Background Lymph nodes often serve as the first site of metastasis in solid tumors despite being specialized peripheral immune organs. Evaluating tumor-immune interactions within distinct architectural regions of the lymph node has the potential to inform the clinical heterogeneity observed in metastatic cancer response to immunotherapy. Here, we applied multiplexed immunofluorescence (MxIF) to melanoma lymph node (LN) tissue specimens to quantify tumor-immune interactions contributing to recurrence risk in patients receiving anti-PD1 immunotherapy.

Methods Whole, excisional lymph node biopsies were obtained from 33 treatment-naïve patients with metastatic melanoma who underwent subsequent anti-PD1 therapy. A single 5μm formalin-fixed paraffin embedded LN tissue section was used to assess a panel of 45 analytes by cyclic MxIF. Fields of view (~1mm²) were selected from pathologist-annotated regions of the tumor core (n=447), tumor-immune interface (n=298) and adjacent lymphoid tissue (n=339). Pixel-based single cell segmentation and a supervised classifier approach was applied to resolve 10 distinct tumor, stromal and immune cell phenotypes and functional states (e.g. PD1, PDL1, LAG3) in 5.5 million cells.

Results Stratification based on responsiveness to anti-PD1 therapy resulted in 15 patients experiencing melanoma recurrence by 12 month follow-up. Single cell quantification of the tumor cell fraction was reflective of the pathologist-annotated histology (tumor: 69.7 ± 21.7%, interface: 20.9 ± 13.0%, lymphoid: 0.7 ± 2.5%). Similarly, the distribution of T cell subsets (cytotoxic T, T helper, Tregs) followed conventional histology patterns (tumor 5.3 ± 7.0%, interface: 32.0 ± 16.2%, lymphoid: 51.6 ± 20.9%). Within tumor regions, B and T cell counts displayed a high concordance with pathologist TIL scores as calculated by ANOVA (p-value=0.012). Functional expression of PD1 on T cells was observed in all histologies (tumor: 15.1%, interface: 8.9%, lymphoid: 2.8%). Notably, the percentage of PD1+ T cells was significantly higher in the interface histology of patients that did not experience recurrence (11.5% vs 5.8%) and lymphoid tissue (4.5% vs 1.4%) and lower in the tumor core (14.3% vs 17.7%) suggesting distinctive spatial localization patterns for PD1+ T cells correlate with clinical outcomes (p<0.001). Ongoing analyses will evaluate the diverse cellular interactions across these histologies to determine unique spatial signatures that correlate with recurrence.

Conclusions Spatial distribution of PD1+ T cells is regionally enriched at the tumor-immune interface among patients that did not experience recurrence following anti-PD1 therapy. The metastatic lymph node represents an ideal tissue landscape to apply cyclic MxIF and study tumor-immune cellular interactions that inform recurrence risk following immunotherapy.

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