Objective: To identify mutational biomarkers using next-generation sequencing (NGS) and endoscopic samples for stratification of Barrett Esophagus (BE) patients at increased risk of dysplasia.
Method: Ion Torrent AmpliSeq Cancer Panel was used to screen for mutations in 46 cancer genes. Sixteen samples were tested: Intestinal metaplasia from 6 patients (IM-P) with concomitant high-grade dysplasia (HGD)/adenocarcinoma (EAC) and HGD/EAC in 3 patients; IM of 7 patients followed for at least 2 years without any dysplasia (IM-N). Ion torrent suite software and ANNOVAR were used for analysis.
Results: The most frequent mutations in IM and HGD/EAC were detected in TP53 spanning codons 150 to 280. Four of 6 IM-P patients had TP53 mutations in IM samples and in HGD/EAC tested. None of the 7 IM-N patients had TP53 mutations. Sensitivity of TP53 mutation for presence of concomitant dysplasia was 67 % and specificity was 100 %.
Conclusion: DNA from routine endoscopic samples can be efficiently used to simultaneously detect multiple mutations by NGS. TP53 mutations were frequently detected in IM of patients with HGD/EAC but not in patients who did not progress to HGD/EAC, suggesting that TP53 mutational testing may be useful to identify IM-P patients who may benefit from closer surveillance.

OFP-03-002
Amplification but not translocation of ALK is a frequent event in esophageal cancer
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Objective: Translocations of ALK have been demonstrated in a variety of human malignancies, and the corresponding fusion-proteins are potential therapeutical targets. Aim of this study was to investigate ALK gene status in a large cohort of esophageal squamous cell carcinoma (SCC) and adenocarcinoma (AC).
Method: ALK status was investigated in 117 SCCs and 136 ACs by fluorescence in situ hybridization (FISH), and ALK protein expression by immunohistochemistry. Data on expression of ALK downstream effector tyrosine −705 phosphorylated STAT3 (pSTAT3) was available from a previous study.
Results: FISH was successfull in 251 cases. No ALK translocations were found, while 14/135 (12.1 %) of SCCs and 14/116 (10.4 %) of ACs showed ALK amplifications. Concomitant EML4 amplifications were present in 27/28 cases with ALK amplifications. Three cases showed EML4 translocations not involving ALK. None of the tumors with ALK amplification showed ALK protein expression, and no correlation with clinical parameters, survival or pSTAT3 expression was observed.
Conclusion: While ALK translocations are not present in esophageal cancer, ALK amplifications are common events. Since ALK amplified breast cancer cells were shown to respond to ALK inhibitors, ALK amplified esophageal cancers might be considered as possible candidates for therapies targeting ALK.

OFP-03-003
MALDI imaging mass spectrometry for proteomic segmentation of tumor heterogeneity in gastric cancer tissues
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Objective: A high clonal diversity within a patient's tumor is an important factor for the evolution of the disease and the clinical management of the patient with regard to cancer relapse and response to therapy (Marusyk, Nat Rev Cancer. 2012 Apr 19;12(5):323–34). The identification and molecular characterization of the tumor's clonal diversity is therefore of high clinical relevance.
Method: In this study, matrix-assisted laser desorption/ionization (MALDI) imaging mass spectrometry was used to identify clinically relevant tumor subpopulations in gastric cancer. MALDI imaging allows the unlabeled in situ measurement of hundreds of molecules (like proteins) within their histomorphological context of tissue sections (Balluff, Gastroenterology. 2012 Sep;143(3):544–9.e1-).
Results: Spatially-resolved, tumor-specific proteomic data was acquired from 63 intestinal-type gastric cancer patients by MALDI imaging. The resulting data underwent a novel statistical procedure (Jones, PLoS One. 2011;6(9):e24913) which results in a spatial segmentation of areas within a tumor section based on their molecular similarity. Correlation of the molecular signatures of the segmented tumor areas with clinical data resulted in the identification of clinically relevant tumor subpopulation in terms of prognosis and metastasis.
Conclusion: Our results highlight the usefulness of MALDI imaging in combination with advanced statistical approaches for detecting novel and clinical relevant information from tumor tissues.

OFP-03-005
Increased tumor-budding/CD8+ lymphocytes ratio is associated with metastasis and venous invasion in Pancreatic Ductal Adenocarcinoma (PDAC)
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Objective: T-Lymphocytes can be a major part of tumor microenvironment, especially at the area of tumor-host interaction where tumor progression, reflected by epithelial-mesenchymal transition (EMT) and its hallmark tumor-budding is taking place. Our aim was to determine the role of CD8+lymphocytes in correlation with tumor-budding in PDAC.
Method: Double immunostaining for AE1-AE3/CD8 was performed on a multipunch tissue microarray of 120 well-characterized PDACs. Tumor-buds, CD8+ and tumor-budding/CD8+ lymphocytes indices were evaluated and associated with clinico-pathological features, follow-up and adjuvant therapy information.
Results: There was a strong negative correlation between the number of buds and CD8+ counts ($p=0.01$). Increased numbers of tumor-buds were associated with venous invasion ($p=0.0272$) and reduced overall survival ($p=0.0147$). Low CD8+ peritumoral lymphocytes showed only a marginal association with metastasis ($p=0.0683$). Tumor-budding/CD8+lymphocytes ratio was strongly associated with venous invasion ($p=0.0078$) and metastasis ($p=0.0452$). No intratumoral CD8+lymphocytes were detected.
Conclusion: Low counts of CD8+ peritumoral lymphocytes in the micro-environment of PDAC promote EMT as reflected by tumor-budding and facilitate tumor progression, since tumor-buds seem to display metastatic potential only when coupled by decreased counts of CD8+lymphocytes. However, unlike to other tumor types, no prognostic effect was found, probably reflecting the fact of the absence of intratumoral CD8+lymphocytes.

OFP-03-006
Various carcinogenetic pathways may be involved in colorectal serrated polyposis
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Objective: Serrated polyposis (SP) is considered as a model leading to colorectal cancer (CRC) through the serrated pathway. However, the precise morphological and molecular steps are still incompletely understood. We aimed to characterize the morphological and molecular abnormalities in a series of SP, in CRC and in precancerous lesions.