signaling. Therefore, inhibition of CD39 and/or CD73 holds exceptional advantages over A2aR blockade as both, A2aR dependent and A2aR independent effects of ATP degradation products are targeted simultaneously.

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P03.03 ORGANIZATION, FUNCTION AND GENE EXPRESSION OF TERTIARY LYMPHOID STRUCTURES IN PDAC RESEMBLES LYMPHOID FOLLICLES IN SECONDARY LYMPHOID ORGANS

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Background Secondary lymphoid organs (SLO) are involved in induction and enhancement of anti-tumor immune responses on different tumor entities. Recent evidence suggests that anti-tumor immune responses may also be induced or enhanced in the tumor microenvironment in so called tertiary lymphoid structures (TLS). It is assumed that TLS represent a hotspot for T cell priming, B cell activation, and differentiation, leading to cellular and humoral anti-tumor immune response.

Methods FFPE-slides of 120 primary pancreatic ductal adenocarcinoma (PDAC) patients were immunohistochemically (IHC) stained for CD20, CD3, CD8 and HLA-ABC to analyze spatial distribution of tumor-infiltrating lymphocytes. 5-color immunofluorescence staining was performed to further investigate structural components of TLS in comparison to lymphoid follicles in SLOs. Microscope-based laser microdissection and Nanostring-base RNA expression analysis were used to compare gene expression in PDAC, TLS, SLOs and normal pancreatic tissue.

Results TLS were frequently detected in PDAC and were mainly localized along the invasive tumor margin. In less than 10% of the cases TLS were infiltrating the tumors. Interestingly, 20% of the patients had no TLS. Results of TLS will be correlated with clinical parameters, Immunoscore and immune escape mechanisms. 5-color Immunofluorescence staining revealed similar organization and function of TLS and SLO. Finally, gene expression analyzed by Nanostring revealed largely overlapping expression patterns in TLS and SLO.

Conclusions The results clearly demonstrate close similarities between SLO and TLS in terms of composition, distribution and gene expression Patterns.

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P03.04 APPLYING MULTISPECTRAL UNMIXING AND SPATIAL ANALYSES TO EXPLORE TUMOR HETEROGENEITY WITH A PRE-OPTIMIZED 7-COLOR IMMUNO-ONCOLOGY WORKFLOW

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Background The tumor microenvironment hosts a myriad of cellular interactions that influence tumor biology and patient outcomes. Multiplex immunofluorescence (mIF) provides the ability to investigate a large number of these interactions in a single tissue section, and has been shown to outperform other testing modalities for predicting response to immunotherapies.¹ Multispectral imaging (MSI) improves the capabilities of mIF by providing the ability to spectrally unmix fluorescence signals. This increases the number of markers that can be probed in the same scan and allows for separation of true immunofluorescence signals from tissue autofluorescence background. Here, we apply MSI to explore spatial interactions observed in lung cancer samples using an end-to-end translational workflow based on the Phenoptics™ platform. The workflow includes a pre-optimized 7-color staining panel kit along with a pre-configured analysis algorithm for cell phenotyping. Using tissue microarrays (TMA), we demonstrate the heterogeneity of spatial interactions observed among different lung cancer samples and the improved sensitivity of detection afforded by unmixing multispectral scans.

Materials and Methods Formalin-fixed paraffin-embedded (FFPE) lung cancer TMA contained 120 cores (1.5 mm diameter, US Biomax, Inc., Derwood, MD). The TMA was stained using the MOTiF™ PD-1/PD-L1 Panel: Auto Lung Cancer Kit and pre-optimized protocol for the Leica BOND RX™. Whole slide 7-color MOTiF multispectral scan was acquired on Vectra Polaris® using pre-defined parameters. Phenochart™ software was used to identify cores for analysis. Scans were unmixed and analyzed with inForm® software using a pre-configured algorithm tailored to the MOTiF™ PD-1/PD-L1 Panel kit. With this algorithm, cells are assigned phenotypes using intensity thresholds for CD8, PD-1, FoxP3, CD68, and PanCK signal levels, subject to pre-defined marker priority rules. The rules limit co-positivity to any combination of CD8, FoxP3, and PD-1, but no combinations of those markers with CD68 or PanCK, and no combination of CD68 with PanCK. When threshold levels generate excluded combinations, priority is given to calls for CD8/FoxP3/PD-1 over CD68, which in turn has priority over PanCK. To explore the dynamic range of PD-L1, it was assessed via expression level (signal intensity), not phenotyping. Spatial analyses and visualizations were performed in R² using the phenoptr and phenoptrReports packages³, and custom scripts.

Results The pre-optimized Opal Polaris 7-Color PD-1/PD-L1 Lung Cancer Panel Kit was able to visualize the panel targets (PD-L1, PD-1, CD8, CD68, FoxP3, and Cytokeratin) across the variety of lung cancer samples in the TMA. Cell phenotyping and spatial analyses revealed core-to-core variations in cell densities and proximities among different markers.