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# Abstracts

## 30th European Congress of Pathology

## Oral Free Paper Sessions

Sunday, 9 September 2018, 08:30 - 12:00, Room A2
**OFP-01 | Joint Session: Molecular Pathology / Haematopathology**

**OFP-01-001**
Microfluidic-based automated multiplex immunophenotyping and imaging
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**Background & Objective:** The tumour microenvironment plays a vital role in cancer development. Multiplex immunostainings allow studying the interaction of different cell types in the tumour microenvironment using a single tissue slide. The objective is to develop a fully automated microscope integrated method for rapid 10-plex fluorescent immunostaining and imaging of tissue sections.
**Method:** FFPE tonsil sections underwent manual dewaxing and antigen retrieval step. All subsequent steps of staining, antibody elution and imaging were automated on the microscope integrated microfluidic device. A single tissue section was stained sequentially for CD3, CD4, CD8, CD20, CD56, CD68, FOXP3, PD-1, PD-L1 and CK with mouse or rabbit primary antibody and corresponding Alexa Fluor labelled secondary antibody. The section was imaged after each staining step and subsequently eluted before staining the next marker.
**Results:** Our microscope integrated microfluidic system allowed automated 10-plex staining with conventional primary and fluorescently labelled secondary antibodies in less than five hours, including image acquisition steps. Protocol optimization resulted in a high signal to background noise ratio for each marker, while fully eluting antibodies from the previous staining step.
**Conclusion:** With the microscope integrated microfluidic system, it is possible to perform fast multiplex stainings including image acquisition without removing the tissue slide. Moreover, due to the sequential nature of the system it would be easily possible to further increase the number of markers in the multiplex staining. We believe that this technique greatly facilitates the execution of high-plex stainings and thereby the discovery of novel tumour-microenvironment interactions.

**OFP-01-002**
**The potential biomarker HR23b regulates sensitivity towards histone deacetylase inhibitors (HDACi) via the NGFR death receptor pathway**
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**Background & Objective:** Deregulation of histone deacetylases (HDACs) plays an important role in tumourigenesis and progression. Restoring a regular acetylation profile by HDAC inhibitors (HDACi) is a promising therapeutic approach. Human Rad Homolog B (HR23b), has been identified as a predictive biomarker of HDACi sensitivity in haematological and hepatocellular tumours. We showed previously that HDACi also exhibit antiproliferative and pro-apoptotic effects in sarcomas in dependence of HR23b expression. We therefore aim to elucidate the regulatory relationship between HR23b expression and sensitivity towards HDACi.
**Method:** A stable knockout of HR23b was generated in a malignant peripheral nerve sheath tumour (MPNST) cell line (ST-8814) using CRISPR /Cas9-technology. An efficient knockout was verified both, on DNA- and expression level. Its influence on proliferation and apoptosis was measured with the ApoTox ™ Glo assay. HDACi vorinostat was adminstered at IC50 concentration to wildtype and HR23bKO cells. Afterwards expression analysis of important signaling pathways was performed with the nCounter PanCancer Pathways panel on a NanoString platform.
**Results:** qPCR and Western Blot analysis confirmed a stable knockout of HR23b in ST-8814 cells. HR23b dependent sensitivity towards HDACi is mediated by apoptosis induction via the NGFR death receptor pathway. In contrast, HR23b loss reduces apoptosis induction and shifts response to TLR2-regulated autophagy.
**Conclusion:** Understanding the key pathways by which HDAC inhibitors affect tumour growth plays a major role for future therapeutic approaches. In particular, the importance of HR23b as a predictive biomarker should help to select patients who may benefit from HDACi therapy.

**OFP-01-003**
**Patient-derived colorectal cancer explants retain the histological and molecular key features of their primary counterparts**
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**Background & Objective:** Colorectal cancer (CRC) 5-year overall survival is 64.9%. Chemotherapy responses are limited. In order to improve clinical outcomes, reliable models predictive of drug response are needed. Patient derived explants (PDE) are ex-vivo cultures that can overcome representability limitations of other models.
**Method:** Our aim is to develop PDEs from CRC samples dissociated and placed in a dynamic system; evaluate their viability by metabolic and morphologic evaluations; characterize their phenotype (architecture, senescent phenotype, stroma cellularity, inflammatory cells); and assess PDE representation of the primary tumour (gland formation, p53 and mismatch repair proteins, microsatellite instability, KRAS exon2 and BRAFV600E mutations).
**Results:** Eleven adenocarcinomas were successfully cultured. All PDEs retained their originals' glandular architecture. There was viable tumour

