QUANTITATIVE EVALUATION OF THE TISSUE MICRO-ENVIRONMENT BY HIGH-RESOLUTION 17-PLEX IMMUNOFLOUORESCENCE REVEALS DISTINCT CELL POPULATIONS

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Background Inflammatory tumor micro-environments contain cells of various types and sub-types. The composition and spatial location of the cell populations reflects the host reaction to the inflammatory stimulus and increasingly is understood to influence responsiveness to tumor immunotherapies. Multiplexed imaging technologies allow identification of cell types and states within the spatial context of tissue architecture. We present here a prototype workflow that combines rapid high-resolution, whole-slide highly multiplexed immunofluorescence imaging with advanced image analysis tools for 1) segmenting tissues, cells, and quantifying cellular phenotypes based on multiple markers and 2) determining regional densities and proximity between cells. We apply the workflow to comparative assessment of three lymphoid tissues: tonsil (follicular hyperplasia); lymph node (quiescence); lymphoma (architectural effacement).

Methods Formalin-fixed, paraffin-embedded 5 micron sections of tonsil, lymph node and chronic lymphocytic leukemia/small lymphocytic lymphoma were deparaffinized, subjected to alkaline pH epitope retrieval, and then manually stained with a 17-plex panel including CD45 (leukocytes); CD20 (B cells); CD3d, CD4, CD8 (T cells); FOXP3 (T reg cells); CD68, CD163 (macrophages); CD45RO (activated cells); PD-L1, PD-1 (checkpoint markers); CD31 (vascular and lymphatic endothelial cells); cytokeratin, E-cadherin (epithelial cells); PCNA, Ki-67 (proliferating cells); and a nuclear dye. Stained slides were coverslipped and imaged on the Orion Instrument (RareCyte) generating .ome tiff image files. The HighPlex FL module of the HALO image analysis platform from Indica Labs with embedded HALO AI performed nuclear and cell segmentation, nuclear phenotyping, and user-defined thresholds were applied to each of the biomarkers to define positivity for the appropriate subcellular localization (nuclear, cytoplasmic, and/or membrane) for phenotypic analysis. H & E images from either the same or serial sections were integrated with the multiplex images using the HALO Serial Stain module.

Results Regional masks that were defined by predominance of B-cells (CD20) or T-cells (CD3d) matched known lymphoid micro-anatomy of follicles and inter-follicular cortex respectively. Within the regions, populations and sub-populations of B-cells, T-cells, macrophages and vessels were measured, and their densities calculated and compared between tissues. Rare cell types of potential importance in immuno-oncology were investigated. The results demonstrate differences between the tissues at a phenotypic level that correspond to the morphologic differences seen by light microscopy.

Conclusions Orion imaging combined with HALO image analysis provides a powerful and intuitive workflow for visualization and quantification of distinct microenvironment populations for use in translational and clinical research.

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