inflammatory diseases as compared to healthy secondary lymphoid organs. The striking link with microsatellite instability and unfavorable tumor features suggest a potential clinical utility of assessing Ki67⁺CD8⁺ in colorectal cancer.

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P03.07 FAST AUTOMATED MICROFLUIDIC-BASED MULTIPLEXED IMMUNOFLUORESCENCE FOR TUMOR MICROENVIRONMENT ANALYSIS

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Background Immuno-oncology and targeted molecular therapies have acquired a central role in the treatment of multiple cancers. Consequently, high-throughput biomarker analysis and tumor immune profiling have seen an increased demand. Multiplexed immuno-assays are a powerful tool to address these needs, but still time- and resource-consuming. Our goal is to develop a fast and automated high-plex fluorescent immunostaining procedure, using a microfluidic-based device, that can be easily implemented as routine assay.

Materials and Methods Protocol optimization has been performed on FFPE sections of human tonsil. Slides were manually deparaffinized before being entirely processed (antigen-retrieval, staining, elution and counterstaining) by Lunaphore's autostainer, LabSatTM. The OPAL[®] tyramide signal amplification (TSA) system was used as detection method. Signal analysis was done on Mantra[®] workstation. The 6-plex panel was composed of FoxP3, PD-L1, PD-1, CD68, CD8 and pan-CK, plus DAPI counterstaining. Protocols were subsequently transferred on NSCLC representative specimens and finally assessed on a TMA cohort.

Results Our platform allowed to reduce drastically the incubation times due to active transport of reagents across the tissue. Thereby, the automated 6-plex assay could be performed in less than 4h30min, within the timeframe of a single IHC standard assay. Protocol optimization resulted in high signal-to-background ratio for each marker and removal of previous step antibodies over 99%. LabSatTM also guaranteed remarkable signal uniformity, even over large tissue sections with less than 10% signal gradient over 1 cm. On NSCLC samples, the detected pattern and expression level for all six biomarkers were comparable to the standard chromogenic stainings performed with standard automated tissue stainer.

Conclusions LabSatTM autostainer enables multistaining runs in a timely manner, opening the perspective of rapid simultaneous detection of multiple markers in their morphological context on a routine-based approach. This versatile analysis tool can offer a better and more quantitative understanding of tumor heterogeneity and microenvironmental interactions, allowing advances in targeted therapy for lung cancer as well as broader spectrum of malignancies.

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P03.08 OMENTAL FAT IN OVARIAN CANCER POTENTIALLY INDUCES LYMPHANGIOGENESIS

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Background Ovarian cancer metastasis occurs by direct multifocal seeding in the peritoneum as well as by migration through the lymphatic system. High grade ovarian carcinoma patients present with distant metastases. Significant risk factors for the development of those are stage, grade, and lymph node involvement. An increase of the number of lymphatic vessels is shown in ovarian tumors and these vessels seem implicated in tumor progression. While the tropism of ovarian cancer cells for fat is well described, the potential impact of a fatty microenvironment on the dissemination of tumor cells via lymphatic vessels has, to our knowledge, never been investigated yet. In this study, we examined the effect of omental fat on lymphangiogenesis in ovarian carcinoma.

Materials and Methods To examine the effect of omental fat on lymphangiogenesis in OC we used a cohort of 80 human specimens. We analysed lymphatic vessels histologically with D2-40 and Lyve-1 markers. We also developed a healthy fat tissue explant culture model and treated explants with ascites of patient with OC before analysis. We analysed by fluorescence stainings the co expression of adipose derived stem cells (ASCs) and lymphatic markers in these explants.

Results We observed a higher density of tumor-associated vessels, especially lymphatic vessels in OC in contact with the omentum; mainly localized along the adipose tissue. We also measured a higher secretion of VEGF-C in tissues with fat compared to tissues without fat. Healthy fat tissues treated with ascites show an increase of number of ASCs, some of them express lymphatic markers such as D2-40 and Lyve-1. In a clinical trial of patients with OC treated by Bevacizumab, we observed a decrease of the number of lymphatic vessels in correlation with a decrease of the inflammation around the fat tissue.

Discussion We saw an increase in the number of lymphatic vessels in ovarian carcinoma infiltrating fat. These vessels are