Clinical Trials Completed

538 COMPREHENSIVE SINGLE CELL TRANSCRIPTOMIC PROFILING OF UNTREATED RESECTABLE LUNG CANCERS

Sydney Connor*, Jiayia Zhang, Justina Caushi, Boyang Zhang, Zhen Zeng, Khaled Sanber, Gavvin Pereira, Valsamo Anagnostou, Ada Tam, Nicholas Ionita, Franck Housseau, Patrick Forde, Hongkai Ji, Andrew Pandoli, Kellie Smith. Johns Hopkins School of Medicine, Baltimore, MD, United States

Background Non-small cell lung cancer (NSCLC) is the leading cause of cancer-related deaths in men and women. The 5-year overall survival rate of metastatic NSCLC is an abysmal 21%, in part since >80% of lung cancers are diagnosed at an advanced stage.1,2 Encouragingly, neoadjuvant immune checkpoint blockade (ICB) plus chemotherapy is now approved as standard of care for resectable lung cancer.3,4 Because ICB success depends on an endogenous anti-tumor T cell response, it is critical that we understand the baseline functional biology of these T cells. To date, however, comprehensive studies of endogenous tumor-reactive TIL in operable NSCLC (Stage I-III) are lacking. Herein we present an integrated single cell immunogenomic profiling of 21 untreated tumor resections from patients who were surveilled for 1-5 years post-surgery.

Methods After acquiring written informed consent, PBMC and resected tissues were obtained from patients with Stage I-III NSCLC undergoing surgical resection. Tissues were enzymatically digested and viably frozen. Cryobanked tissues were thawed, T cells (CD3+CD45+) and non-T cells (CD3-) were sorted, and prepared for single cell RNA sequencing. Single Cell 5′ V(D)J and 5′ DGE kits (10X Genomics) were used to capture immune repertoire and gene expression information for the T cell fraction, DGE libraries were prepared for the non-T cell fraction.

Results Transcriptomic profiles were defined for CD3+ TIL from 21 untreated surgically-resected tumors (527,062 cells). Refined UMAP projection of CD8+ TIL (183,375 cells) uncovered 14 CD8+ T cell subsets. We observed four distinct tissue resident memory (TRM) clusters. This is notable, as our previous work defined the TRM subsets as being enriched in tumor-reactive TIL.5,6,8 Apropos of this notion, two of these TRM clusters were significantly enriched in the tumor tissue compared to adjacent normal lung and lymph node (adj.p-value=1.3x10^-4 and 3.6x10^-3), with one of these TRM clusters expressing high levels of markers previously shown to mark tumor-reactive TIL, including HOBIT, CXCL13, and CD39 [5,7,8]. Further supporting our hypothesis that this cluster harbors tumor-reactive TIL, cross-reference with public TCR databases showed almost no overlap of the TCRs in this cluster with TCRs corresponding to virus-specific T cells, whereas 726 EBV- and 177 flu-specific TIL were readily detected within other TRM and T Effector clusters.

Conclusions This study is one of the first to evaluate the transcriptional programming of tumor-reactive TIL in treatment-naïve lung cancers. Understanding the baseline functional biology of these cells has significant implications for biomarker and novel therapy development for the treatment of lung cancers resistant to currently-approved therapies.

Trial Registration For the ICB treated NSCLC patients enrolled under NA_00092076 at JHU (NCT02259621), samples have already been collected, stored, and published.