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DO-001
Whole exome sequencing revealed frequent mutations in esophageal cancer

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Aims. Esophageal cancer is one of the most common malignancies in the Western world with increasing incidence of esophageal adenocarcinoma (EAC). Despite improvements in staging, surgical procedures, and post-operative treatments, the overall survival of patients with esophageal cancer remains low. In order to evaluate the mutation status of EAC and squamous esophageal cancer (SEC) we performed next generation sequencing (NGS) approaches on a wide set of tumor-derived DNA from histologically classified EAC and SEC biopsies.
Methods. Whole exome analysis was performed on 16 DNA samples from histologically characterised esophageal cancer (N=8) and the corresponding non-tumor biopsies (N=8). Extracted DNA was applied to NimbleGen capture exon hybridisation, adapter ligation and subsequent deep sequencing on a Illumina HySeq platform. After tumor macrodissection, DNA from additional 147 formalin-fixed and paraffin-embedded (FFPE) EAC and SEC biopsies was extracted using the Qiagen M48 robotic system. After DNA quality control, multiplex PCR libraries, representing tumor-relevant genetic loci, were prepared from 50 quality controlled EAC and SEC DNA samples. Multiplex libraries were analysed for more than 2000 putative driver mutations by next generation sequencing on the MySeq Illumina platform.
Results. 745 putative driver mutations in 657 genetic loci were found in a first whole exome screening step. p53 hot spot mutations occurred in two third of the esophageal cancers. In addition to the p53 mutations, whole exome analysis identified more than two mutation hits in genes for the regulatory phosphatase unit and cycline kinase 12. These mutations were also addressed by conventional Sanger sequencing. Subsequently, DNA samples from 147 SEC and EAC were studied. Analyses of a hot spot cancer panel in 50 samples, that had passed the quality control, confirmed high frequency of p53 mutations, but a low occurrence of K-Ras mutations. In addition, a set of further mutations such as in PIC3CA, PP2R1B, and PPP1R1B were shown, whose clinical relevance has to be addressed in future studies.

Conclusions. Next generation sequencing is a sensitive method in evaluation of the mutation status of esophageal cancer, providing the opportunity to detect a wide range of genetic alterations, which have to be linked to cancer progression and therapeutic outcome in future studies.

DO-002
A specific expression profile of heat shock proteins and glucose regulated proteins is associated with response to neoadjuvant chemotherapy in esophageal adenocarcinomas

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Aims. Heat-shock proteins (HSPs) and glucose-regulated proteins (GRPs) are molecular chaperones that play an important role in cancer biology and response to anti-tumoral therapy. Esophageal adenocarcinomas are aggressive tumors with a significant resistance to chemotherapy.
Methods. We performed a comprehensive investigation of HSP and GRP expression in 90 formalin fixed paraffin embedded biopsies of locally advanced adenocarcinomas of the esophagus prior to a cisplatin/5FU based neoadjuvant chemotherapy (CTX). Expression of HSP27, phosphorylated (p)-HSP27(Ser15), p-HSP27(Ser78), p-HSP27(Ser82), HSP60, HSP70, HSP90, GRP78 and GRP94 was performed using reverse phase protein arrays (RPPA), immunohistochemistry (IHC) and real-time quantitative RT-PCR (qPCR). Results were correlated with CTX response.
Results. Two distinct groups of tumors with specific protein expression patterns determined by RPPA were identified by unsupervised hierarchical clustering: the first group (pattern A) was characterized by low expression of HSP90, HSP27 and p-HSP27(Ser15, Ser78, Ser82) and high expression of GRP78, GRP94, HSP70 and HSP60. The second group (pattern B) showed the inverse pattern with high expression of HSP90, HSP27 and p-HSP27 and low expression of GRP78, GRP94, HSP70 and HSP60. Tumors with HSP/GRP-pattern A were more likely to show response to neoadjuvant chemotherapy with histopathological tumor regression (p=0.041) and posttherapeutic downstaging (p=0.040). High pretreatment HSP60 expression assessed by IHC (p=0.01) and high HSP60 mRNA levels (p=0.004) were also associated with tumor downstaging.
Conclusions. In summary, RPPA analysis could detect two distinct HSP/GRP protein expression patterns in pretherapeutic biopsies of

esophageal adenocarcinomas, which were associated with different response behaviour to neoadjuvant CTX. Additional findings of IHC and qRT-PCR underline an important role of HSP60 in the context of CTX response. Our findings may offer new insights in the biology of this aggressive cancer entity, may be helpful for the understanding of the mechanisms of chemotherapy response, and may also serve as base for the development of strategies to overcome chemotherapy resistance.

DO-003

Predicting lymph node metastases in early esophageal adenocarcinoma: derivation and validation of a simple scoring system

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Aims. Endoscopic mucosal resection and endoscopic submucosal dissection have advanced the treatment of early esophageal adenocarcinoma. These two techniques are indicated in patients with negligible risk of concurrent lymph node metastasis (LNM). The purpose of the study, therefore, was to design a simple predictive scoring system to uniquely categorize patients' risk of LNM and to validate it in an independent cohort.

Methods. 113 patients with primary esophagectomy for pT1 esophageal adenocarcinoma without neoadjuvant therapy (performed at four academic centres from 2000–2011) were enrolled in the derivation set and 145 patients from a fifth institution in the validation set. Clinical and pathological features were determined and compared between patients with LNM and those without LNM using multivariate analysis in the derivation set. Results were then externally validated in the validation set.

Results. The incidence of LNM was 21/113 (19%) in the derivation set and 23/145 (16%) in the validation set. Tumor size and depth of invasion were significantly associated with LNM in the derivation set and independent cohort. A weighted scoring system was derived from the multivariate model proposing the following four variables to estimate the risk of LNM: tumor size (1 point per cm), depth of invasion (2 points for pT1b), differentiation (2 points for each step of dedifferentiation) and lymphatic invasion (4 points if present). Total number of points then estimated the probability of LNM (0–1: <4%; 2–3: 7%; 4+: 17%). This scoring system showed high accuracy in the validation set (observed/expected ratio=0.90, p=0.88).

Conclusions. We developed and externally validated an accurate and simple scoring system in order to better estimate the risk of LNM, thus contributing to optimal therapeutic decision-making in patients with pT1 esophageal adenocarcinoma.

DO-004

Distinct EGFR, HER2 and HER3 dimerization define the effects of receptor tyrosine kinase inhibitors in esophageal squamous cell carcinoma and Barrett's adenocarcinoma cells

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Aims. Dimerization of specific receptor tyrosine kinases (RTK) of the EGFR-family influences cancer cell biology and may hence guide efficacy of and predict response to RTK inhibitors. Here, we investigated EGFR, HER2 and HER3 expression, co-localization and dimerization in context of the eligibility of esophageal squamous cell carcinoma (ESCC) and Barrett's adenocarcinoma (BAC) for RTK inhibitors -small molecular tyrosine kinase inhibitors (TKI) and humanized monoclonal antibodies (mAB).

Methods. From 110 immunohistochemically pre-screened cases, EGFR/ EGFR, EGFR/HER2 or HER2/HER3 co-expression and dimerization was analyzed in immediate serial tissue sections in ESCCs (n=5) and BACs (n=10) by double immunofluorescence staining (dIF) and in situ proximity ligation assay (in situ PLA). In ESCC (OE21), BAC (OE33) and normal esophageal epithelial (Het-1A) cell lines EGFR/EGFR, EGFR/ HER2 and HER2/HER3 co-expression and dimerization was similarly examined. Effects of the TKIs Lapatinib, Erlotinib and Gefitinib on cell proliferation, survival and signalling were determined by EdU proliferation assay, AnnexinV staining and Western Blot. The mechanism of action of Trastuzumab and Pertuzumab was similarly assessed, but additionally included investigation of antibody-dependent cell mediated cytotoxicity (ADCC).

Results. Reflecting the prevalent expression of EGFR in ESCCs and of HER2 and HER3 in BACs, dIF and in situ PLA revealed preferential EGFR/EGFR homodimers and HER2/HER3 heterodimers in the selected ESCCs and BACs, respectively. Similar expression and dimerization patterns were seen in ESCC (OE21) and BAC (OE33) cells in vitro. Accordingly, Erlotinib and Gefitinib were most effective OE21 cells with EGFR/EGFR homodimers, whereas Lapatinib primarily affected OE33 cells with EGFR/HER2 and HER2/HER3 heterodimers. The mABs Trastuzumab and Pertuzumab alone were ineffective in OE33 cells (HER2+, HER3+). However, upon co-culturing with peripheral blood mononuclear cells (PBMCs) these two inhibitors induced cell cytotoxicity via ADCC in BAC cells exclusively.

Conclusions. Our study shows that distinct RTK co-expression and dimerization occurs in the two histotypes of esophageal carcinomas, determining their eligibility for RTK inhibitors. Thus, EGFR-targeting TKIs may act preferentially in ESCCs and HER2-targeting mABs or Lapatinib in BACs, according to their specific RTK co-expression and dimerization.

DO-005

Whole genome and whole exome sequencing of gastric cancer

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Aims. Gastric cancer (GC) is one of the most common cancers worldwide. In recent decades we witnessed major advancements in the understanding of the epidemiology, pathology and pathogenesis of GC. However, compared with colon and lung cancer, phenotypic and genotypic characterization of GC is still in its infancies and a more in depth view is urgently needed. To further unravel the genetic basis of gastric cancer, we sequenced the whole genome of a microsatellite stable and unstable

gastric carcinoma and validated the prevalence of mutations in a cohort of 454 gastric cancer patients.

Methods. Whole genome sequencing was done on the Illumina HiSeq with an average coverage of 49x. Whole exome sequencing was done using the Agilent SureSelect Human All Exon v2 kit followed by sequencing on the Solid 4 and received an additional average coverage of 55x. Single nucleotide variants (SNVs) and structural variants (SVs) like insertions, deletions, translocations, inversions and tandem duplications were sought. All variants occurring in the normal samples were subtracted from the tumor samples. To identify somatic mutations, all variants found in the dbSNP or 1000 Genomes Project databases were excluded. After an additional quality check the results were compared with common databases like OMIM, HGMD and GWAS in order to find known cancer associated mutations. The main SNV and small indel candidates were selected by quality, genomic region, damaging prediction (Sift, PolyPhen), base conservation (PhyloP) and gene conservation. We performed pathway (KEGG) and functional (GO) analysis of the remaining SNV and SVs using 1087 whole genomes from the 1000 Genomes project as a normal SNV matrix.

Results. We identified a multitude of novel potentially damaging mutations that were validated independently by conventional sequencing, including a mutation of GNAS. The overall prevalence of GNAS-mutations was assessed in an independent cohort of 454 gastric cancer patients.

Conclusions. Our study highlights the strategy of an exemplary tumour genome analysis, which combines both exome and whole genome sequencing with two different NGS platforms, uses population-based whole genome resources as a novel pathway-based filter and integrates SNV as well as structural variant analysis.

