Conclusions These studies indicate that the semi-syngeneic a-PD-1 IgG1e3 ab might be a more efficient and translatable a-PD-1 ab for preclinical in vivo studies, which is important for the future investigation of immune checkpoint inhibitor therapy.

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P02.06 ABSTRACT WITHDRAWN

P03 Tumor Microenvironment

P03.01 PREVALENCE OF CD112R$^+$IMMUNE CELLS IN NORMAL LYMPHATIC TISSUES, INFLAMMATION AND THE CANCER MICROENVIRONMENT

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Background CD112R is an inhibitory immune checkpoint receptor and a putative target for novel immune therapies, but little is known about its molecular epidemiology in healthy and diseased tissues.

Materials and Methods To study the prevalence and expression level of CD112R$^+$ immune cells, we analyzed more than 200 samples of normal lymphatic, inflamed and cancerous tissues in a microenvironment tissue microarray format (4 mm tissue spot diameter) and large sections using fluorescent multiplex immunohistochemistry.

Results CD112R expression was detected at variable intensity levels in 47% of CD8$^+$ cytotoxic lymphocytes, 49% of CD4$^+$ T helper cells, 30% of FOXP3$^+$ regulatory T helper cells and in 25% of CD56$^+$ natural killer cells, but no expression was seen in CD11c$^+$ dendritic cells and CD68$^+$ macrophages. All analyzed compartments across normal and diseased tissues showed a small subset (CD8: 9±18%, CD4: 5±15%, FOXP3: 2±5%) of immune cells with supramaximal CD112R expression. The highest fraction of cells with supramaximal CD112R expression was found in the subset of CD8$^+$ cytotoxic T cells in the Peyer's patches of ileum (62%), the intergranuloma area of lymph node sarcoidosis (27%) and in ovarian cancer (37%). In cancerous tissues, the density and the fraction cytotoxic T cells with supramaximal CD112R expression was highly variable and ranged from 5% in bladder cancer to 3% in lung cancer and 36% in ovarian cancer. A high variability of the number of cells with supramaximal CD112R expression was also seen within every tumor entity.

Conclusions In summary, our analysis shows that CD112R expression is abundant in various subsets of immune cells but identifies a small fraction of cells with exceedingly high CD112R levels. The widespread occurrence of CD112R$^+$ cytotoxic T cells in the cancer microenvironment may suggest considerable opportunities for checkpoint inhibitors targeting CD112R.

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P03.02 SUPPRESSION OF T-CELL PROLIFERATION AND CYTOKINE RELEASE BY THE ADENOSINE AXIS ARE MEDIATED BY DIFFERENT MECHANISMS

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Background The so-called adenosine axis has emerged as a promising therapeutic target pathway as high adenosine levels in the tumor microenvironment contribute to the suppression of antitumor immune responses. The ectonucleotidases CD39 and CD73 act in concert to degrade extracellular immune-stimulating adenosine triphosphate (ATP) to immunosuppressive adenosine. According to the current model, subsequent suppression of effector immune cell function is caused by binding of adenosine to adenosine receptors like the A2a receptor (A2aR). The ectonucleotidases CD39 and CD73 as well as the A2aR have emerged as molecular targets within the adenosine axis with currently more than 20 clinical trials investigating antitumor effects of CD39-, CD73- or A2aR blockade. We aimed to perform a direct comparison of these targets with regard to their roles in regulating T-cell proliferation and IFN-γ secretion.

Materials and Methods CD39 and CD73 expression was suppressed using LNAplus^{TM} antisense oligonucleotides (ASOs). ASOs were synthesized as gapmers with flanking locked nucleic acids (LNA) to increase stability and affinity to the target RNA, leaving a central gap for recruitment of the RNA-degrading enzyme RNaseH I. Knockdown efficacy of ASOs on mRNA and protein level was investigated in primary human T cells. Furthermore, the effects of ATP, AMP and adenosine analogues on T–cell proliferation and IFN–γ secretion were investigated. A2aR was blocked using small molecule inhibitors that are currently under clinical investigation.

Results Treatment of human T cells with LNA-modified ASOs specific for human CD39 and CD73 resulted in potent target knockdown *in vitro* without the use of a transfection reagent. T-cell proliferation was reduced after addition of ATP to activated T cells that was completely reverted by ASO-mediated suppression of CD39 and/or CD73 expression but not A2aR inhibition. Adenosine analogues inhibited IFN–γ secretion of activated T cells, however, they did not suppress T-cell proliferation. Blockade of the adenosine kinase was able to revert the anti-proliferative effect of ATP degradation products, arguing for downstream metabolites of adenosine, but not A2aR signaling, being responsible for the suppression of T-cell proliferation.

Conclusions Cytokine secretion and proliferation of T cells might be differentially regulated by the adenosine axis. Adenosine might primarily affect cytokine secretion via A2aR signaling, whereas adenosine metabolites might especially impair proliferation of activated T cells independent from A2aR

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 R [2] using the phenoptr and phenoptrReports packages[3], 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.