124 LOCATION, LOCATION, LOCATION: SPATIAL ANALYSIS OF ULTRAHIGH-PLEX IMMUNOFLUORESCENCE PANEL IN HEAD AND NECK CANCER

[1]Quyen Vu*, [2]Niyati Jhaveri, [2]Aditya Pratapa, [4]Christopher Paustian, [4]Tarsem Moudgil, [3]Venkatesh Rajamanickam, [4]Noah Simons, [2]Oliver Braubach, [4]Rom S Leidner, [4]Bernard A Fox, [4]Shawn M Jensen. *[1]Providence Cancer Center, Portland, OR, USA; [2]Akoya Biosciences, Menlo Park, CA, USA; [3]Providence Health, Portland, OR, USA; [4]Earle A. Chiles Research Institute, Providence Cancer Institute, Portland, OR, USA*

Background By integrating genomics, transcriptomics, proteomics, and metabolomics, researchers have gained unprecedented insights into the complex interplay between tumors and the immune system. This wealth of data has fueled the discovery of novel biomarkers for early cancer detection, prognostic stratification, and prediction of treatment response. Ultrahigh-plex multispectral immunohistochemistry enables the ability to analyze a large set of biomarkers simultaneously while maintaining the context of tissue architecture. By obtaining spatial information, researchers can understand the contact interactions and proximity between different cell types, leading to a deeper understanding of cellular dynamics and disease progression. This approach enables the identification of complex cellular patterns, such as immune cell infiltration, cell-cell interactions, or tumor heterogeneity. Ultimately, these findings may contribute to the development of more effective therapies and diagnostics for cancer patients.

Methods We performed whole-slide spatial phenotyping with an ultrahigh-plex PhenoCycler-Fusion panel on FFPE human head and neck tumor biopsies (pre-treatment and post-treatment) from a patient receiving immunotherapy. Enumeration of cell phenotypes and spatial location analysis was performed using QuPath,[1] R, and proprietary software.

Results Within the invasive margin of the tumor (+/- 20mm of the tumor edge) we measured baseline densities of $CD8^+$ T cells (264 cells/mm^2) and $CD4^+$ T cells (458 cells/mm^2) at the pretreatment timepoint, with the densities substantially increased following treatment (1042 cells/mm^2 CD8; 652 cells/mm^2 CD4). The $CD8^+$ T cells present within the invasive margin also displayed functionality as evidenced by their expression of GranzymeB (109 vs 397 cells/mm^2, pre vs post). In addition, differential enrichment was observed within the invasive margin compared to the entire biopsy (41% vs 58%, pre vs post). In conjunction tumor cells at the post-treatment timepoint showed higher expression of the pro-apoptotic BAX protein (34% vs 58%, pre vs post) with reduced expression of the anti-apoptotic Bcl-2 protein (32% vs 12%, pre vs post).

Conclusions Data from this ultrahigh-plex multispectral panel has provided a number of potential biomarkers for investigation in patients with head and neck cancer receiving immunotherapy. These findings inform development of smaller, more efficient multispectral panels to be designed and run on larger cohorts of patients. Additionally, examining cell populations residing within specific spatial tumor microenvironments (ie., invasive margin) uncovers additional metrics that should be examined in association with a patient's response to treatment.

Acknowledgements Support: Providence Medical Foundation, The Chiles Foundation, The Harder Family, Nancy Lematta, Robert W. Franz and Elsie Franz Finley, The Murdoch Memorial Trust

REFERENCE

1. Bankhead P, *et al.* QuPath: Open source software for digital pathology image analysis. *Scientific Reports* 2017.

Ethics Approval PSJH IRB# 06–108

http://dx.doi.org/10.1136/jitc-2023-SITC2023.0124