DO-006

Gene expression analysis identifies overexpression of myosin light-chain kinase and aurora kinase in a transgenic gastric cancer model by laser capture microdissection

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Aims. Our previous studies have shown associations between Helicobacter pylori infection, strong up-regulation of cathepsin X (CTSX), and the development of gastric cancer. In a gastritis model, CTSX-deficient mice developed significantly more severe spasmolytic metaplasia (SPEM) with higher infiltration of macrophages compared to wild-type mice. Laser-microdissection (LCM) combined with PCR-Array-technology represents an ideal model, indentifying potential candidate genes possibly involved in regulating cytoskeleton and epithelial-mesenchymal transition (EMT) depending on CTSX.

Methods. Wild-type and ctsx-/--mice were infected with H. pylori SS1 for 0–50 weeks. Freshly frozen stomach tissues from these mice were cut into 10 µm sections in a cryostat. After staining, the sections were laser-microdissected using Zeiss PALM system to image, cut and catapult target cells. After procurement of 300 cells, total RNA was extracted. 50 ng of amplified RNA was converted to cDNA. Differential gene expression profiles were analyzed between the two mice groups and weeks post infection. Mouse Cytoskeleton Regulators RT2-Profiler-TM PCR-Array profiles the expression of 84 genes controlling epithelial dynamics in gastric cancer development.

Results. Comparing 4 different stomach cell populations and 3 different time points (24, 36, 50 weeks after H. pylori), we identified 6 cytoskeleton-associated genes, showing differential expression patterns in ctsx-/-- versus WT cell populations (MLCK, AURK, TAU, WASP, Cdk5R1, Cyfip2). Expression levels of AURK and MLCK as well as their co-expression and interaction with CTSX were further analyzed by double immunofluorescence and chemiluminescent co-immunoprecipitation system. This analysis validated the results from the microarray analysis

as the expression of these two genes was significantly higher in SPEM-tissue compared with the normal tissue and that CTSX is involved in these processes.

Conclusions. We successfully obtained expression profiles of gastric cancer cells and identified potential biomarkers interacting with CTSX in gastric cancer. Our results represent the baseline for future structure-/functional analyses for clarifying the functional meaning of CTSX in gastric carcinogenesis.

DO-007

Exome sequencing reveals novel mutations in a GIST and a paraganglioma occurring simultaneously in a patient with complete form of Carney triad

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Aims. Carney triad (CT) is a rare syndrome which is defined by the occurrence of multifocal gastric GIST, paraganglioma and pulmonary chondroma. In contrast to the closely-related Carney-Stratakis syndrome (CSS), CT is not inherited. In both syndromes, the tumors are characterized by a loss of Succinate Dehydrogenase B protein (so-called SDHB-deficient tumors). Only recently, inactivating mutations in the SDH subunits A, B, C, and D have been found to be present in the majority of SDHB-deficient GISTs of CSS, but not in GISTs in the setting of CT.

Methods. We performed exome sequencing of DNA samples derived from blood as well as of the GIST and the paraganglioma from a female patient with a complete form of CT. Additionally, the genome from the blood DNA was sequenced at high coverage, and low coverage genomes of both tumor DNAs were evaluated for copy number variations. SDHA and SDHB mRNA and protein expression were analysed by qRT-PCR and Western Blot analysis, respectively.

Results. Both the GIST and the paraganglioma from the Carney patient were negative for SDHB by immunohistochemistry, and did not display noticeable levels of SDHB in the Western Blot analysis. In contrast, SDHB mRNA levels were equal to SDHA levels, and also to GISTs from sporadic GISTs with KIT mutations. Exome sequencing revealed 10 and 18 somatic missense mutations in the DNA from the GIST and the paraganglioma, which were not present in the DNA from the blood. Additionally, one somatic insertion/deletion was found in each tumor.

Conclusions. These novel mutations will provide better insights in our understanding of this rare albeit fascinating disease, and might also be suitable as targets for future therapies.

DO-008

The lymphangiogenic growth factor VEGF-C participates in the regulation of mTOR Complex 1 and mTORC1 dependent autophagy in ductal adenocarcinoma of the pancreas

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Aims. Recent studies in exocrine pancreatic adenocarcinoma demonstrate a high level of basal autophagy in the cells. This might be responsible for the rapid progression and the therapy resistance of this disease. Accordingly, blocking autophagy might be a future therapeutic

avenue. We have already shown that VEGF-C can induce autophagy and therapy resistance by binding to the non-tyrosine kinase receptor Neuropilin-2. One of the inducers of autophagy is the inhibition of the mTOR protein complex 1 (mTORC1). Therefore, we evaluated the role of VEGF-C in regulating mTORC1 in pancreatic cancer.

Methods. To investigate the role of VEGF-C and its receptor Neuropilin-2 in regulating mTORC1 we reduced protein levels of VEGF-C and Neuropilin-2 by RNA interference in VEGF-C and Neuropilin-2 expressing pancreatic cancer cells lines (CaPan1 and Hs766T). In a parallel approach, we inhibited VEGF-C and Neuropilin-2 signaling by blocking antibodies. After treatment the activation of mTORC1 was measured by evaluating the activation/phosphorylation of its downstream targets, S6K1 and 4EBP1. Upstream signaling was further characterized by investigating the activation of Akt1. The role of VEGF-C induced mTORC1 inhibition in autophagy was measured by using pharmaceutical modifiers of mTORC1 activity.

Results. Blocking VEGF-C and Neuropilin-2 activates mTORC1 and its downstream targets, 4EBP1 and S6K1. Interestingly, this activation is independent of the protein kinase Akt1. mTORC1 activation by VEGF-C/Neuropilin-2 inhibition reduces autophagy significantly. Treatment with Rapamycin, a mTORC1 inhibitor, reverses the effects of mTORC1 activation on autophagy after blocking VEGF-C related signaling pathways. To study the significance of this pathway human tissue samples of ductal adenocarcinoma of the pancreas are evaluated.

Conclusions. Together, these data elucidate signaling pathways that contribute to the induction of autophagy by VEGF-C and its receptor Neuropilin-2. Understanding autophagy related pathways might lead to the development of new cancer therapies.

DO-009
Phospho-ERK expression in erlotinib-treated patients with advanced pancreatic cancer: a translational subgroup analysis from the randomized AIO-PK0104 phase III trial

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Aims. Biomarkers for patients (pts) with advanced pancreatic cancer (APC) receiving anti-EGFR agents are still lacking. Previous translational analyses of AIO-PK0104 found an association of KRAS mutation status with overall survival (OS). ERK 1/2 represent the downstream targets of the RAS-RAF-MEK cascade and are phosphorylated upon MAPK activation. pERK expression might serve as potential prognostic biomarker.

Methods. In the AIO-PK0104 trial 281 pts with treatment-naive APC were randomized between gemcitabine/erlotinib followed by capecitabine and capecitabine/erlotinib followed by gemcitabine. Formalin fixed paraffin embedded (FFPE) tumor tissue for pERK expression analysis by immunohistochemistry (IHC) was available from 153 pts. Within a retrospective analysis pERK data (either as dichotomous or continuous variable) were correlated with AIO-PK0104 biomarker results on KRAS mutation status and EGFR expression and also with efficacy study endpoints using a Cox regression model.

Results. An IHC score for cytoplasmic and nuclear pERK expression was developed (score 0–12). Samples with a score of 6–12 were defined as pERKhigh. 98/153 pts were classified as pERKhigh and 55/153 pts as pERKlow. The median pERK score was 7 (range 0–12). No significant correlation of pERK with baseline pt characteristics like stage of disease, gender, age, KPS or serum CA 19-9 levels was detected. Median OS in pERKhigh pts was estimated with 5.7 months and in pERKlow pts with 6.2 months (HR 1.29, 95% CI 0.90–1.83, p=0.16). When analysing pERK expression as continuous variable, a significant association bet-

ween the pERK score and OS was found (HR 1.06, 95% CI 1.0–1.12, p [log rank] =0.050, p [likelihood ratio] =0.047) indicating an increase in the hazard for death by a factor of 1.06 for each score level of pERK expression. No correlation of pERK expression with the objective disease control rate (OR 0.99, p=0.91) or the occurrence of skin rash (OR 0.93, p=0.41) was detected. Pts with a KRAS mutation and KRAS wildtype patients had similar rates of pERKhigh expression (61% vs. 71%, p=0.32). EGFR expression determined by either IHC (p=0.59) or FISH (p=0.97) had no impact on pERK expression levels.

Conclusions. In concordance to current pre-clinical data, pERK expression seems to have a potential impact on OS of APC pts treated with the anti-EGFR agent erlotinib. pERK expression levels did not correlate with KRAS mutation status. Prospective validation of these results is necessary.

DO-010
Differentially expressed microRNAs as a prognostic marker of liver allograft survival in Hepatitis C patients

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Aims. Reinfection of the liver allograft by Hepatitis C virus is currently inevitable. Yet there are different clinical courses of patients concerning recurrent disease and progression to graft failure. This study aims to evaluate microRNA expression analysis as a prognostic tool for allograft survival in Hepatitis C patients.

Methods. Post transplant liver biopsies of Hepatitis C patients with favourable (n=5) and poor (n=5) clinical course were subjected to RNA extraction. MicroRNA expression patterns were determined by quantitative real time PCR. Altogether the expression of 754 microRNAs was analysed. Relative gene expression was calculated using the $2-\Delta Ct$-method. Statistical analysis was performed using Wilcoxon-test.

Results. In total 590/754 microRNAs were detectable. Of these 33 were found to be differentially expressed between the cohorts (p<0.05). Eleven microRNAs showed a≥2.5-fold difference of the mean relative expression (hsa-miR-944, -197, -296, -1227, -616, -138, -886, -219, -520b, -520e, -328). As to yet no specific function in the pathogenesis of Hepatitis C or transplant rejection has been ascribed to these differentially expressed microRNAs.

Conclusions. Differentially expressed microRNAs could provide a prognostic marker for liver allograft survival of Hepatitis C patients and this new diagnostic tool should be further explored in future studies.

DO-011
IL6 deficiency leads to reduced liver damage and tumorigenesis in Mcl-1Δhep mice

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Aims. Many human chronic liver diseases (e.g. viral hepatitis) are characterized by increased (apoptotic) cell death and proliferation of hepatocytes. Mcl-1Δhep mice, mice with a hepatocyte-specific knock-out of the anti-apoptotic Bcl-2 protein Mcl-1, show chronic loss of hepatocytes and compensatory hyper-proliferation, finally leading to hepatocarcinogenesis and thus are a suitable model for studying human hepatocarcinogenesis.

Methods. Here, Mcl-1Δhep mice were backcrossed to mice deficient of IL6 (IL6-/-), a key driver of hepatocyte proliferation, in order to investigate how hepatocyte-specific deletion of Mcl-1 is leading to liver carcinogenesis during chronic tissue regeneration.

Results. First, by analysing liver damage in Mcl-1Δhep mice at 2 months of age we observed a significant reduction of liver cell damage (reflected by aminotransferase levels). In addition, histological and morphological analyses revealed a significant decrease of apoptotic and proliferative hepatocytes. Second, Mcl-1Δhep -IL6-/- mice developed significantly less liver tumors compared to Mcl-1Δhep mice at 12 months of age.

Conclusions. Il6 deficiency partially rescues liver damage and tumorigenesis in the Mcl-1Δhep mouse model. Our findings illustrate the intimate interplay of cell death and regeneration in hepatocarcinogenesis.

