immunotherapy could be adapted. Further investigations required to map the immune evasion machinery for each type of cancer and design the best targets for personalised immunotherapy of human malignancies.

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P02 Tumor microenvironment and microbiome in immunotherapy

**P02.01 A BIOINFORMATIC ANALYSIS: THE OVEREXPRESSION AND CLINICAL SIGNIFICANCE OF HVEM IN LIVER HEPATOCELLULAR CARCINOMA**

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**Background** The occurrence and development of liver cancer is related to the immune evasion caused by abnormal expression of immune costimulatory molecules in liver cancer cells. High expression of herpesvirus entry mediator (HVEM) was associated with tumor progression and reduced patient overall survival in multiple cancers. Overexpression of HVEM may lead to binding with inhibitory co-receptor B- and T-Lymphocyte Attenuator, downregulating T cell activation and proliferation, and mediating immune evasion in tumor immune microenvironment. However, clinical significance of HVEM overexpression in liver hepatocellular carcinoma (LIHC) and its relation to immunity remains unknown.

**Materials and Methods** We obtained expression profiles of HVEM in pan-cancer and LIHC through The Cancer Genome Atlas (TCGA) and Genotype-Tissue Expression. Expression of HVEM in LIHC was then validated by immunohistochemical staining in Human Protein Atlas (HPA). Additionally, we evaluated the prognostic value and association of clinicopathological factors to HVEM in LIHC with clinical survival data from TCGA. A risk prognostic nomogram model using Cox regression analysis was constructed to identify important LIHC prognostic indicators. Finally, we evaluated the correlation of HVEM with 24 immune cell infiltrates based on single sample gene set enrichment analysis.

**Results** Paired data from TCGA LIHC dataset indicated HVEM overexpression in LIHC compared to normal liver tissues (p < 0.001, normal = 50, tumor = 50). Unpaired data showed similar results (p = 0.001, normal = 160, tumor = 371). Additionally, immunohistochemical staining of HVEM in normal liver tissues was weak in HPA database but strong in LIHC tissues. High HVEM expression was strongly associated with worse overall survival [Hazard Ratio (HR) = 1.60 (1.13–2.26), p = 0.008], disease-specific survival [HR = 1.63 (1.03–2.26), p = 0.035], and progress free interval [HR = 1.35 (1.00–1.82), p = 0.048] in LIHC patients. Alpha-fetoprotein levels, Child-Pugh score, fibrosis ishak score, histological grade, T stage, M stage, and N stage are benchmarks associated with cancer progression. Logistic regression analysis revealed significantly higher HVEM expression in LIHC patients with greater cancer progression according to those benchmarks. Additionally, Cox regression analysis indicated that HVEM might be an independent prognostic factor for LIHC patients (p = 0.016). Nomogram calibration was performed on the constructed nomogram, revealing a C-index of 0.67, indicating good predictive ability. Functional enrichment and Gene Ontology analyses and Kyoto Encyclopedia of Genes and Genomes pathway analysis showed HVEM association with immune-related genes and pathways respectively. HVEM was positively correlated with the abundance of tumor-infiltrating immune cells like DC, B cells, CD8 T cells, macrophages, NK cells, Th1 cells, and Th2 cells, and negatively correlated with the abundance of TCM and TEM, suggesting association between HVEM and infiltration by immune cells.

**Conclusions** In conclusion, our findings demonstrated high expression of HVEM in LIHC patients and is a potentially dangerous prognostic predictor of LIHC. We also found a correlation between HVEM immune-related signaling pathways and immune cell abundance, which may affect progression and prognosis of LIHC patients.

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**P02.02 EASY AND SCALABLE TRANSFER OF AN IMMUNO-ONCOLOGY PANEL ACROSS A WIDE RANGE OF CANCER TISSUE TYPES**

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**Background** Therapies based on checkpoint inhibitors have emerged as promising strategies in the treatment of cancer patients. The selection of the most efficient combination of immunotherapies along with the accurate prediction of cancer prognosis are unmet endeavors. Thus, scientists and clinicians need deeper knowledge of the immune profile of each patient as well as a detailed characterization of the tumor microenvironment (TME) to develop targeted therapies. Multiplex immunofluorescence (mIF) has become an important tool in the immune profiling of the TME. Ideally, multiplex IF assays should allow for the study of multiple markers in the same tissue sample while preserving the spatial information of tissue morphology. Despite a fast-growing need of multiplex methods, the broad implementation of mIF still remains challenging due to several technical barriers. Assays require robust validation but also a large degree of flexibility to be used across multiple tissue types.

**Materials and Methods** Here we present COMET™, an innovative solution to easily develop mIF assays that work as a fully automated staining and imaging platform allowing for hyperplex staining with up to 40-markers in just a few hours, without human intervention. COMET™ performs sequential immunofluorescence assays, which consist of sequential cycles of staining, imaging, and elution of two markers per cycle. With the aim of phenotyping different immune and cancer cells and evaluating the prognosis of epithelial tumors, we...
developed a core immuno-oncology panel that allows the simultaneous detection of ten biomarkers on the same tissue slide.

**Results and Conclusions** This core panel was initially developed and validated on formaldehyde-fixed paraffin-embedded tissue (FFPE) human tonsil as positive control tissue. In the next steps, we employed the same validated panel in different tissue contexts, such as breast, lung and head and neck carcinomas. Thanks to an assisted workflow of optimization and the fast staining cycles lasting approximately 30 minutes, the optimal staining conditions for each tumor type were rapidly identified in a few steps. Our data proves that immuno-oncology panels developed on COMET™ can be easily transferred from one tissue type to another, enabling researchers to identify core biomarkers across different tumors for the development of novel targeted immunotherapies.


**P02.03 ORGANIZATION, FUNCTION AND GENE EXPRESSION OF TERTIARY LYMPHOID STRUCTURES IN PANCREATIC CANCER RESEMBLES LYMPHOID FOLLICLES IN SECONDARY LYMPHOID ORGANS AND THEIR ABUNDANCE IS RELATED TO SUPERIOR SURVIVAL**

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**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.


**P02.04 HIGH-PLEX SPATIAL IMMUNE CELL PROFILING OF THE TUMOR MICROENVIRONMENT WITH CHIPCYTOMETRY**

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**Background** Understanding the spatial distribution of key immune cell populations is critical in advancing our understanding of cancer to inform the development of novel therapeutics. Historically, the spatial analysis of the tumor microenvironment has been limited to relatively low-plex immunohistochemical (IHC) or immunofluorescent (IF) assays, which are inadequate for deep immune cell profiling of the tumor microenvironment.

**Materials and Methods** Here we present the analysis of human breast carcinoma samples with a 60-plex protein panel using a novel precise spatial multiplexing technology called ChipCytometry, which combines iterative immuno-fluorescent staining with high dynamic range imaging to facilitate quantitative phenotyping with single-cell resolution. Standard FCS files are generated from multichannel OME-TIFF images, enabling identification and quantification of cellular phenotypes via flow cytometry-like hierarchical gating.