AACR 2022 Abstract#627

Custom PhenoImager™ fluorescent multiplex IHC panel identifies mature tertiary lymphoid structures in colorectal cancer

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Background: Tertiary lymphoid structures (TLS) are promising prognostic indicators of positive outcomes for patients with solid tumors including colorectal cancer (CRC) [1]. Large-scale retrospective analysis shows patients with mature TLS in particular respond to PD-1/PD-L1 antibody treatment with improved objective response, progression-free and overall survival [2]. Since not all patients respond to PD-1/PD-L1 antibody treatment, identifying patients with mature TLS is clinically relevant as it enables selection of patients likely to respond to immune checkpoint blockade.
Mature TLS are composed of T cells (CD3+), follicular B cells (CD20+), germinal center B cells (CD23+), and follicular dendritic cells (CD21+) [3]. The ability to identify and evaluate TLS is limited by detection methods which traditionally employ hematoxylin and eosin (H&E) staining for visual quantification of immune aggregates, potentially significantly underestimating their quantity [4]. Multiplexed fluorescent immunohistochemistry (IHC) detection assays have the capability to precisely quantify mature TLS within the TME.

Methods: Colorectal cancer specimens expressing a dynamic range of TLS were stained with a novel custom PhenoImager multiplex immunofluorescence panel detecting CD20, CD21, CD23, CD3 and pan-cytokeratin. Identification of mature TLS with CD3+CD20+CD21+ and CD3+CD20+CD23+ expression in the TME is reported.

Results: PhenoImager TLS multiplex immunofluorescence panel successfully identified mature TLS in the TME of colorectal cancer patient samples. TLS were quantified via custom analytics algorithms generated with Indica HALO software.

Conclusions: Characteristics of TME immunity in colorectal cancer differentially impact an individual patient's odds of survival [5]. The novel PhenoImager multiplex assay in this study shows a detailed picture of mature TLS in patient cancer samples. Future applications of this panel include investigations of TLS associated with successful anti-tumor immune development and therapeutic response to treatment.

[1] Oncoimmunology. 2020; 9(1): 1724763
[2] Nat Cancer. 2, 794–802 (2021)
[3] Oncoimmunology. 2021; 10(1): 1900508.
[4] Mod Pathol 2017 Sep;30(9):1204-1212. doi: 10.1038/modpathol.2017.43.
[5] Cell. 2020 Sep 3;182(5):1341-1359.e19. doi: 10.1016/j.cell.2020.07.005

PhenoImager Assay Workflow and Biomarker Panel

Panel Biomarkers	
CD3	CD23
CD20	panCK
CD21	

Co-expression	Phenotype
CD3+CD20+CD21+	Mature TLS
CD3+CD20+CD23+	

Figure 1. PhenoImager Workflow and Panel Composition. Colorectal cancer samples were stained with a 5-marker, 6-Color panel including CD3, CD20, CD21, CD23, panCK and DAPI for nuclear detection. Staining was performed with Akoya Automated IHC Detection Kit run on Leica Bond RX Autostainer. Samples imaged on the Akoya Biosciences PhenoImager HT system were visualized using Phenochart and inForm software for ROI selection, spectral unmixing and autofluorescence removal. Images imported to Indica™ HALO and quantification algorithms were generated to identify mature TLS phenotypes.

Identifying Tertiary Lymphoid Structures using 5-plex PhenoImager Panel

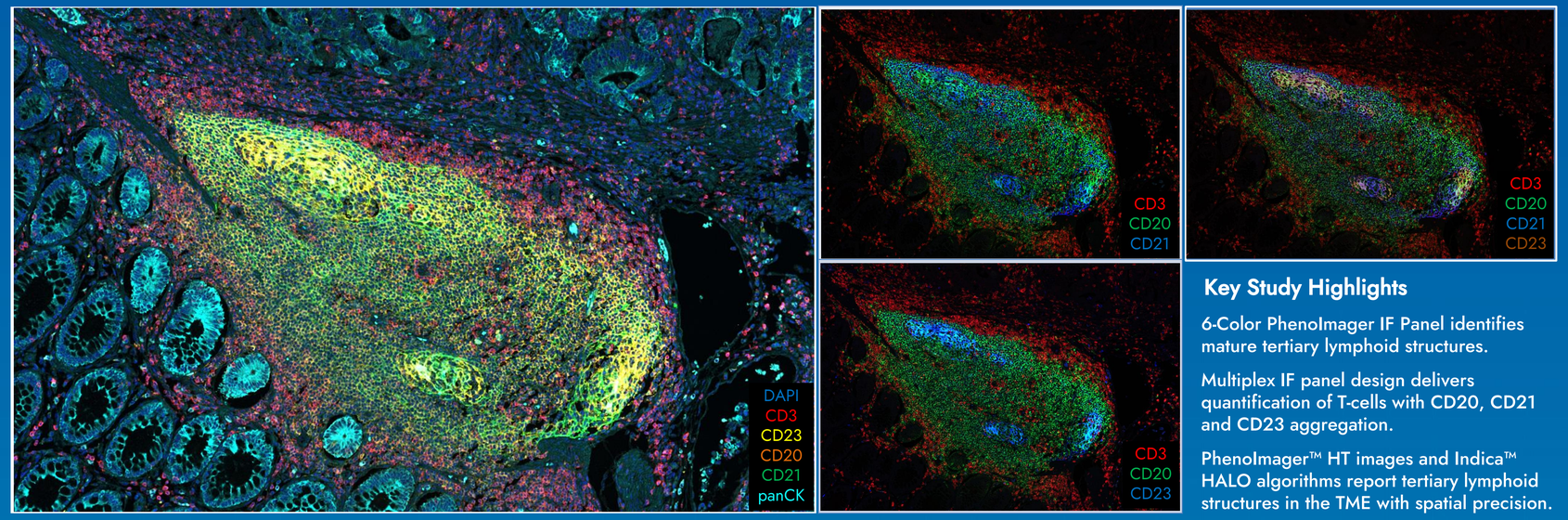

Figure 2. Detection of mature TLS in CRC sample using PhenoImager 5-plex assay

Key Study Highlights

6-Color PhenoImager IF Panel identifies mature tertiary lymphoid structures.