DO-012
HSF1 has a prognostic role and provides growth advantages via DNA-PKcs in human hepatocellular carcinoma

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Aims. Activation of heat shock factor 1 (HSF1) has been previously detected in various cancer types. Here, we investigated the functional role of HSF1 in human hepatocellular carcinoma (HCC).

Methods. HSF1 status was assessed in a collection of human HCC specimens by immunoblotting, real-time RT-PCR, activity assay, and immunohistochemistry. Effects of HSF1 on HCC cell growth were evaluated by inhibition and forced overexpression experiments in human HCC cell lines.

Results. HSF1 and its downstream target, DNA-dependent protein kinase catalytic subunit (DNA-PKcs), were progressively induced from non-tumorous surrounding liver tissue to HCC, reaching the highest expression in the most aggressive tumors. Activated/phosphorylated HSF1 positively correlated with HCC proliferation, genomic instability, and microvessel density, and negatively with apoptosis. Noticeably, activated/phosphorylated HSF1 was a strong predictor of patient's survival length. HSF1 overexpression significantly increased proliferation and survival, whereas a strong decline in cell proliferation and induction of massive apoptosis followed HSF1 silencing via specific siRNA in HCC but not in non-transformed liver cell lines. Massive apoptosis and extensive DNA damage occurred in HCC cells when HSF1 silencing was coupled to treatment with the DNA damaging agent doxorubicin. Effects of HSF1 on HCC cell growth were independent of p53 status, and almost completely blunted by suppression of DNA-PKcs.

Conclusions. HSF1 contributes to multiple aspects of hepatocarcinogenesis and represents a prognostic factor of HCC. Therapeutic approaches aimed at suppressing HSF1 and/or DNA-PKcs might thus be highly beneficial in human HCC.

DO-013
Nup153 is involved in hepatocarcinogenesis

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Aims. Bidirectional transport between the nucleus and the cytoplasm occurs exclusively through the nuclear pore complex (NPC). The NPC is embedded in the nuclear envelope and consists of approximately 30 different nucleoporins (Nups). We could previously show that Nup98 is linked to the p53 pathway and downregulated in a significant fraction of hepatocellular carcinomas (HCCs). Here, we investigated the

expression of Nup153 in HCC patient samples and the biological effects of Nup153 depletion in HCC cell lines.

Methods. Nup153 mRNA and protein levels were determined by using gene expression arrays (40 HCCs and 5 controls) and immunohistochemical staining (IHC) of tissue microarrays (100 HCCs and 20 controls). Two different siRNAs were used to deplete Nup153 for functional assays in HLE and HepG2 cells.

Results. We observed that Nup153 (in contrast to Nup98) was significantly higher expressed on mRNA and protein level in HCC samples. Consistent with this observation Nup153 depletion in HLE and HepG2 cells diminished tumor cell viability. We are currently analyzing the impact of Nup153 knockdown on cell cycle distribution, apoptosis and the expression of genes involved in hepatocarcinogenesis.

Conclusions. We conclude that Nup153 is upregulated in HCC and required for maintaining tumor cell viability in hepatoma cell lines. Although Nup98 and Nup153 share functional and structural similarities it seems that both Nups are differentially expressed in HCC.

DO-014
EEF1A2-PI3K-AKT-mTOR axis contributes to functional inactivation of p53 by stabilizing MDM4 in human hepatocellular carcinoma

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Aims. We aimed at the identification of the molecular mechanisms involved in the upregulation of Mouse Double Minute homolog 4 (MDM4) in human hepatocellular carcinoma (HCC).

Methods. To investigate a potential role of PI3K-AKT-mTOR signaling on MDM4 activity we treated HCC cell lines with small molecule inhibitors and siRNAs and recorded expression changes as well as functional consequences. Expression changes of central pathway components were determined in human liver samples (normal liver, peritumorous liver tissue, and HCC). An AKT transgenic mouse model was used to demonstrate that AKT signaling alters the MDM4 expression in vivo.

Results. Both, inhibition of the PI3K-AKT and mTOR signaling pathways resulted in reduced MDM4 protein levels in HCC cell lines, which was associated with the transcriptional activation of p53-target genes. Biochemical assays revealed that both AKT-mediated phosphorylation and ubiquitin-specific protease 2a-mediated deubiquitination protect MDM4 from proteasomal in human HCC cell lines. The PI3K-AKT-mTOR cascade can be activated by the eukaryotic elongation factor 1A2 (EEF1A2), which is frequently upregulated in human HCC. AKT transgenic mice revealed increased MDM4 protein levels indicating that AKT signaling enhances the MDM4 protein stability in vivo. In human HCCs, we observed a strong positive correlation between the expression of EEF1A2, pAKT, and MDM4, in which strong upregulation was associated with shorter survival of HCC patients.

Conclusions. The protumorigenic function of MDM4 is supported by EEF1A2-mediated activation of the PI3K-AKT-mTOR axis, which involves AKT-mediated phosphorylation of MDM4 and USP2a. Sustained activation of the EEF1A2-PI3K-AKT-mTOR-MDM4 axis negatively affects the survival of HCC patients in vivo, thus the PI3K-AKT-mTOR axis represents a promising molecular target.

Abstracts

DO-019
HMGB1 controls Warburg metabolism in colon cancer cells

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Aims. Energy metabolism is decisive for the resistance of colon cancer cells to chemotherapeutic agents as well as for immunological surveillance. Thus it is important to identify the cellular mechanisms that control the utilization of energy substrates. We have shown in the past that the HMGB1 (high mobility group box 1) protein induces a new form of cell death associated with a massive decline of cellular ATP in cancer cells. We now investigated the effects of HMGB1 on the main cellular energy pathways in colon cancer.

Methods. 13C-NMR measurements, 14C-, 3H2O-tracer experiments, ATP luciferase assay, OXPHOS (oxidative phosphorylation) flux, western blot, immunoprecipitation, generation of rho zero cells, subcellular fractionation, liposome transfection, stable Flag-/Myc-tagged-HMGB1 overexpressing cells, O2-consumption, vibratome generated colon carcinoma tissue slice culture.

Results. Different colon carcinoma cell lines showed high (SW480A), intermediate (HCT116) or low (HT29) sensitivity to the cytotoxic effects of HMGB1. Stable isotope and radio isotope tracer experiments revealed that HMGB1 inhibited the oxygen dependent break-down of glucose favouring its fermentation to lactate. Simultaneously, HMGB1 inhibited the turn-over of oxygen and oxidative phosphorylation in all colon carcinoma cell lines tested. Poor HMGB1 responder colon carcinoma cells could efficiently up-regulate glutaminolysis that rescued them from HMGB1-induced cell death. The inhibition of glutaminolysis with the anti-metabolite L-DON restored the HMGB1 cytotoxicity. The disruptive effects of HMGB1 on the production of energy equivalents was caused by a blockage of tetrameric PKM2 (pyruvate kinase) without affection of the dimeric PKM2, and by an inhibition of cytochrome c oxidase. Consistently, the key enzyme of the rescuing pathway glutaminolysis, malic enzyme 1, was strongly up-regulated in the resistant colon cancer cells.

Conclusions. HMGB1 blocks the utilization of oxygen and the channelling of glucose to the citric acid cycle, thereby inducing a Warburg phenotype in surviving colon cancer cells. Cells resistant to the cytotoxic effects of HMGB1 show a high plasticity in energy metabolism, particularly a high glutaminolysis:glycolysis ratio after HMGB1 treatment. This resistance can be overcome by the simultaneous administration of glutamine-analogues and HMGB1. Targeting energy metabolism with HMGB1 and anti-metabolites might be a promising approach in treating colon cancer.

DO-020
Epithelial layer damage primes the recognition of bacterial products by human intestinal lamina propria cells

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Aims. Human resident intestinal lamina propria cells express only low levels of pattern recognition receptors (PRR). Their mode of activation in the case of intestinal infections therefore remains obscure. Here, we investigated the interrelationship between epithelial layer damage, bacterial stimuli and inflammatory response of colonic mucosal cells using an organ culture model.

Methods. Punches of intact intestinal mucosa or injured mucosa denuded of epithelial cells were cultured in the presence or absence of E. coli lysates. After 12 h, cytokine and surface receptor expression were determined in mucosal tissue as well as in leukocytes emigrated from the injured tissue.

Results. Intact mucosa constitutively released no or only low amounts of IL-1beta, TNF-alpha, IFN-gamma, and IL-22 into the organ culture supernatant. Exposure to E. coli lysates did not affect production of these cyokines. In contrast, injured mucosa denuded of epithelial cells released detectable levels of IL-22, which were further increased in the presence of bacterial lysates. Secretion of IL-1beta, TNF-alpha, and IFN-gamma by the injured mucosa was low /undetectable in the absence but clearly induced in the presence of E. coli lysates. Mucosal expression of CD14, TLR2, and CD86 was up-regulated following epithelial layer damage. It was also increased in mononuclear cells emigrated from the damaged tissue when compared to resting mononuclear mucosal cells. Exposure to E. coli lysates did not significantly further enhance expression of these receptors.

Conclusions. In conclusion, epithelial layer damage is required for the recognition of bacterial products by human lamina propria cells. It may promote recognition of bacterial products by up-regulating PRR in these cells.

DO-021
Stem cell hypothesis and function of Lgr5 in the development of colon cancer

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Aims. Similar to normal colonic epithelium, colorectal carcinomas (CRC) comprise subpopulations of cells with stem-like properties. Leucine-rich-repeat-containing G-protein-coupled receptor (Lgr5) is associated with these stem cells in the normal colonic epithelium and CRC.

Methods. Here, we analyzed the function of Lgr5 in CRC by shRNA-mediated knockdown of Lgr5 in CRC cell lines SW480 and HT-29. Additionally, we exposed Lgr5-EGFP-IRES-Cre-ERT2 mice to azoxymethane/dextrane sodium sulfate which induces inflammation-driven colon tumors. Tumors were then flow-sorted into Lgr5 high and low fractions and molecularly characterized using gene expression profiling and array comparative genomic hybridization.

Results. Silencing of Lgr5 in SW480 resulted in a depletion of spheres, yet did not affect the adherently growing cell population. It also reduced proliferation, migration, and colony formation in vitro as well as tumorigenicity in vivo, consistent with a down-regulation of Notch signaling. Microarray gene expression profiling of flow-sorted mouse colon tumors revealed that Lgr5 high tumor cells have higher Wnt signaling activity compared to both Lgr5 low tumor cells as well as Lgr5 high normal stem cells. Lgr5 high and low tumor cells were both chromosomally stable.

Conclusions. In conclusion, our data suggest Lgr5 as a functionally important marker for stem-like cells in CRC, thus representing a potential therapeutic target.

DO-022
Un-coupling of ACSL5-related mortalin (HSPA9) expression in colorectal adenocarcinomas

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Aims. ACSL5 activities are associated with changes in lipid metabolism as well as expression of mitochondrial proteins. Mortalin (HSPA9) was recently identified as an ACSL5-related stress-response molecule. Aim

of the present study was to characterize mortalin dependent ACSL5 expression in colorectal adenocarcinomas.

Methods. Expression and synthesis of ACSL5, mortalin, and p53 were molecular analyzed in a cell culture approach. The findings were further validated with normal and tumour affected human intestinal tissues.

