autologous Tumor-Infiltrating Lymphocytes and T-cell Receptor-Engineered T Cells Targeting Common p53 Neoantigens in Human Solid Tumors. *Cancer Immunol Res* 2022;**10**:932–946. https://doi.org:10.1158/2326–6066.CIR-22–0040
5. Leidner R, *et al.* Neoantigen T-Cell Receptor Gene Therapy in Pancreatic Cancer. *N Engl J Med* 2022;**386**:2112–2119. https://doi.org:10.1056/NEJMoa2119662
6. Scheper W, *et al.* Low and variable tumor reactivity of the intratumoral TCR repertoire in human cancers. *Nat Med* 2019;**25**:89–94. https://doi.org:10.1038/s41591–018-0266–5
7. Lowery FJ, *et al.* Molecular signatures of antitumor neoantigen-reactive T cells from metastatic human cancers. *Science* 2022;**375**:877–884. https://doi.org:10.1126/science.abl5447
8. Simoni Y, *et al.* Bystander CD8(+) T cells are abundant and phenotypically distinct in human tumour infiltrates. *Nature* 2018;**557**:575–579. https://doi.org:10.1038/s41586–018-0130–2
9. Chacon JA, *et al.* Co-stimulation through 4–1BB/CD137 improves the expansion and function of CD8(+) melanoma tumor-infiltrating lymphocytes for adoptive T-cell therapy. *PLoS One* 2013;**8**:e60031. https://doi.org:10.1371/journal.pone.0060031
10. Hernandez-Chacon JA, *et al.* Costimulation through the CD137/4–1BB pathway protects human melanoma tumor-infiltrating lymphocytes from activation-induced cell death and enhances antitumor effector function. *J Immunother* 2011;**34**:236–250. https://doi.org:10.1097/CJI.0b013e318209e7ec
11. Lak S, *et al.* Combined PD-L1 and TIM3 blockade improves expansion of fit human CD8(+) antigen-specific T cells for adoptive immunotherapy. *Mol Ther Methods Clin Dev* 2022;**27**:230–245. https://doi.org:10.1016/j.omtm.2022.09.016
12. Levin N, *et al.* Identification and Validation of T-cell Receptors Targeting RAS Hotspot Mutations in Human Cancers for Use in Cell-based Immunotherapy. *Clin Cancer Res* 2021;**27**:5084–5095. https://doi.org:10.1158/1078–0432.CCR-21–0849
13. Parkhurst MR, *et al.* Unique Neoantigens Arise from Somatic Mutations in Patients with Gastrointestinal Cancers. *Cancer Discov* 2019;**9**:1022–1035. https://doi.org:10.1158/2159–8290.CD-18–1494

http://dx.doi.org/10.1136/jitc-2023-SITC2023.0366