Background Colorectal carcinoma (CRC) represents a major worldwide health burden and shows an increasing incidence particularly in younger patients. The majority of metastatic CRC (microsatellite stable, MSS) do not respond to current immunotherapies in contrast to the small subset of microsatellite unstable (MSI) patients. However, responses to immunotherapy were recently observed in subsets of primary MSS tumors indicating their potential vulnerability to such therapeutic approaches. Here, we use multiplex imaging coupled with powerful image analysis tools to highlight important phenotypic differences between MSI and MSS.

Methods 100 human CRC sections (30 MSI, 70 MSS) were examined on the COMET™ platform (Lunaphore) with a multiplex sequential immunofluorescence (seqIF) panel of 12 biomarkers (CD3, CD31, CD4, CD8, FoxP3, TCF-1, TOX, EOMES, CK, CD45RO, S100, D2-40).

Images were preprocessed by background subtraction and local contrast enhancement. Cell segmentation was done with the Stardist algorithm. Cell phenotypes (CT) were defined by threshold-based classifiers. The epithelium was detected based on CK expression. The density of CT was assessed per area of interest. Cell neighborhoods (CN) were defined as the composition of the 10 closest CTS within 300um. CNs were clustered into CN-classes using DB-SCAN.

Results Cell segmentation and phenotyping algorithms resulted in precise cell detection (figure 1). We compared CT infiltration patterns in MSI vs MSS in the tumor-epithelium and epithelium-stroma interfaces. While we observed no significant differences at the interface, CD3 cell density was significantly higher in MSI vs MSS patients in the tumor-epithelium (figure 2), with MSS more heterogeneously distributed with CD3-high and CD3-low outliers.

Differences in cell interactions CRC patients were examined using CN analysis. We observed a CN composed of CD3, CD8 cytotoxic T cells, CD3+CD8+ and tumor cells to be differentially enriched between MSI and MSS patients (figure 3). A more precise characterization of the immune response to cancers is essential to leverage the advantages of immune modulations in the treatment of cancers. We confirm here important differences between MSI and MSS CRCs in part systematic due to the higher antigen load in MSI CRCs but also highlight heterogeneity amongst the MSS tumors.

Characterization of the phenotype of the T-lymphocyte population and its localization with respect to elements such as epithelial cells and tertiary lymphoid structures may help to define both the prognosis of tumors and the possibility of a response to immune checkpoint therapy.

REFERENCES