Results. In enterocytes, positive correlation of ACSL5 and mortalin expression was found. In normal intestinal mucosa, both molecules were predominantly expressed in the most apical located epithelial cells. In colorectal pre-invasive lesions and adenocarcinomas strong expression of mortalin and p53 was found, but ACSL5 was diminished.

Conclusions. ACSL5-related mortalin expression is a physiological phenomenon which is un-coupled in colorectal neoplasias by the p53 pathway.

DO-023

Activator of S-phase Kinase (ASK) is significantly overexpressed in colorectal carcinoma (CRC) and is regulated by miR-30 family

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Aims. Colorectal cancer is the second most common cancer in Germany. Hence there remains an increasing demand for further novel biomarkers in CRC. The aim of this study was to identify aberrant microRNAs (miRNAs) regulating ASK in CRC that might be of potential use as diagnostic marker in CRC.

Methods. In a macro-dissected formalin-fixed paraffin-embedded (FFPE) CRC collective (n=61), and normal colorectal mucosa (n=59), we performed qReal-time PCR based analysis of expression of ASK and miR-30a, b, c, d and e. Post siRNA mediated knockdown of ASK in HCT116 cells we performed cell viability assay (MTT). Using luciferase reporter assay with 3'-UTR of ASK+Luc2P construct and miR-30a, b, c, d and e mimics, we investigated ASK regulation by miR-30 family. Post plasmid based transient ectopic expression of miR-30a, c and e in HCT116 cells, we investigated the expression of ASK by qReal-time PCR and its nuclear localization by immunofluorescence.

Results. ASK is significantly over-expressed in analysed CRC specimens as opposed to normal colonic mucosa ($p<0.05$) and this up-regulation is associated with a significantly decreased expression of miR-30a, c, e in the same patient collective ($p<0.05$). ASK knock down by siRNA in HCT116 caused a decrease in cell viability. Further, miR-30a, c, e mimics significantly abrogated luciferase activity from 3'-UTR of ASK ($p<0.05$). Transient ectopic expression of miR-30a in HCT116 cells decreased cell survival and nuclear localization of ASK.

Conclusions. ASK confers proliferative advantage and might be a novel biomarker in CRC. MiR-30a, c, e that abrogate ASK expression is down-regulated in CRC to maintain ASK over-expression.

DO-024

Detection of miR-34a promoter methylation in combination with elevated expression of c-Met and β-catenin predicts distant metastases of colon cancer

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Aims. After potentially curative resection colon cancer-related death and prognosis mainly depend on occurrence of distant metastases. To prevent distant metastases and to improve survival adjuvant chemot-

herapy is applied. However, a subset of patients does not display distant spread. Therefore, reliable prognostic markers are needed to avoid unnecessary chemotherapy. Here we determined whether the epigenetic inactivation of miR-34a and miR-34b/c genes may serve as a prognostic marker for distant metastases in colon cancer.

Methods. Using a case control study design of 94 primary colon cancer samples with and without metastases to the liver we determined CpG methylation frequencies of the miR-34a and miR-34b/c promoters, expression of the respective mature miRNAs and expression of the miR-34 targets c-Met, Snail and β-catenin. Furthermore, we evaluated whether these parameters may have a prognostic value.

Results. miR-34a methylation was detected in 45.1% (n=42/93) of the samples and was strongly associated with metastases to the liver (p=0.003) and lymph nodes (p=0.006). miR-34b/c methylation was detected in 91.9% of the usable samples (n=79/86). A significant inverse correlation between miR-34a promoter methylation and expression of mature miR-34a (p=0.018) was detected. Decreased miR-34a expression was associated with up-regulation of c-Met, Snail and β-catenin protein expression (p=0.031, 0.132 and 0.004). Increased c-Met, Snail and β-catenin levels were associated with distant metastases (p=0.001, 0.017 and 0.005). In a confounder adjusted multivariate regression model miR-34a methylation, high c-Met and β-catenin levels provided the most significant prognostic information about metastases to the liver (p=0.014, 0.031 and 0.058) and a significant larger proportion of matched pairs showed a higher prevalence of these risk factors in the respective samples with distant spread (p=0.029).

Conclusions. Here we show that the p53-independent inactivation of miR-34a by promoter methylation in primary tumors is associated with loco-regional and distant spread of colon cancer at a high frequency presumably by mediating up-regulation of c-Met, Snail and β-catenin expression. Furthermore, detection of miR-34a silencing in resected primary colon cancer may be of prognostic value, especially in combination with detection of c-Met and β-catenin expression.

DO-025
Salinomycin, an old drug with new antitumor and pro-autophagic activities

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Aims. Salinomycin is a polyether ionophore antibiotic, which has recently been shown to induce cell death in human cancer cells displaying multiple mechanisms of drug resistance including tumor stem cells. The mechanisms by which salinomycin treatment causes cell death have not yet been completely elucidated. In the present work, we investigated the mechanisms by which salinomycin causes caspase dependent and independent cell death in colon cancer cell lines.

Methods. We tested colon cancer cell lines (SW480, SW620, RKO, HCT116) of differing TP53 status, where RKO and HCT116 are TP53 wild type, and SW480 and SW620 carry the hot-spot mutation for colon cancer pR273H. In addition to the TP53 wild-type HCT116, the congenic TP53 knock-out strain was also studied. We applied Western blot, caspase activity, and flow cytometry assays to study the relative contribution of different cell death pathways.

Results. Salinomycin reduced the cell viability in all tested cell lines, but caspases were only strongly activated in RKO cells. In contrast, all investigated cell lines showed characteristics of autophagy such as cytoplasmic vacuolization, uptake of monodansyl cadaverine and LC3 processing. The level of TP53 was increased after salinomycin treatment, but TP53 expression was not required for the induction of autophagy. Instead, TP53 knock-out cells showed stronger LC3B processing than the corresponding TP53 wild type cells. In addition to TP53 activation, salinomycin also led to the formation of reactive oxygen species leading

to JNK1 activation and induction of the downstream transcription factor JUN.

Conclusions. Salinomycin, a novel anti-tumor drug is able to induce autophagic and apoptotic cell death depending on cellular background.

AG Pneumopathologie

DO-027
Evaluation of ERCC1 as a prognostic and predictive marker in early stage NSCLC

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Aims. Excision repair cross-complementing rodent repair deficiency, complementation group 1 protein (ERCC1), is a component of a multi-enzyme complex involved in DNA repair. Operated NSCLC patients with increased ERCC1 concentrations in the tumor were reported to have a superior prognosis compared to patients with low ERCC1. In contrast, ERCC1 is thought to interfere with adjuvant Platinum-based chemotherapy and might therefore act as a negative predictive and prognostic factor in this setting. The aim was to evaluate the prognostic/predictive value of ERCC1 in early NSCLC.

Methods. We retrospectively analyzed immunohistochemically stained FFPE sections of 298 completely resected (R0) NSCLC patients (136 surgery alone, 162 plus adjuvant platinum-based chemotherapy) operated between 2003 and 2008. The slides were stained with two antibodies raised against ERCC1 (8F1 and SP68; provided by Medac, Wedel). An established scoring system (Olaussen et al., 2006) was applied to evaluate the staining pattern. The median value was used as a cut-off to differentiate between high and low ERCC1 staining. Comparison between staining patterns and relation to recurrence-free (RFS) and overall survival (OS) was done with SPSS 20.0 (Munich).

Results. The median score for both antibodies was 2 (range 0–3). However, the agreement between both antibodies was only weak as shown by Spearman rank correlation (r=0.531) and a Cohen's kappa coefficient of 0.34. Both antibodies did not differentiate prognostic groups in patients with surgery alone. However, there was a trend for better RFS in stage I (p=0.133) or squamous cell histology (p=0.2) with high ERCC1 (SP68-staining). No significant difference was seen for OS for either antibody. In patients with platinum-based adjuvant chemotherapy low ERCC1 expression scores (≤2) for both antibodies predicted a significantly better RFS (8F1, p=0.019; SP68, p=0.032) and low scores (≤2) of SP68 also a better OS (p=0.02) in squamous cell carcinomas. No significant differences in RFS and OS were seen for the total of adjuvant treated patients or with regard to tumor stages.

Conclusions. Clone SP68 showed a more distinct staining and was found to be superior compared to the established clone 8F1 with regard to its predictive potential. The most prominent effect concerning prognosis and therapy prediction was seen for patients with squamous cell carcinoma. Therefore, ERCC1 staining with SP68 might aid to select suitable therapy regimens for these patients.

DO-028

Incidence of non-typical EGFR mutations in advanced NSCLC patients—a single center experience

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Aims. Due to the recent development of targeted therapies survival of non-small cell lung cancer (NSCLC) patients can be significantly prolonged while quality of life is preserved. Among these, small molecules inhibiting the epidermal growth factor receptor (EGFR) kinase domain are the most prominent. Thus, characterization of EGFR status is performed routinely in advanced NSCLC patients to evaluate this therapeutic option. Since therapy with tyrosine kinase inhibitors (TKI) is dependent on specific activating mutations within the EGFR gene we retrospectively evaluated EGFR mutation analyses performed in our institute for the presence of non-typical EGFR mutations.

Methods. Retrospectively all EGFR mutation analyses performed at the Institute of Pathology, University Medical Center Freiburg, between January 2009 and July 2012 have been reevaluated. Genetic areas investigated included exons 18, 19, 20 and 21 of the EGFR gene. Mutation analysis was performed by Sanger sequencing. Deletions in exon 19 starting in between aminoacids 745 and 750 as well as L858R mutation in exon 21 were regarded as typical activating mutations. Any mutation occurring at other sites of exon 19 or 21 as well as those which have previously not been characterized with respect to their activating properties in exons 18 and 20 were regarded as non-typical EGFR mutations.

Results. Out of 1050 analyses of the EGFR gene 108 (10.3%) tumors were bearing mutations. 93 (86.1%) were classified as typical mutations. Within this group, three patients revealed typical mutations in both exon 19 and exon 21. Two mutations in exon 21 and two in exon 19 were detected in each of two patients, respectively. All in all 17 (15.7%) non-typical mutations were detected and beside two double mutations 15 (13.9%) patients only revealed a non-typical mutation. Thus, 1.6% of advanced NSCLC patients harbour non-typical EGFR mutations.

Conclusions. To our experience uncommon EGFR mutations occur in 1.6% of advanced NSCLC patients and represent approximately 16% of EGFR mutations. Since activating properties and responsiveness to EGFR-TKI therapy of most non-typical EGFR mutations have not been described in current literature so far careful evaluation of EGFR mutation analyses need to be performed with complete sequencing of the mutation hotspots and specific comments about a possible change in TK-activity should to be included in molecular-pathological reports.

DO-029

comprehensive assessment of diagnostic, prognostic, and predictive clinicopathologic characteristics of pulmonary adenocarcinoma

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Aims. Pulmonary adenocarcinomas (ADC) are a complex, heterogeneous disease with various clinicopathologic and molecular characteristics with relevant prognostic and predictive impact affecting daily treatment decisions. Selected patients have a significantly improved outcome with biomarker-based, tumor-specific therapies compared to standard platinum-based chemotherapy. Linkage of relevant histomorphological, immunohistochemical, molecular, and clinical characteristics by comprehensive data analyses may allow for an optimized patient stratification.