Multiplex IF panel design delivers quantification of T-cells with CD20, CD21 and CD23 aggregation.

PhenoImager™ HT images and Indica™ HALO algorithms report tertiary lymphoid structures in the TME with spatial precision.

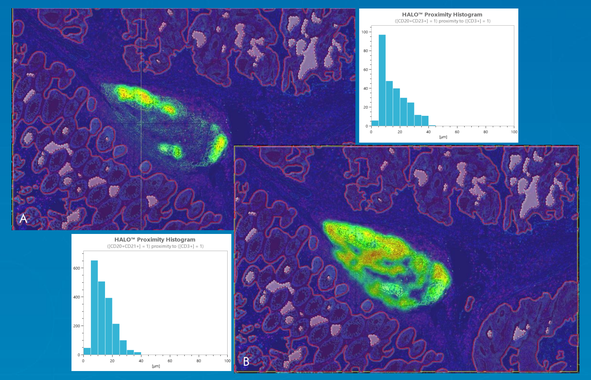

Figure 3. Proximity histogram of A) CD23 containing and B) CD21 containing hotspots detecting tissue area containing panel biomarkers

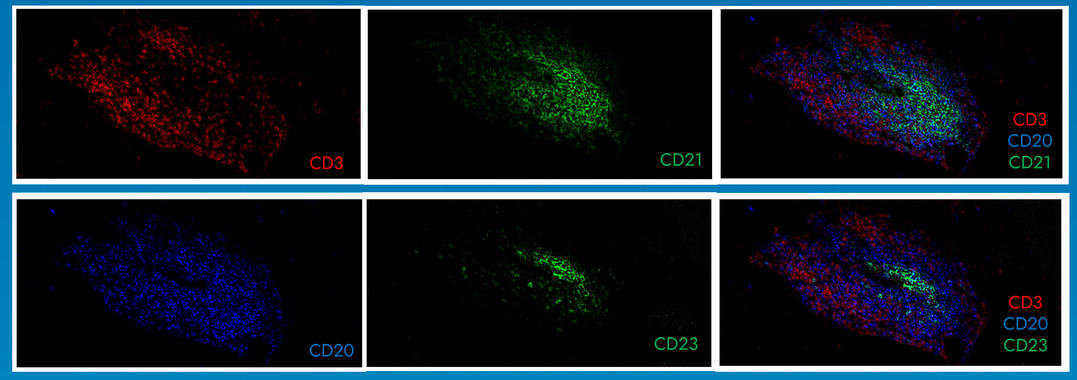

Figure 4. Representative 20X Images of Tertiary Lymphoid Structures in Colorectal Cancer

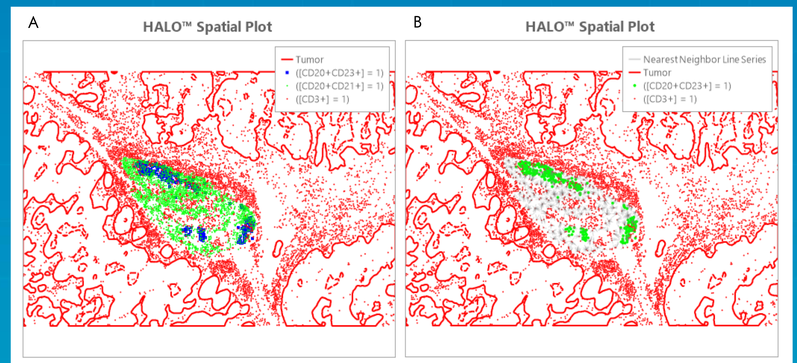

Figure 5. A. Labeling mature TLS phenotypes with spatial information preserved. B. Nearest neighbor analysis of B-cells CD20+CD23+ and CD3+ T-cells in a mature TLS

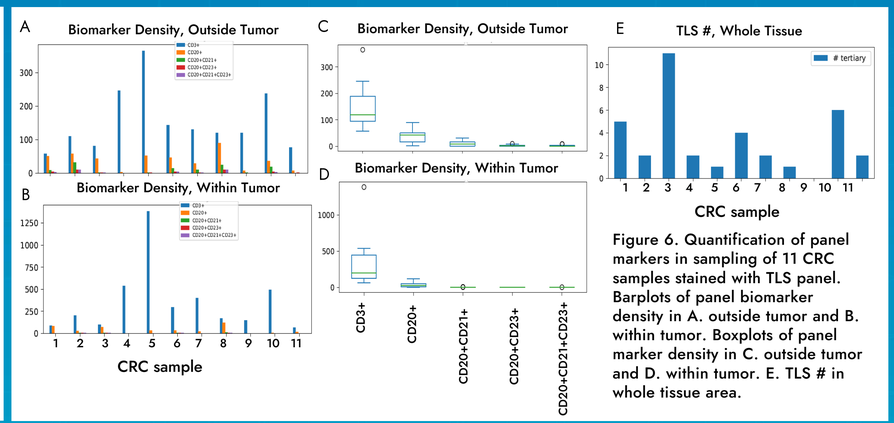

Figure 6. Quantification of panel markers in sampling of 11 CRC samples stained with TLS panel. Barplots of panel biomarker density in A. outside tumor and B. within tumor. Boxplots of panel marker density in C. outside tumor and D. within tumor. E. TLS # in whole tissue area.

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