COMBINED IL-2, AGONISTIC CD3 AND 4-1BB STIMULATION PRESERVE CLONOTYPE HIERARCHY IN PROPAGATED NON-SMALL CELL LUNG CANCER TUMOR-INFILTRATING LYMPHOCYTES

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Background While immune checkpoint blockade is regarded as standard of care for treatment of non-small cell lung cancer (NSCLC), up to 50% of patients with metastatic NSCLC do not achieve an optimal response.¹⁻³ Previous work by our group and others in adoptive cell therapy (ACT) of metastatic melanoma (MM) has shown that infusion of a CD8+ TIL product significantly improved clinical outcomes, yet traditional IL-2 expansion methods have resulted in a predominantly CD4+ NSCLC TIL expansion product.⁷⁻¹² This preclinical study explores the feasibility of producing a tumor-specific, CD8+-enriched NSCLC TIL product for ACT with an improved culture method.

Methods TIL from resected NSCLC tumors were cultured using 1) the traditional method using IL-2 alone in 24-well plates (TIL 1.0) or 2) IL-2 in combination with agonistic antibodies against CD3 and 4-1BB (Urelumab) in a G-Rex flask (TIL 3.0). Expanded TIL were phenotyped using flow cytometry for CD4 and CD8 subset assessment and the CDR3-beta variable region of the T-cell receptor (TCR) involved in antigen binding was sequenced to assess the T-cell repertoire.

Results In a shorter manufacturing time (median of 14 days vs 27.5 days), TIL 3.0 expanded on average 5.3-times more NSCLC TIL (95% CI= 4.3-6.2, $p<0.0001$) and achieved a higher expansion success rate than the traditional TIL 1.0 method (100% vs 62.5%, respectively, $p<0.0001$). Additionally, TIL 3.0 greatly enriched for CD3+CD8+ TIL (81.8% vs 36.9%, $p=0.001$) and expanded a larger breadth of clonotypes ($p=0.039$) which shared greater homology with the total clonotypes found in the repertoire of the resected tumor ($p=0.0007$), and contained a greater fraction of the clones found at high frequency in the tumor ($p<0.00001$). TIL 3.0 also retained a higher proportion of putative tumor-specific TCR when compared to TIL 1.0 ($p=0.0039$), defined based on exclusion of known viral-specific TCR and other TCR found in the paired uninvolved lung tissue.

Conclusions This study reports the feasibility of using the TIL 3.0 methodology to robustly expand a CD8+ T-cell repertoire which maintains the respective clonal hierarchy in NSCLC tumors and enriches for putative tumor-specific TIL clones. The robustness and speed of the new process may facilitate testing and implementing effective TIL ACT in NSCLC.

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Ethics Approval This study was performed on NSCLC tumor tissue resected from 16 patients enrolled, following informed consent, in the Immunogenomic prOfiling of early-stage NSCLC (ICON) project. This study was approved by the University of Texas MD Anderson Cancer Center's Institutional Review Board (protocol number PA15-1112_MODCR001).

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