Methods. In order to comprehensively assess yet identified relevant clinicopathologic ADC characteristics by means of the daily routine setting we retrospectively analysed an unselected Caucasian cohort of 425 subsequently resected ADC with available clinical data for histomorphology and diagnostic immunohistochemistry according to the IASLC/ATS/ERS classification, genetic alterations of KRAS, EGFR, B-Raf, and ALK, as well as protein expression of TS and ERCC1.

Results. Besides others we identified significant correlations between morphological and immunohistochemical parameters (e.g. micropapillary/napsin), morphology and predictive biomarkers (lepidic/EGFR mutations, lepidic/ERCC-1 expression, micropapillary/EGFR mutations, invasive mucinous/KRAS mutations), and immunomarkers and predictive biomarkers (TTF-1/TS expression, TTF-1/ERCC1 expression). Furthermore, TS and ERCC1 expression as well as B-Raf mutations were significantly associated with the patients' outcome.

Conclusions. Identification of significantly associated or mutually exclusive criteria by comprehensive data analyses may allow for the establishment of tissue-sparing, time- and cost-effective treatment algorithms and optimized outcomes.

DO-030

Circulating miRNAs as prognostic markers in operable lung adenocarcinoma patients

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Aims. microRNAs (miRNA) are short non-coding RNAs that emerge as a new class of markers for diagnosis and prognosis in non-small-cell lung cancer (NSCLC). miRNAs cannot only be stably quantified in tissues but as well in body fluids like serum uncovering their potential as noninvasive blood-based fingerprints in cancer. However, little is known about circulating miRNAs as prognostic markers in lung adenocarcinoma patients. The aim of this study was to identify novel miRNAs as prognostic markers involved in disease recurrence in serum and tissue of patients with early-stage lung adenocarcinoma.

Methods. Total RNA was isolated from serum samples and tissue specimen and matched non-tumour samples from early-stage NSCLC patients. Serum miRNAs were screened using qRT-PCR based low-density arrays comparing adenocarcinoma patients with and without recurrence 24 months after surgery. Selected serum miRNAs associated with disease recurrence were validated in an independent patient cohort.

Results. Two circulating miRNAs were identified in the screening and confirmed in the validation cohort to be increased in sera of early-stage NSCLC patients suffering from recurrence within 24 months. Elevated levels of the miRNAs were exclusively observed in the group of high-risk patients diagnosed for operable adenocarcinoma compared with benign diagnosis or advanced tumour disease. The differentiation between lung adenocarcinoma patients with low and high risk for recurrence was improved by accounting for the miRNA levels and tumour stage together. The expression of miRNA levels in NSCLC tissues did not reveal an association with metastatic spread as was observed for the circulating form in the sera of early-stage NSCLC patients. Currently, we aim to identify the source of the elevated serum miRNA in formalin-fixed, paraffin embedded (FFPE) tissue of NSCLC patients using miRNA in-situ hybridization.

Conclusions. In conclusion, circulating miRNAs were found to be associated with a high risk of recurrence in early-stage lung adenocarcinoma patients and may serve as putative non-invasive prognostic markers.

DO-031

Long non-coding RNA profiling identifies novel oncogenes and therapy response genes in lung cancer

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Aims. Non-coding RNA profiles in cancer are largely unknown which greatly impedes the discovery of functionally important ncRNAs in tumorigenesis as well as the generation of genome-wide libraries.

Methods. Here, we define the ncRNA expression landscape of lung adenocarcinoma and normal lung as well as for breast and liver cancer in a large set of primary patient samples (n=150).

Results. We provide the first comprehensive map of 17000+ long ncRNAs and discovered hundreds of new ncRNAs associated with lung cancer. The significant ncRNA signatures in this study demonstrate specific ncRNA patterns that are unlikely to be transcriptional background but rather the result of concerted regulation. To mimic the response to cytotoxic chemotherapy, we have also treated lung cancer cells with the DNA damaging agents Cisplatin and Etoposide and found significant deregulation of long ncRNAs—reversing in part the differences seen between normal and malignant lung tissue. Importantly, one of the Cisplatin-regulated ncRNAs interacts with DNA repair factors linking it immediately to the DNA damage response. The so far largest ncRNA expression map is also exploited in two other ways: first, we generate an siRNA library specifically targeting over 600 tumor-associated ncRNAs based on our profiling landscape. This comprehensive library will elucidate the role of ncRNAs in tumorigenesis, viability, apoptosis and the DNA damage response. Additionally, we explore the ncRNA and mRNA landscape using the Guilt-by-Association method to bioinformatically predict ncRNA functions based on their coregulation with protein-coding genes of known function.

Conclusions. In summary, we provide the first global comprehensive map of long ncRNA expression in a broad range of human tumor and normal tissue samples and discovered many new lncRNAs which are characterized at the cellular and molecular level.

DO-034

Differential miRNA expression in neuroendocrine tumours of the lung

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Aims. The differentiation of atypical from typical carcinoids or large cell neuroendocrine carcinomas from small cell carcinomas is essential for treatment decisions and prognosis. However, for pathologists it is often a challenge to establish a reliable differential diagnosis with an accurate prognosis which is restricted in terms of limited specificity of immunohistochemical markers and possible artifacts. Thus we investigated three types of miRNAs as an additional tool for differential diagnosis and possible molecular targets.

Methods. A collective of 38 patients suffering from well to poorly differentiated neuroendocrine lung tumours were examined. Three different miRNAs (miR-21, miR-34a and miR-155) were investigated in four distinct subtypes of pulmonary neuroendocrine tumours by comparative gene expression analysis.

Results. miR-21 expression was increased in high grade neuroendocrine tumours (p<0.000). Contrarily to high grade neuroendocrine tumours, miR-34a showed high expression values in low grade neuroendocrine carcinomas (p=0.008). As for miR-155, a tendency towards increased expression levels in high grade tumours is predictable.

Conclusions. miRNAs seem to play an important role in tumorgenesis of neuroendocrine tumours of the lung. A close association is implicated between the elevated miR-21 in high-grade and miR-34a in low grade NE lung carcinomas which could potentially be exploited as practical biomarkers for early and differential lung cancer diagnosis. However, some additional research and validation studies are needed to utilize them as routine markers or potential targets for personalized medicine.

DO-035

Prognostic morphologic features in small cell lung cancer

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Aims. Small cell lung cancer (SCLC) is the second most frequent entity in lung cancer patients. Although generally prognosis of SCLC still remains poor, patients having survival times of 5 to 10 years are not too rare. In order to evaluate if morphologic features can be of prognostic value we analyzed specimens gathered by primary resection or mediastinoscopy of a cohort of SCLC patients by light microscopy.

Methods. Hematoxilin-eosin stained sections of 67 chemotherapy naïve patients suffering from SCLC were reviewed for specific morphologic features. These included necrosis and pattern of necrosis, nuclear size, shape and chromatin, presence of nucleoli, infiltrating pattern, amount of stromal lymphocytes, stromal fibrosis and mitotic count. Median follow-up was 20 months.

Results. Necrosis and pattern of necrosis did not show significant correlation with overall survival. The same results were observed for pattern of infiltrative growth, nuclear shape and appearance of chromatin. Nuclear size reached a statistical trend for correlation with overall survival (p=0.086). Observation of nucleoli (p=0.033) was significantly correlated with patient's survival as well as the amount of stromal lymphocytes (p<0.001) and mitotic count (p=0.007). Using these three statistically significant morphological features we could calculate a three-tiered morphological score. This score has a highly significant prognostic value (p=0.002) with regard to overall survival.

Conclusions. Delicate morphological analysis of SCLC gives rise to prognostic features, i.e. presence of nucleoli, amount of stromal lymphocytes and mitotic count. These features can be combined to a grading score for SCLC patients with prognostic significance.

DO-036

The proteasome subunits PSMA5 and PSMB4 are potent markers to discriminate between typical and atypical carcinoid tumors of the lung

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Aims. 40S proteasome is an important player in tumorgenesis, cancer-induced immunoreaction and apoptosis. Differences in expression levels of different PSM subunits, especially of the catalytic chains, might be also important for drug sensitivity/resistance and is a potential drugable therapeutic target. A study was designed to test the expression levels of PSM subunits in different pulmonary neuroendocrine tumors, potentially resulting in a feasible predictive or diagnostic marker.

Methods. 80 pulmonary tumors with neuroendocrine features (20 typical carcinoids, 20 atypical carcinoids, 20 LCLC, 20 SCLC) were tested for gene expression levels of PSMA1, PSMA5, PSMB4, PSMB5 and PSMD1. As internal reference genes, ACTB and GAPDH were used. Expression was determined by commercially available TaqMan-Assays (AoD).

Results. All tested enzymes showed a strong correlation in expression pattern to each other (p≤0.01). No correlation to clinical data (age, gender) could be determined except for PSMB4/sex (p=0.022). PSMB4 obviously has the power to divide all different entities (p=0.043), PSMA5 (p=0.024 with exact "Wilcoxon Mann-Whitney Rank Sum Test") and PSMB4 (p=0.050 with exact "Wilcoxon Mann-Whitney Rank Sum Test") show statistical significance to discriminate between typical and atypical carcinoid tumors.

Conclusions. PSMA5 and PSMB4 seem to be powerful diagnostic tools to differentiate the aggressive and the non-aggressive neuroendocrine pulmonary carcinoids from each other. This may result in a useful diagnostic marker, lacking in the nowadays pathology.

DO-037
The non-coding RNA MALAT1 is a critical regulator of lung cancer metastasis

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Aims. Metastasis formation is a critical hallmark capability and the major cause of cancer-related death. The role of long non-coding RNAs (ncRNAs) in this process is largely unknown. Our study aimed at characterizing the role of MALAT1, a highly expressed and ubiquitously expressed ncRNA, in the process of lung cancer metastasis.

Methods. We uncovered the functional importance of the abundant ncRNA MALAT1 in this process by applying a novel human knockout strategy. Zinc Finger Nuclease-mediated site-directed targeting and integration of RNA destabilizing elements into the MALAT1 locus of human A549 lung cancer cells specifically decreased MALAT1 expression about 1000-fold.

Results. Gene expression profiling identified a metastatic gene signature whose expression but not alternative splicing is regulated by MALAT1. Consequently, MALAT1-deficient cells showed a reduced motility in vitro and a dramatic reduction of their metastatic potential in vivo. Moreover, targeting MALAT1 with "free-uptake" Antisense-Oligonucleotides in EBC-1 xenograft tumors impaired lung cancer metastasis in mice.

Conclusions. Therefore, MALAT1 is not only a prognostic marker but also an active player in lung cancer metastasis and a promising target in cancer therapy.

AG Urologische Pathologie

DO-040
Decoy Receptor 3 is regulated by a PI3K-dependent mechanism and promotes migration and invasion in renal cell carcinoma

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Aims. Overexpression of Decoy Receptor 3 (DcR3), a soluble member of the tumor necrosis factor receptor superfamily, is a common event in several types of cancer. In renal cell carcinoma (RCC), DcR3 overexpression is associated with lymph node and distant metastasis as well as a poor prognosis. However, the functional relevance of DcR3 overexpression in renal cancer is unknown. The aim of the project was to elucidate the functional role of DcR3 in renal cell carcinoma.

