

1466

## NOVEL CELL PHENOTYPES CORRELATED WITH CHECKPOINT IMMUNOTHERAPY RESPONSES IDENTIFIED IN THE TUMOR AND IMMUNE MICROENVIRONMENT OF PATIENTS WITH METASTATIC MELANOMA

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**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.<sup>1, 2</sup> These populations include ectoenzyme expressing Tcells (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, SOX10S100, 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 phenoptr 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 SOX10S100+/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;  $R^2=0.16$ ,  $p=0.2$ ). We also identified CD83-expressing Tcells (CD3+/CD8+/CD83+ and CD3+/CD4+/CD83+) and their frequencies were positively correlated in the stroma compartment of patients that responded favorably to immunotherapy ( $R^2=0.98$ ,  $p<0.000001$ ). Within the stroma compartment of the same patient cohort, similar increases in frequencies are seen with SOX10S100+/CD83+ cells and the two CD83-expressing Tcells 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 Tcells, and their presence are associated with response to anti-PD1 therapy.

### REFERENCES

1. Woods D, et al. Nivolumab and ipilimumab are associated with distinct immune landscape changes and response-associated immunophenotypes. *JCI Insight*. 2020;5(11):e137066.

2. Amato C, et al. Pre-Treatment Mutational and Transcriptomic Landscape of Responding Metastatic Melanoma Patients to Anti-PD1 Immunotherapy Cancers (Basel). 2020 Jul 17;12(7):1943.

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