Background Tertiary lymphoid structures (TLS) are ectopic B cell clusters, which have been described in close proximity to tumor areas in a variety of cancer types. Abundance of TLS is related to cancer-specific survival and also susceptibility to immune checkpoint inhibition. TLS in the tumor microenvironment are assumed to represent hotspots for T cell and B cell activation leading to tumor-specific humoral and cellular immune responses. We aim to identify shared and distinct features of TLS and lymphoid follicles in secondary lymphoid organs (SLOs) to elucidate their functional overlap.

Material and Methods We performed immunohistochemistry staining of 163 primary pancreatic ductal adenocarcinoma (PDAC) patients for CD20, CD3, CD8 to analyze spatial distribution of tumor-infiltrating lymphocytes and to calculate the Immunoscore. Comparison of structural components of lymphoid follicles between TLS and SLOs was done by 5-color immunofluorescence staining. Tissue extraction by laser microdissection and Nanostring-based RNA expression analysis was conducted to compare gene expression in TLS, PDAC, SLOs and normal pancreatic tissue. Gene expression in tumor tissue of patients with high and low TLS abundance was analyzed to identify factors promoting TLS formation. Sequential immunostainings were performed to identify predominant immunoglobulin classes arising of TLS. To identify possible overlap of the B cell receptor repertoire between TLS and SLOs, B cell receptor sequencing will be performed.

Results Tumor samples of 95% of all analyzed patients contained TLS and their abundance was heterogeneous. TLS were mainly localized in a 2000 μm invasive margin adjacent to the tumor. In 49% of samples also intratumoral TLS were present. Patients with high abundance of TLS inside and surrounding the tumor had significantly improved overall survival. Correlation of TLS abundance and T cell abundance (Immunoscore) as well-established prognostic factor will be provided. Most B cells implemented in TLS were IgG+ positive proving class switching and affinity maturation in TLS. Five-color Immunofluorescence revealed high similarities regarding composition and spatial distribution of immune cells and structural components. Nanostring analysis of 12 patients confirmed functional similarities of TLS and SLOs by largely overlapping expression patterns in a variety of immune related gene clusters. However, differences in expression levels between TLS and SLOs were found for some genes. We will also provide a comparison of gene expression in tumor tissue from TLS high vs low patients to identify factors that promote or inhibit TLS formation.

Conclusion Our results confirm beneficial impact of TLS abundance in the tumor microenvironment on the clinical outcome of PDAC patients. The largely overlapping composition, structural organization and gene expression patterns of SLOs and TLS in PDAC further suggest similar function. Our results indicate a role of TLS in cancer immunosurveillance of PDAC, which may be susceptible to therapeutic targeting in this highly aggressive and immunotherapy-resistant disease.
