Background Visualizing and profiling cells within the tumor microenvironment (TME) is an important component of translational studies, particularly with the advent of immunotherapies. Successful optimization and utilization of multiplex immunofluorescence (mIF) allows researchers to identify cell populations and interactions involved in cancer biology. In separate publications, we describe novel immunophenotypes in the peripheral blood or tumors of patients with metastatic melanoma receiving immunotherapy. These populations include ectoenzyme expressing T cells (CD38 and CD39), and melanoma cells that express CD83, a marker for dendritic cells. The aim of this current study is to create an mIF panel capable of interrogating the TME of melanoma samples for these phenotypes.

Methods Using the Vectra Polaris platform, a nine-parameter immunofluorescence panel was designed and optimized to detect distinct cell phenotypes in paraffin tissues (CD3, CD4, CD8, SOX10/S100, Ki67, CD38, CD39, and CD83). We implemented this panel to identify unique population(s) of cells within FFPE specimens from patients with melanoma undergoing anti-PD1 immunotherapy. Thirty-four samples from 23 patients were analyzed (including 11 responders and 12 non-responders). Based on specimen availability, we assessed a variety of tissue types collected across anatomic location and/or time points (ie primary or metastatic lesion; before or on-treatment). InForm and phenostr software was used to discern tumor versus stroma compartments and distinguish phenotypes.

Results We detected the previously described immunophenotypes (CD3+/CD4+/CD38+/CD39+, CD3+/CD8+/CD38+/CD39+, and SOX10/S100+/CD83+) in all 34 specimens. After cell segmentation, the frequency of CD3+/CD4+/CD38+/CD39+ cells were higher in the tumor compartment compared to stroma (p=0.0045). The frequency of CD3+/CD4+/CD38+/CD39+ cells was positively correlated with the frequency of Tregs in the tumor compartment of patients that were non-responsive to immunotherapy (no statistical significance; R²=0.16, p=0.2). We also identified CD83-expressing T cells (CD3+/CD8+/CD38+ and CD3+/CD4+/CD83+) and their frequencies were positively correlated in the stroma compartment of patients that responded favorably to immunotherapy (R²=0.98, p<0.000001). Within the stroma compartment of the same patient cohort, similar increases in frequencies are seen with SOX10/S100+/CD83+ cells and the two CD83-expressing T cells phenotypes.

Conclusions We designed an mIF panel that identifies novel immunophenotypes and validated these populations in the TME of patients with metastatic melanoma. Notably, we detected populations that express CD83, a molecule often overlooked in solid tumors. Here we show CD83 expression on melanoma cells and T cells, and their presence are associated with response to anti-PD1 therapy.

REFERENCES