

665

## TRANSCRIPTIONAL LANDSCAPE OF TUMOR-REACTIVE TIL IN LUNG CANCER AND MELANOMA

Jiajia Zhang\*, Justina Caushi, Boyang Zhang, Zhicheng Ji, Taibo Li, Hongkai Ji, Andrew Pardoll, Kellie Smith. *Johns Hopkins University, Baltimore, MD, United States*

**Background** Melanoma and lung cancers have two of the highest response rates to immune checkpoint inhibitors (ICIs).<sup>1</sup> However, patients may respond unpredictably, partly due to heterogeneity in the quantity and quality of tumor-specific T cells. In this study, we performed an integrated transcriptomic analysis of anti-tumor CD8+ TIL from non-small cell lung cancer (NSCLC) and melanoma. Our goal was to study the global transcriptomic landscape of tumor-specific T cells and to compare their functional programming in lung cancer vs. melanoma.

**Methods** TIL from 19 patients (15 NSCLC and 3 melanoma) were sequenced using combined single-cell (sc) RNA-seq/TCR-seq. All NSCLC patients received neoadjuvant anti-PD-1 (nivolumab, NCT02259621) whereas melanoma patients received a personal neoantigen vaccine (NCT01970358). Neoantigen-, tumor-associated antigen-, and viral-specific CD8+ T cell clonotypes were identified using functional assays and were validated by TCR cloning as previously described.<sup>2 3</sup> Transcriptional profiles of antigen-specific T cells were identified using the TCRβ CDR3 as a barcode to link with the antigen specificity output from the functional assays. The prevalence, phenotype, and differentiation trajectory of tumor-specific T cells were compared between the two cancer types.

**Results** A total of 175,826 CD8+ TIL were analyzed, of which 30,174 single cells were from the melanoma cohort and 145,652 were from the NSCLC cohort. Tumor-specific T cells were detected at variable frequencies among CD8+ TIL (median=1.2%, range 0.01%–35.8%) across nine patients, with melanoma having more clonal tumor-specific T cells as compared to NSCLC. CD8+ TIL from melanoma were more enriched in an activated tissue resident T cell (TRM) cluster characterized by upregulated expression of CXCL13, CRTAM, 4-1BB, XCL1/2, and FABP5, whereas those from NSCLC have a greater representation of a cytotoxic TRM cluster with an exhaustion signature (coexpression of GZMB, GZMH, PDCD1, and CTLA4). Distinct from EBV-specific T cells and flu-specific T cells, tumor-specific T cells primarily resided in TRM clusters in both cancers. More MANA-specific TIL from melanoma presented with an effector phenotype and were more proliferative as compared to those from NSCLC. To reveal the differentiation trajectory and regulatory programs of tumor-specific T cells upon tumor recognition and association with response to ICIs, pseudotime/velocity analysis of tumor-specific TIL is underway.

**Conclusions** This is the first analysis to inform on the global transcriptomic landscape of tumor-specific CD8+ TIL in lung cancer and melanoma at single cell resolution. This provides a useful framework to study the underlying mechanisms of T cell exhaustion and dysfunction in human cancer.

**Trial Registration** NCT02259621, NCT01970358

### REFERENCES

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**Ethics Approval** The melanoma clinical trial was approved by the Dana-Farber/Harvard Cancer Center Institutional Review Board (IRB) (NCT01970358). The NSCLC clinical trial was approved by the Institutional Review Boards (IRB) at Johns Hopkins University (JHU) and Memorial Sloan Kettering Cancer Center (NCT02259621). All participants gave informed consent before taking part.

**Consent** Written informed consent was obtained from the patient for publication of this abstract and any accompanying images. A copy of the written consent is available for review by the Editor of this journal.

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