Methods. Cell culture, siRNA transfection, ectopic transgene expression, Luciferase assays, migration assays, invasion assays, adhesion assays, ex vivo tissue technique, in vivo experiments, Western blotting, quantitative RT-PCR, immunohistochemistry.

Results. In our studies we show that DcR3 strongly promotes adhesion, migration, and invasiveness of RCC cells in vitro. In a mouse xenograft model, this phenotype is confirmed in vivo. Further, we identified the

signaling pathway regulating DcR3 expression in RCC. The expression of DcR3 is regulated in a PI3K/AKT-dependent manner involving the transcription factor NFAT. This mechanism is demonstrated in vitro as well as in an ex vivo model of RCC using slice culture of freshly obtained human RCC tissue.

Conclusions. Our results identify DcR3 as a key driver of tumor cell dissemination and designate DcR3 as a promising marker for rational therapy of renal cell carcinoma.

DO-041
Application of FISH analyses for the detection of translocation tumours on renal cell carcinoma tissue micro arrays

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Aims. Translocation tumours were acknowledged as a new renal cell carcinoma (RCC) subtype in the 2004 WHO classification. Diagnosis is usually performed by IHC analysis for transcription factor E3 (TFE3) and/or Cathepsin K (CTSK) overexpression. As not every translocation variant results in overexpression, several cases might have remained undiscovered. To overcome this problem, a dual color break-apart FISH assay was recently developed for the TFE3 locus. Another translocation tumour variant was discovered in RCC which involves rearrangements of ALK and EML4, a genetic alteration predominantly described in NSCLC, and can be detected by a three color FISH assay. Here, we tested the applicability of both FISH methods on small tissue specimens of a cohort of RCC that had been characterized for TFE3 and CTSK-overexpression. (Project of the German Network Renal Cell Carcinoma)

Methods. Two sets of tissue micro arrays [(TMA), FFPE material], that were already described earlier and comprised 252 renal cell carcinoma punches from patients ≤35 yrs and ≥80 yrs, or 169 punches ≤45 yrs and ≥75 yrs, respectively, were analysed for breaks or rearrangements of TFE3 and ALK/EML4. TFE3 status was analysed by ZytoLight® SPEC TFE3 Dual Color Break Apart Probe and ALK/EML4 status by ZytoLight® SPEC ALK/EML4 TriCheck™ Probe (ZytoVision, Bremerhaven). The resulting fluorescent signals were evaluated and compared to the IHC results we had obtained earlier.

Results. TMA evaluation was compromised by tissue limitations. None of the 6 RCC punches with strong and only one of the 5 punches with weak positivity for TFE3 in IHC could be confirmed by TFE3-FISH. This case had also demonstrated strong CTSK positivity. The remaining 13 CTSK positive (weak to strong staining) cases were TFE3 translocation negative. One papillary tumour, pT1a N0 M0 G2 R0, demonstrated rearrangement of ALK.

Conclusions. Results of IHC were not substantially confirmed using FISH on TMA. On the one hand, the TFE3 or ALK/EML4 status of many cases was not available due to tissue limitations. On the other hand, relying on IHC alone might create false positive TFE3 translocation tumors. Therefore, application of FISH in this context is recommen-

ded to detect genomic rearrangements directly. Moreover, by detecting one case with ALK rearrangement among the evaluable cases, we confirm that this type of translocation is very rarely found in RCC patients.

DO-042
Expression of EpCAM and KIT in renal tumors: diagnostic and prognostic implications in 948 cases

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Aims. Epithelial cell adhesion molecule (EpCAM) is a membrane bound protein with oncogenic properties that is expressed on different normal human epithelia and corresponding malignant tumors. EpCAM expression frequently correlates with more aggressive tumor behaviour. But, expression of EpCAM and prognosis in renal cell carcinoma (RCC) produced conflicting results. Thus, we performed a retrospective study of the expression and prognostic value of EpCAM in RCC using a tissue microarray (TMA) approach. Furthermore the expression of Kit was studied and correlated with EpCAM expression and histopathology.

Methods. We studied the immunohistochemical expression of EpCAM and Kit on TMA containing formalin-fixed, paraffin-embedded samples of 948 patients with documented renal tumors. The tumor diagnosis was made in the period between January 1998 and July 2012.

Results. EpCAM-expression was found in 221/607 (36.4%), 124/152 (81.6%), 54/68 (79.4%), 15/51 (29.4%), 17/45 (37.8%), 12/17 (70.6%) of clear cell RCC, papillary RCC, chromophobe RCC, sarcomatoid RCC, oncocytomas and other rare tumor types, respectively. Log rank tests showed EpCAM-expression to be significantly associated with a longer overall survival (OS, p=0.047). There was a trend toward a longer recurrence-free survival (RFS, p=0.125) in EpCAM-positive RCC. EpCAM expression was significantly correlated with a better RFS in chromophobe carcinomas (p=0.010). Kit-expression was found in 4/281 (0.1%), 27/48 (56.2%), 0/43 (0%), 1/17 (5.9%), 34/41 (82.9%) of clear cell RCC, chromophobe RCC, sarcomatoid RCC, papillary RCC and oncocytomas, respectively. In chromophobe carcinomas Kit-immunoreactivity was significantly linked to EpCAM-expression (p<0.005).

Conclusions. This retrospective analysis suggests a longer OS and RFS in all major RCC subtypes depending on EpCAM expression. There was a significant prognostic value of EpCAM expression in patients with chromophobe carcinomas. Histologic subtypes associated with a higher rate of EpCAM expression were chromophobe carcinomas and papillary carcinomas. Combined EpCAM and Kit-expression analysis may be of diagnostic value to differentiate chromophobe carcinomas from oncocytomas.

DO-043
Relationship between the mTOR signaling pathway and apoptosis regulation in renal cell carcinoma

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Aims. The mTOR signaling pathway is a central regulator of protein synthesis, cell cycle and proliferation. In renal cell carcinoma (RCC) this pathway plays an important role as a novel therapeutic target. Everolimus and other mTOR inhibitors are already established as standard therapeutics for patients with RCC. However, the development of resistance to mTOR inhibition is an emerging clinical problem counteracting the success of these novel targeted therapies. In this study, we

investigated the molecular crosstalk between the mTOR pathway and apoptosis. Moreover, we evaluate mTOR signaling components as novel prognostic and predictive markers for RCC.

Methods. Cell culture, tissue slice culture, Western blotting, apoptosis assays, siRNA transfection, cytotoxicity assays, ectopic transgene expression, proliferation and clonogenicity assays, immunohistochemistry, S35-methionine assay.

Results. In a panel of six RCC cell lines, the effects of Everolimus on apoptosis, cell cycle, proliferation, clonogenicity, and migration were characterized. Treatment with Everolimus resulted in a substantial inhibition of proliferation and clonogenicity. This was paralleled by a strongly diminished protein synthesis, as assessed by S35-methionine assays both in vitro in long term-cultered RCC cell lines and ex vivo in freshly obtained human RCC tissue slices. Importantly, Everolimus alone did not induce apoptosis in RCC cells. The combined treatment of RCC cells with Everolimus and apoptosis-inducing agents, such as the death ligand TRAIL or the Bcl-2/Bcl-XL inhibitor ABT-737, resulted in an additive induction of cell death. We screened for potential predictive markers for response to Everolimus by siRNA-mediated downregulation of several mTOR pathway components. In RCC cells with down-regulated S6 the proliferation-inhibiting effect of Everolimus was abolished suggesting S6 as a candidate for a predictive biomarker. Finally, expression of S6 and pS6 was studied by immunohistochemistry in a large collection of RCC samples and correlated with pathological and clinical data.

Conclusions. The mTOR signaling pathway is active both in long-term cultured RCC cell lines and in ex vivo cultured RCC tissue slices. Everolimus alone strongly inhibits proliferation and clonogenicity, but does not induce apoptosis. In combination with apoptosis-inducing drugs, Everolimus acts in an additive manner. The mTOR pathway components S6 and/or pS6 are promising candidates for prognostic and predictive markers in patients with RCC.

DO-044

Differential hypoxia-associated gene transcripts in patients with acquired chronic kidney disease (CKD)

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Aims. Most chronic kidney diseases (CKD) are initiated as glomerular damage with loss of glomerular capillaries. The pathogenesis of the glomerular insult can be manifold including diabetes and hypertension. The best morphologic indicator of disease progression and development of end-stage renal disease, however, is interstitial fibrosis accompanied by a reduction of capillary density. As hypoxia—a potential consequence of the capillary rarefication—has been associated with fibrosis the question arises whether renal cells indeed face hypoxia in CKD and respond with a transcriptional program which could lead to progression of renal disease. Aim of the study was the systematic protein expression analysis using immunohistochemistry based on gene expression datasets.

Methods. Gene expression profiles from human glomeruli and tubulointerstitium were obtained from more than 160 renal biopsies from patients with different CKD stages using Affymetrix arrays. Renal paraffin tissue samples and clinical parameters of patients with chronic renal failure (IgA Nephropathy, diabetic nephropathy, rapidly progressive glomerulonephritis etc.) were systematically collected for immunohistochemical analysis (n=150). Tumor nephrectomy specimens (n=60) of patients with neoplastic kidney disease were used as reference tissue.

Results. Expression of hypoxia-associated genes was assessed in genome-wide expression profiles. From a total of 84 established HIF-target genes expression levels of 27 correlated with renal function (eGFR) in the cortical tubulointerstitium and 22 in glomerular samples, respectively. To validate these results on protein level we are currently establishing immunohistochemistry of HIF-target genes in human biopsies from patients with a wide range of renal function as measured by eGFR.

Conclusions. Our expression studies in acquired CKD may help to identify the relevant cell types and to potentially establish the stainings as prognostic indicator for renal outcome. Our findings may lead to novel biomarkers and potential targets for CKD with the aim to transform these scientific discoveries into new clinical tools and application for patient benefit.

DO-045

Endocan is upregulated on tumor vessels in invasive bladder cancer and mediates VEGF-A-induced angiogenesis

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Aims. Tumor-associated blood vessels differ from normal vessels at the morphological and molecular level. Proteins that are only present on tumor vessels may serve as biomarkers and as therapeutic targets for inhibition of angiogenesis in cancer.

Methods. To identify factors contributed by the tumor-associated endothelium, we performed immuno-laser capture microdissection (i-LCM) of blood vascular endothelial cells in conjunction with transcriptional profiling from surgically harvested cancer and normal matched bladder tissue of five patients with invasive urothelial bladder cancer.

Results. Comparing the transcriptional profiles of blood vascular endothelium from human invasive bladder cancer and from normal bladder tissue, we found that endocan (endothelial cell-specific molecule-1) was highly elevated on tumor vessels. This upregulation was confirmed by RT-qPCR and immunohistochemistry. Moreover, endocan associated with the filopodia of angiogenic endothelial tip cells in invasive bladder cancer. Notably, endocan expression on tumor vessels strongly correlated with the tumor stage and invasiveness and predicted a shorter recurrence-free survival time in non-invasive bladder cancers. In addition, endocan and VEGF-A levels were significantly higher in plasma of patients with invasive bladder cancer (n=53) compared to healthy individuals (n=60). Accordingly, we found—using cultured blood vascular endothelial cells and an in vivo transgenic mouse model—that endocan expression is strongly upregulated by VEGF-A activation of VEGFR-2. RNA interference-mediated knockdown of endocan in cultured blood vascular endothelial cells revealed that endocan was required for the promotion of cell migration and tube formation by VEGF-A. Furthermore, we found that endocan potentiates VEGFR-2 phosphorylation in response to VEGF-A.

