Background PD-1/PD-L1 pathway blockade has improved survival in patients with advanced NSCLC. Neoadjuvant (pre-operative) anti-PD-1 plus chemotherapy was also recently approved for patients with resectable stage II/III NSCLC. However, among patients receiving neoadjuvant anti-PD-1-based therapy, only 33–45% achieved a major pathologic response (MPR, ≤10% of residual viable tumor), highlighting the need for biomarkers predicting response. Based upon recent results in advanced melanoma showing that CD8+FoxP3+ cells were strongly associated with therapeutic response, we hypothesized that these cells would also be predictive of response in resectable NSCLC. Additionally, we performed single-cell RNA sequencing (scRNAseq) to define the phenotype of CD8+FoxP3+ cells, given reports suggesting an immunosuppressive role.

Methods Pre-treatment formalin-fixed paraffin-embedded tumor specimens from the first-in-human clinical trial of neoadjuvant anti-PD-1 (nivolumab) +/- anti-CTLA-4 (ipilimumab) in NSCLC (NCT02259621) were stained with a 6-marker multiplex immunofluorescence mIF panel (PD-1, PD-L1, CD8, CD163, FoxP3, and cytokeratin). Eight specimens were from patients demonstrating MPRs and 17 were from patients with non-MPRs. The densities of immune cell populations within the tumor microenvironment (TME) were analyzed using the AstroPath platform and the area under the receiver operating characteristic curve (AUC) for each possible cell phenotype was calculated for predicting MPR. The association for cell phenotypes with event-free survival (EFS) and overall survival (OS) was determined using the log-rank test. scRNAseq analyses were performed on freshly collected CD+ TIL from 15 of the same NSCLC patients. T-cells were clustered by UMAP and were queried for co-expression of CD8 and FoxP3.

Results The density of CD8+FoxP3+ T-cells was significantly elevated in patients achieving MPR (AUC=0.78, p=0.014, N=25). This association was strongest in the PD-1(+) (AUC=0.83, p=0.004) and PD-L1(+) (AUC=0.81, p=0.007) subsets. The AUCs for CD8+FoxP3+ cells were stronger than any other cell phenotype labeled by this 6-plex mIF assay. Patients whose TMEs contained CD8+FoxP3+ cells (n=18) when compared to those lacking this phenotype (n=7) had improved EFS and OS (41 vs. 8 months, p=0.041; and 26 vs. 8 months, p=0.074, respectively). scRNAseq studies of the CD8+FoxP3+ T-cell subset revealed a transcriptome compatible with a highly-activated, cytotoxic phenotype (CCL5, CD8A, GZMB, NKG7, CTSW, CD8B, LINC02446, GZMK) highly expressed).

Conclusions CD8+FoxP3+ T-cells in the NSCLC TME do not represent immunosuppressive cells, as has been previously reported, but instead represent highly-potent early, effector T-cells. When detected by mIF in pre-treatment NSCLC tumor specimens, these cells associate with major pathologic response and improved survival outcomes following neoadjuvant anti-PD-1.

REFERENCES

Ethics Approval This study was conducted in accordance with the Declaration of Helsinki and was performed following Johns Hopkins University IRB approval (#NA 0008595). This protocol allows for the retrieval of tissue from archives from patients who signed an informed written consent or with waiver of consent.