93 ORION™: 15-PLEX SINGLE-STEP STAIN AND IMAGING OF TONSIL TISSUE WITH REACTIVE LYMPHOID HYPERPLASIA

Eric Kaldjian, Selena Larkin*, Tad George. *RarCyte, Inc., Seattle, WA, USA*

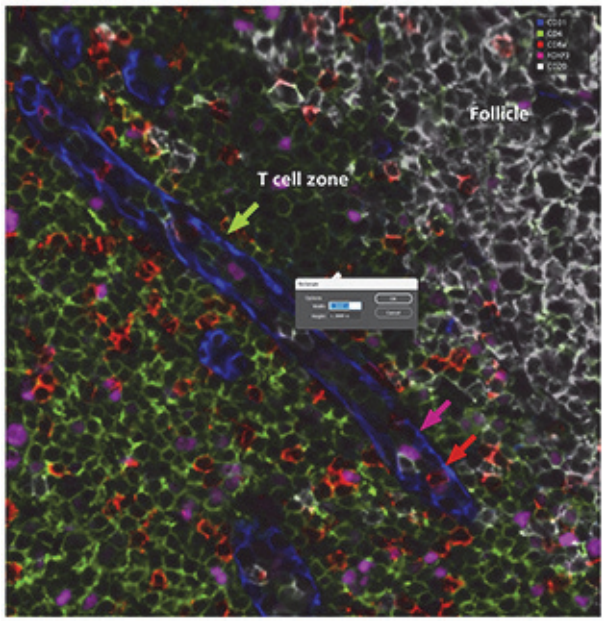
Background In order to understand the subcellular nature of the tumor microenvironment (TME), high quality imaging at single-cell resolution is needed as a basis for downstream biomarker quantitation and predicting patient outcomes. Here we investigate a sample of tonsil tissue with reactive lymphoid hyperplasia using whole slide, single-step stain and imaging at single-cell resolution, and same section immunofluorescence (IF) and H&E.

Methods These images profiled a whole slide tissue section of a tonsil with reactive lymphoid hyperplasia, stained with a 15-plex immuno-oncology biomarker panel. In this profile, we designed a high-plex panel of 15 biomarkers where the tissue autofluorescence was imaged and isolated as an additional fluorescence channel. Whole slide spatial staining and imaging was conducted on the Orion spatial biology platform, and H&E staining was performed after IF imaging on the same section and imaged by brightfield microscopy. The full protocol is fairly quick and simple, using standard histology tools:

- Mount sections on glass slides
- De-paraffinize and perform antigen retrieval
- Quench autofluorescence
- Stain slides with a panel of ArgoFluor™ conjugated antibodies
- Coverslip with ArgoFluor Mounting Medium and cure overnight
- Image whole slides at 20X magnification using the Orion instrument
- Process to ome.TIFF and analyze
- De-coverslip in aqueous solution
- Perform H&E staining and scanning on same section

Results Multiplexed imaging revealed a follicle showing a highly proliferative germinal center with a surrounding mantle zone, and T cell zone (figure 1), each with varying expressions of PD-L1. PD-L1 and PD-1 are cognate immune checkpoint receptors involved in immune system tumor evasion, and their expression on tumor cells, and within the TME, impacts checkpoint inhibitor therapy response. Imaging uncovered that in this follicle, germinal center macrophages express CD68 alone, where just outside the follicle in the T cell zone, macrophages co-express CD163 and CD68.

Conclusions A benefit of Orion collecting all markers in a single scan is that localization of individual markers may be robustly interrogated at subcellular resolution as singletons or in combination. Here multiplexed imaging by Orion identified distinct cell proliferation seen in the follicle, PD-L1 expression, and macrophage subsets which may be mapped spatially to their location within a tissue; thus, confirming the biological differences in tissue composition and cellular interactions between these tissues and provides crucial information for future spatial quantitation. The morphology of cells and spatial location with biomarker phenotype may be correlated by staining the same tissue section with multiplexed IF and H&E, an additional benefit of Orion.



Abstract 93 Figure 1 Longitudinal section of the HEV lumen showing CD4 (green arrow) T cells, FoxP3 (magenta arrow) T regulatory cells, CD8 (red arrow) T cells, and CD20 (white arrow) B cells.

http://dx.doi.org/10.1136/jitc-2023-SITC2023.0093