Background Lung cancer is the most common cause of cancer-related death and approximately 85% is non-small cell lung cancer (NSCLC). Although first-line immune checkpoint inhibitors (ICIs) exhibit remarkable clinical benefits, most patients never respond or fail to respond long-term. In order to better utilize ICI, we aim to determine which immune cell populations are contributing to ICI resistance with a focus on tumor-associated neutrophils (TANs) as they have an important role in modulating immune response in the tumor microenvironment. Here, we performed in vivo studies to investigate TANs that potentially contribute to resistance to anti-PD1 immunotherapy.

Methods 6~8-week-old 129/Sv mice were implanted with 1x10⁶ KRAS/p53 (KP) mutant murine lung tumor cells subcutaneously. Starting at week 1, tumors were measured and treated with anti-PD1 antibody (2mg/ml) or IgG control (2mg/ml) i.p. weekly. Tumors were then collected at weeks 5 (T1) and 7 (T2). Tumor samples were processed and stained for multiparameter flow cytometry to analyze immune cell populations. Fixed cells were acquired on a Cytek Aurora flow cytometer. Tumors from T1 and T2 were also submitted for single-cell RNA sequencing (scRNA-seq).

Results Anti-PD1 treatment reduced tumor size at the early stage of the treatment (T1); however, tumors treated with anti-PD-1 rapidly acquired resistance, with no significant difference between treatment groups by T2. With FACS analysis, we found that the CD11b⁺Ly6G⁺ neutrophils were significantly upregulated in anti-PD1 treated tumors at T2, compared to that of T1, supporting the role of these cells in contributing to acquired resistance. The abundance of TANs was consistent with tumor single-cell RNA sequencing data. Differentially expressed gene (DEG) analysis from the scRNA-seq data demonstrated elevated expression of S100a8/a9 in the TAN cluster from the T2-resistant tumor compared to T1 sensitive tumor. These data were confirmed by RT-qPCR. Together, these data are supportive of the involvement of TANs in contributing to acquired resistance to ICI, and ongoing efforts will focus on further investigating TAN subsets and the mechanism(s) in which they promote resistance to ICI.

Conclusions Anti-PD1 immunotherapy treatment in the NSCLC KP syngeneic model showed sensitivity at T1 with resistance developing by T2. We have confirmed that neutrophil cells are significantly increased within tumors that are resistant to immunotherapy via scRNA-seq and flow cytometry analyses. Understanding the mechanism by which TANs contribute to the ICI resistance will provide evidence for the development of TAN-targeting reagents that potentially synergize with immunotherapy for NSCLC patients.

http://dx.doi.org/10.1136/jitc-2023-SITC2023.0552