Conclusions. Therefore, blocking the interaction of endocan to either VEGFR-2 or VEGF-A might represent a promising approach for inhibiting tumor angiogenesis. Endocan might also serve as a novel biomarker for monitoring disease progression and the efficacy of VEGF-A-targeting therapies in patients with bladder cancer.

DO-046
Characterization of specific genetic aberrations in squamous bladder tumours

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Aims. Little is known about specific genetic changes of non-Schistosoma associated squamous carcinoma of the bladder. Therefore, in this study we investigated squamous carcinomas, mixed tumours and urothelial cancers for structural genetic differences.
Methods. Urovysion®-FISH was performed on tissue microarray slides. Fifty nuclei of n=25 pure squamous cancers (SCC), n=31 mixed tumours (MIX) and n=19 urothelial carcinomas (UC) could be successfully analysed. Additionally, comparative genomic hybridization of n=35 SCCs, n=40 MIXed and n=22 UC samples was implemented.
Results. All tumours scored positive according to the Vysis FISH criteria. Overall means for CEP3 (1.98±0.67 SCC, 2.6±0.99 MIX, 2.17±0.7 UC), CEP7 (1.70±0.81 SCC, 1.7±0.96 MIX, 1.6±0.68 UC), CEP17 (1.91±0.65 SCC, 2.41±0.69 MIX, 1.97±0.55 UC) and LSIp16 (1.15±1.01 SCC, 0.98±0.80 MIX, 0.98±0.48 UC) showed slight differences of chromosome numbers of squamous and mixed/urothelial carcinomas at chromosome 3 and 17. Mixed tumours presented highest levels of chromosome copies, SCCs showed lowest numbers of chromosome 3 and 17. Comparative genomic hybridization revealed significant differences between the three groups regarding chromosome 3p (p=0.004), 5p (p=0.020), 6q (p=0.028), 11p (p=0.023) and 21q (p=0.018). Significant changes in pure squamous cell carcinomas comprised loss of genetic information at chromosome 3p and 5p. Overall, pure squamous cell carcinoma held the lowest mean number of aberrations per tumour, i.e. 5.34 changes (MIX 6.75 and UC 7.74).
Conclusions. In our non-Schistosoma associated cohort SCCs showed less chromosome copy number changes than mixed and urothelial carcinomas, especially at chromosomes 3 and 17. Comparative genomic hybridization also showed fewest genetic aberrations in pure squamous tumours, and indicated losses at chromosome 3p and 5p as characteristic changes in non-Schistosoma associated squamous bladder cancers.

DO-047
VEGF/mTOR/AKT pathway activation and prognosis in advanced urothelial carcinoma of the bladder

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Aims. Activation of the mTOR-pathway has been described in various tumors. Its role in advanced urothelial carcinoma of the bladder (BCA) is still unclear. Several specific therapy options could be used in the treatment of urothelial carcinomas with mTOR pathway activation. The aim of the present study was to assess the activation of the mTOR-pathway in BCA and correlate it with survival data.
Methods. Tumor tissue of 268 patients with BCA undergoing radical cystectomy was collected in a tissue-microarray (TMA). Histopathological features of all cases were reviewed. Using TMA-based immunohistochemistry diagnostic and prognostic value of mTOR, HIF-1-alpha, p4EB-P1, pAKT and VEGF were assessed.
Results. Overexpression of mTOR pathway enzymes was frequently detected for HIF1-alpha (29.8%), pAKT (53.2%), mTOR (43.3%), p4EB-P1

(28.4%) and VEGF (58.5%). In pT1- (p=0.026) and pT2-tumors (p=0.018) overexpression of pAKT was a significant predictor of progression (PFS). In this subgroup overall survival (OAS) was significantly shorter for patients with pT1- (p=0.021) and pT2-tumors (p=0.026). Overexpression of HIF-1-alpha was associated with significantly earlier recurrence of BCA after radical cystectomy in the subgroup of pT3-tumors (p=0.046). In pT2-tumors high levels of mTOR were predictive for cancer related death (p=0.021).
Conclusions. In patients with advanced BCA activation of the mTOR-pathway, especially pAKT is of prognostic value and might be useful for stratification of patients with potential benefit from targeted therapies against.

DO-048
Expression of TIP60 (tat-interactive protein) and MRE11 (meiotic recombination 11 homolog) predict treatment-specific outcome of localised invasive bladder cancer

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Aims. To determine the association between the proteins: tat-interactive protein 60 kDa (TIP60), p16, meiotic recombination 11 homolog (MRE11), phosphorylated ataxia telangiectasia mutated (ATM), retinoblastoma protein (Rb), Ki67, and p53 and clinical outcome in invasive lymph node-negative bladder cancer.
Methods. Protein expression was measured by immunohistochemistry in cancer specimens from two independent cohorts of patients with bladder cancer treated with cystectomy (162 patients and 273) and one cohort of patients receiving radiotherapy (148). Disease-specific survival (DSS) was used as the outcome measure, and patients with no disease-specific death were followed for a minimum of 36 months.
Results. TIP60 was significantly correlated with DSS in both cystectomy cohorts (hazard ratio [HR] 0.42, 95% confidence interval [CI] 0.26–0.68, p<0.001 and HR 0.45, 95% CI 0.28–0.72, p=0.001). MRE11 was significantly correlated with DSS in the cohort receiving radiotherapy (HR 0.64, 95% CI 0.47–0.86, p=0.005). P16 was significantly correlated with DSS in all three cohorts (HR 0.46, 95% CI 0.30–0.75, p=0.032; HR 0.60, 95% CI 0.37–0.97, p=0.032; HR 0.52, 95% CI 0.28–0.96, p=0.001). Rb was significantly correlated with DSS in one cystectomy cohort (HR 1.71, 95% CI 1.13–2.75, p=0.017). Ki67, p53, and pATM were not significantly correlated with DSS in any of the cohorts.
Conclusions. TIP60 protein expression was a predictive marker for DSS after cystectomy in two independent cohorts. This novel marker was the strongest predictive factor in multivariate analysis in patients receiving cystectomy. MRE11 was shown to be a predictive marker for DSS after radiotherapy. We have shown that TIP60 and MRE11 hold the potential to guide patients with invasive bladder cancer to either cystectomy or radiotherapy. This study was based on retrospective material and consequently we suggest that these markers should be validated in a prospective study.

DO-049
Loss of MTUS1 expression is associated with worse outcome in advanced bladder carcinomas

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Aims. Chromosome 8p deletions are a hallmark of bladder cancer and associated with progression and poor prognosis. However, no distinct target genes at chromosome 8p could be identified to date, which could be used as reliable biomarkers to predict disease outcome and progression. Therefore we compared 8p deletions in 9 pTa and 10 pT1 tumors using Array-CGH (aCGH) and investigated one potential deleted target gene at 8p, MTUS1, in cell culture experiments and in different bladder tumor cohorts.

Methods. DNA from 9 papillary non-invasive pTa and 10 non-muscle-invasive pT1 tumors was isolated and aCGH was performed with Genome-Wide SNP Array 6.0 from Affymetrix. Chromosomal alterations were analyzed using Genotyping Console Software. One potential target gene, MTUS1 was chosen for further analysis. Several bladder cell lines were screened for MTUS1 expression and RT112 was chosen for functional experiments using viability, proliferation and wound-healing assay after MTUS1 overexpression. Additionally, one papillary bladder tumor cohort and one cohort with advanced bladder tumors was stained immunohistochemically for MTUS1 and protein expression was correlated with survival and histopathological parameters.

Results. aCGH analysis revealed that pT1 tumors showed more deletions at 8p than pTa tumors; in pTa tumors, however, also distinct microdeletions could be detected at 8p. Several deleted target genes could be identified including microtubule-associated tumor suppressor 1 (MTUS1). In IHC analysis it could be shown that in papillary non-muscle invasive bladder cancers retained MTUS1 expression was not correlated with survival, but inversely correlated with tumor stage and grade as well as with proliferation (Ki67) and de-differentiation (CK20). However in locally advanced bladder tumors retained MTUS1 expression was associated with better overall and tumor-specific survival. MTUS1 was expressed in all cell lines investigated. After overexpression of MTUS1 in RT112, decreased viability and wound-healing was detected.

Conclusions. Our preliminary functional data show that MTUS1 overexpression has the potential to reduce the malignant potential of urothelial carcinoma cells, MTUS1 influences survival only in advanced (mostly solid) bladder carcinomas, but not in non-muscle invasive papillary

bladder cancers. Thus it is likely that MTUS1 acts as a tumor suppressor gene in advanced bladder cancer and might therefore be suitable as predictive biomarker.

DO-052

Whole exome sequencing identifies YWHAZ and PTK2 as potential therapeutic targets in castration resistant prostate cancer

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Aims. Castration resistant prostate cancer (CRPC) is the most aggressive form of prostate cancer (PCa) with a poor prognosis and remains a therapeutic challenge. The key to the development of novel therapeutic targets for CRPC is to decipher the molecular alterations underlying this lethal disease. The aim of our study was to identify therapeutic targets for CRPC by assessing somatic copy number alterations (SCNA) by whole exome sequencing on five CRPC/normal paired formalin fixed paraffin embedded (FFPE) samples.

Methods. Genomic DNA was extracted from 5 CRPC/normal paired FFPE samples and sequenced using the SOLiD4 next generation sequencing (NGS) platform. The sequencing output was mapped, sorted, filtered and annotated with the help of well-known human genome databases. The results were validated by fluorescence in-situ hybridization (FISH) on a PCa progression cohort containing 137 localized PCa samples, 105 primary tumors, 71 corresponding lymph node metastases (LN) and 39 CRPC samples. Furthermore gene copy number amplification status, mRNA and protein expression were determined in selected PCa cell lines. In vitro functional assays were performed with specific inhibition or siRNA knockdown in PCa cell lines.

Results. Among a set of known amplified/deleted genes in PCa (PTEN, AR, cMYC, NKX3.1), whole exome sequencing identified the 8q12.2–24.22 region including PTK2 and YWHAZ to be amplified. FISH analysis of this region showed an increasing amplification frequency of PTK2 and YWHAZ with disease progression. PTK2 was amplified in 1% of the localized PCa and 35% of CRPC samples. YWHAZ was amplified in 4% of the localized PCa and 48% of the CRPC samples. High level amplification of PTK2/YWHAZ in PC3 cells was revealed by FISH analysis. PTK2 inhibition using the specific inhibitor TAE226 and YWHAZ siRNA knockdown significantly reduced proliferation and migration in PC3 cells.

Conclusions. Our findings suggest that inhibition of PTK2 and YWHAZ could delay disease progression in CRPC patients harbouring amplification of the latter genes. The validated sequencing data show that FFPE tissue could be a promising alternative for SCNA screening using NGS technologies.