**PS-20-042**

**Study of expression of calprotectin and myeloperoxidase in clinically diagnosed inflammatory bowel disease**

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**Background & Objective:** Calprotectin and Myeloperoxidase have been employed as diagnostic fecal markers of inflammatory bowel disease (IBD). This study was performed to investigate the diagnostic role of calprotectin and myeloperoxidase immunohistochemical expression in colonic mucosal biopsies.
**Method:** Calprotectin and Myeloperoxidase immunostaining was performed in colonoscopic biopsies of 50 patients diagnosed clinically and/or endoscopically as IBD but with inconclusive diagnosis on histopathological examination. Also, 10 positive control cases proved clinically, endoscopically and histopathologically as IBD were similarly studied.
**Results:** Epithelial calprotectin expression was encountered in 38% and 60% of histopathologically inconclusive cases and positive control cases respectively. The image optical density (IOD) of calprotectin epithelial immunostaining showed no significant difference between confirmed IBD cases and histopathologically inconclusive cases (p=0.459). In addition, most of the values of IOD of the calprotectin epithelial immunostaining of the inconclusive cases fell within the range of the confirmed IBD cases. Myeloperoxidase score showed a significant difference between the two studied groups as a whole (p=0.001), whereas it showed no significant difference between the subset of cases demonstrating epithelial calprotectin staining in both groups (p=0.127) ; in which most of the values of myeloperoxidase score in the inconclusive cases fell within the range of the confirmed IBD cases.
**Conclusion:** Epithelial immunostaining of calprotectin in tissue biopsy was able to point to the cases with inconclusive histopathological diagnosis that showed agreement with the confirmed IBD cases, and thus would help in the diagnosis of IBD. Additional consideration of myeloperoxidase score, besides epithelial calprotectin, would further refine the diagnosis.

**PS-20-043**

**The immune microenvironment of various histological types of EBV-associated gastric cancer**

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**Background & Objective:** EBV-associated cancer is one of the molecular subtypes of gastric adenocarcinomas classified by mutation profile analysis. There is very few data on its IHC-characteristics and its immune microenvironment. The distribution of immune cells might appear to be an important diagnostic criterion of poor prognosis.
**Method:** Samples of 26 gastric adenocarcinomas (surgical material) were included in this study. IHC-staining for LMP-1 protein and IHC-staining for CD4, CD8, CD68, CD1a were used for EBV identification and immune cells detection, respectively. The tumour tissue and normal glands microenvironment was investigated separately.
**Results:** We identified 3 EBV-positive, 10 EBV-negative adenocarcinomas and 13 cases with significant expression of LMP-1 in normal glands of lamina propria. EBV-negative cancers had poorer prognosis than other two groups. CD68+ cells infiltration was significantly higher in tumour tissue in cases with EBV-positive normal glands than in EBV-negative and EBV-positive cases (p<0,05), but in normal glands microenvironment macrophages predominated in EBV-negative cases. We found out statistically significant differences in the number of dendritic cells: it prevailed in cases with EBV-positive normal glands (p<0,05) in the tumour tissue and normal glands microenvironment. In normal glands microenvironment significant correlation between the number of CD4+ cells in the signet ring cell carcinomas and in the highly differentiated adenocarcinomas (R=0,91) was found.
**Conclusion:** Immunological properties of tumour tissue and normal glands in gastric adenocarcinomas with EBV-positive normal glands of lamina propria differ significantly from EBV+ and EBV- cancers. We assume that these facts indicate a premalignant process in these glands different from the classical metaplasia-dysplasia-cancer pathway.

**PS-20-044**

**STAT6 immunoexpression in samples from a metastatic model of colon adenocarcinomas to the liver reveals a group of patients with better prognosis**

A. Alonso Fernández-Velasco*, D. Corte-Torres, A. Vallina, I. Husain Calzada, S. J. Sánchez Luizaga, L. Pérez Pavón, M. B. Álvarez González, R. Ugalde Herrá, I. Fernández Vega
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**Background & Objective:** STAT6 protein plays a central role in exerting IL4 mediated biological responses. Moderate to strong cytoplasmic and nuclear staining was observed in most tumour cells, such as colon cancer cells. The main objective was to study the expression of STAT6 in samples of colon adenocarcinoma and in their respective hepatic metastases and to study their clinical relevance with survival rates.
**Method:** Fifty-two consecutive patients with colon cancer and subsequent hepatic metastasis surgically removed between 2007 and 2017 were studied. Tissue arrays were produced using a 2 mm diameter needle. Immunohistochemical studies were conducted, where the positivity was classified from 1 to 3 degrees and the extension was graded between 0 and 100%. A global score was obtained by multiplying both values. Statistical analysis of these findings was carried out using the SPSSv23; p<0.05 program.
**Results:** Almost all colon adenocarcinoma samples and 90% of metastatic samples showed different degrees of STAT6 positive immunoexpression. No significant differences in STAT6 immunoexpression were noted between primary tumours and their metastatic samples (74.41 vs 73.48 p=0.835). Interestingly, metastatic samples of colon adenocarcinomas with more than 100 points of STAT6 immunoexpression reveals a group of patients with significantly better overall survival, disease-free time and post metastatic survival (p=0,001; p=0,006; p=0,004 respectively).
**Conclusion:** No significant differences for STAT6 were found between primary and metastatic adenocarcinoma samples. Considering metastatic adenocarcinoma samples, those with more than 100 points of immunoexpression for STAT6 reveals a group of patients with better prognosis.

**PS-20-045**

**STATB2 reveals a group of patients with better prognosis in a metastatic model of colon adenocarcinomas to the liver**

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**Background & Objective:** The expression of SATB2 protein is primarily preserved in cancer cells of colorectal origin, indicating that SATB2 could function as a clinically useful diagnostic marker to distinguish colorectal cancers from others. To study the expression of SATB2 in samples of colon adenocarcinoma (52) and their respective hepatic metastases and to study their clinical relevance with survival rates.
**Method:** Fifty-two consecutive patients with colon cancer and subsequent hepatic metastasis surgically removed between 2007 and 2017 were studied. Tissue arrays were produced using a 2 mm diameter needle. Immunohistochemical studies were conducted, where the positivity was