DO-053

The biological relevance of the amyloid precursor protein (APP) in prostate cancer

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Aims. In previous studies, we and others showed that overexpression of the amyloid precursor protein (APP) is a recurrent theme in several human malignancies and is thought to cause cell proliferation, migra-

tion, aggressive phenotypes and resistance to standard therapy. In the current study, we identified APP as a novel and specific androgen receptor (AR) target gene in prostate cancer (PCa). Using newly established in-vitro-model-systems as well as gain-of-function and loss-of-function studies, we showed that APP expression is androgen-inducible and significantly correlates with cell growth and chemoresistance.

Methods. Following PCa cell lines were used: LNCaP, VCaP (both AR-positive), DU-145 and PC-3 (both AR-negative) as well as stably transfected LNCaP cell lines expressing either AR in different expression levels (ARmo, modest) and (ARhi, high) or mock vector (pcDNA3.1). APP expression was stably down-regulated using target-specific shRNA approach in both LNCaP and LNCaP ARhi cell lines. PC-3 cells were stably transfected with AR (PC-3 AR) or mock-vector (PC-3 neo). Following functional assays such as cell proliferation (MTT-assay), cytotoxicity (LDH-assay), colony forming assays, qRT-PCR and Western blot analyses were performed.

Results. In the present study, we showed that APP expression is only regulated in the presence of AR and significantly contributes to the pathophysiology of prostate cancer hormone dependently. AR is known to be overexpressed in castration-resistant prostate cancer, allowing to become sensitive to low or no androgen receptor. AR level directly correlated with cell growth and colony forming ability in established LNCaP cell sublines (expressing various AR levels). In this regard, bicalutamide (Bic) treatment significantly down-regulated AR protein levels in both LNCaP and VCaP cells. Only LNCaP responded to Bic in terms of cell growth and clonality. However, endogenous AR overexpression (VCaP) and ectopic AR-overexpression in LNCaP sublines showed no significant effects on Bic treatment. To interrogate the functional significance of APP, we stably down-regulated APP in LNCaP and LNCaP ARhi. Loss of APP decreased proliferation and clonality and promoted apoptosis and chemosensitivity also under AR overexpressing condition.

Conclusions. In conclusion, our results highlight the functions of APP in an AR-dependent fashion. Furthermore, it also represents a novel molecular target structure and potential treatment strategy in castration-resistant prostate cancer.

DO-054

The genomic evolution of prostate cancer under the selective pressure of anti-androgen therapy

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Aims. The implementation of novel technologies such as array comparative genomic hybridization (aCGH) and next-generation sequencing has led to a deeper understanding of the genomic nature of cancer. However, these analyses have classically been done without respecting intra-tumor heterogeneity. Here, we applied a methodology that allows us analyzing the genomic profile of distinct tumor populations from individual tumors and their clonal evolution during the progression to castration-resistant disease.

Methods. Matched pre- and post-hormone treated fresh frozen and/or formalin fixed prostate cancer samples were selected from our biobank. Clonal tumor populations were flow-sorted according to their nuclear DNA content. Sorted tumor populations were subjected to whole genome CGH and to full exome sequencing analyses by the use of Agilent SurePrint 2x400k microarrays and the SureSelect All Exon Kit, respectively.

Results. The genomic analyses of the tumor samples underscore the presence of significant intra-tumoral heterogeneity. The analysis of matched tumor specimens allowed us to identify two particular patterns of tumor evolution during the progression after treatment: first, a more

parallel pattern of tumor evolution, in which the ancestor population is breeding multiple aneuploid tumor clones. In this case, only the 2N ancestor population is able to withstand therapy by the acquisition of few specific genomic aberrations whereas the aneuploid populations are eradicated. Second, a more sequential pattern of tumor evolution with a tumor population that evolves out of a continuous line of clones. This population shows increasing signs of genomic instability over time, with a punctual event of chromothripsis (a massive destruction and rearrangement of chromosomal structures) resulting in castration-resistant clonal tumor populations.

Conclusions. Genomic profiling of distinct clonal tumor populations during prostate cancer progression allows for analysis of intra-tumoral heterogeneity and the underlying clonal evolution. Importantly, this approach identifies genomic aberrations that were selected for under the pressure of hormone ablation therapy.

DO-055

Everolimus as first-line therapy in metastatic castration resistant prostate cancer: results from a phase II trial (SAKK 08/08)

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Aims. To evaluate everolimus, an oral inhibitor of the mammalian target of rapamycin, in patients with metastatic castration resistant prostate cancer (mCRPC).

Methods. Patients with PSA progression during androgen deprivation therapy and no prior chemotherapy were eligible. Everolimus was administered continuously at a dose of 10 mg daily. The primary endpoint was progression free survival (PFS) at 12 weeks defined as absence of PSA, radiographic or clinical progression. 32 evaluable patients were needed with a power of 90% and a significance level of 10% to test a PFS rate at 12 weeks of ≤15% (H0) vs. ≥35% (H1). Potential predictive serum biomarkers were evaluated by proteomics. PTEN status was investigated using PTEN FISH and a combined dual-color chromogenic and silver in situ hybridization assay in tumor tissue.

Results. Of 37 enrolled patients, 13 (35%; 95% CI 20–53%) met the primary endpoint. Confirmed PSA response ≥50% was seen in 2 (5%), and 2 (5%) further patients had a PSA decline ≥30%. Median PFS was 2.8 months (95% CI 1.9–3.6 months). Serum abundance levels of biomarkers were predictive for achieving the primary endpoint. Deletion of the tumor suppressor PTEN was associated with longer PFS. Everolimus treatment was associated with dose-dependent decrease of CD3, CD4 and CD8 T lymphocyte counts and CD8 proliferation and increase in regulatory T-cells.

Conclusions. Everolimus shows some activity in mCRPC but should not be further evaluated in unselected patients. Prolonged treatment with everolimus decreases T lymphocytes and increases regulatory T-cells.

DO-056

An improved ex vivo model for hypoxic microenvironment investigation of prostate cancer

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Aims. Intratumoral hypoxia plays an important role with regard to tumor biology and susceptibility to radio- and chemotherapy. For further investigation of hypoxia-related changes, areas of certain hypoxia must be reliably detected within cancer tissue. Pimonidazole, a 2-nitroimidazole, accumulates in hypoxic tissue and can be easily visualized using immunohistochemistry. To improve detection of highly hypoxic versus normoxic areas in prostate cancer, immunoreactivity of pimonidazole and known hypoxia-related proteins was used.

Methods. In total, 53 patients with localized prostate cancer (pT2a-pT3b, age range 47–73 years, Gleason score 6–9) were enrolled in our study. Pimonidazole was intravenously administered before radical prostatectomy was performed, using the da Vinci robot-assisted surgical system. Prostatectomy specimens were immediately transferred into buffered formaldehyde, fixed overnight and completely embedded in paraffin. Pimonidazole accumulation, hypoxia-related proteins and the degree of vascularization were detected using immunohistochemistry.

Results. In vitro, specific pimonidazole immunoreactivity could be shown by incubating LNCaP prostate cancer cell lines under hypoxia, whereas no immunoreactivity was observed under normoxia. Based on pimonidazole staining, other hypoxia-related proteins and the degree of vascularization in human prostatectomy specimens, maps of oxygen supply in prostate cancer were created.

Conclusions. Here, we describe a combined ex vivo model for an accurate detection of oxygen supply in human prostate cancer tissue. This platform can be used for precise colocalization of novel candidate hypoxia-related proteins in a representative and large number of prostate cancer cases. Furthermore, this study provides a source for further in situ tests and biochemical investigations.

DO-057

Down-regulation of hsa-mir-675 expression during prostate cancer progression

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Aims. This project aims at the identification of microRNA genes deregulated during development and progression of prostate cancer and the elucidation of the underlying epigenetic mechanisms and the functional consequences.

Methods. DNA and RNA were isolated from archival prostate specimens from 19 patients after manual microdissection of normal prostate tissue adjacent to carcinoma (n=19), atypical adenomatous hyperplasia (n=2), atypical small acinar proliferation (n=5), low grade (n=6), and high grade (n=18) prostatic intraepithelial neoplasia, and prostate carcinoma (n=22). microRNA expression was quantified in altogether 72 specimens by probe based stem-loop real-time RT-PCR. mRNA and ncRNA expression were quantified by random primed cDNA synthesis and subsequent real-time PCR using SybrGreen for PCR product detection. For quantitative high resolution DNA methylation studies

pyrosequencing was employed. Protein expression was analysed using immunohistochemical staining of tissue micro arrays.

Results. Hsa-mir-675 and has-mir-675* showed a four- and eightfold decrease, respectively, in prostate carcinoma compared to adjacent normal prostate tissue. The expression gradually decreases in parallel to the morphological progression (from AAH to carcinoma). This down-regulation is accompanied by complex changes in methylation and expression patterns of the IGF2/H19 locus encoding has-mir-675. Hsa-mir-182 and hsa-mir-375 showed down-regulation and no change in expression, respectively, which contradicts published results reporting up-regulation of both microRNAs in prostate carcinoma compared to normal prostate tissue.

Conclusions. Downregulation of microRNA hsa-mir-675 is already detectable in AAH and becomes more pronounced during prostate cancer progression. It represents a very early event in prostate cancer development. Loss of proper imprint control of the IGF2/H19 locus is involved in this deregulation of hsa-mir-675.

DO-058
FOXA1 expression is an independent predictor of early PSA recurrence in ERG negative prostate cancers treated by radical prostatectomy

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Aims. FOXA1 interacts with the androgen receptor and has a potential prognostic role in prostate cancer. The aim of this study was to analyze FOXA1 in a large cohort of prostate cancer specimens and compare these results with clinical and molecular data.

Methods. FOXA1 expression was analyzed by immunohistochemistry on a tissue microarray containing 11,152 prostate cancer specimens. Results were compared to tumor phenotype, biochemical recurrence, ERG status and deletions on PTEN, 3p13, 5q21 and 6q15.

Results. FOXA1 expression was detectable in 97.6% of 8,235 interpretable cancers. FOXA1 expression was considered strong in 28.5%, moderate in 46.2% and weak in 22.9% of cases. High FOXA1 expression was strongly associated with both TMPRSS2-ERG rearrangement and ERG expression (p<0.0001 each). High FOXA1 expression was also tightly linked to high Gleason grade, advanced pathological tumor stage and early PSA recurrence in ERG negative cancers (p<0.0001 each), while these associations were either weak or absent in ERG positive cancers. In ERG negative cancers, the prognostic role of FOXA1 expression was independent of Gleason grade, pT stage, pN stage, surgical margin status and preoperative PSA. Independent prognostic value became even more evident if the analysis was limited to preoperatively available features such as biopsy Gleason grade (p<0.0001), preoperative PSA (p<0.0001), cT stage (p<0.0001) and FOXA1 expression (p<0.0001). Within ERG negative cancers, FOXA1 expression was associated with PTEN and 5q21 deletions (p<0.0001 each).

Conclusions. High expression of FOXA1 is an independent prognostic parameter in ERG negative prostate cancer. FOXA1 measurement (alone or in combination with other molecular parameters) might thus provide clinically useful information in prostate cancer. The significant associations with key genomic alterations of prostate cancer such as TMPRSS2-ERG fusions and PTEN/5q21 deletions suggest interaction with several pivotal pathways involved in prostate cancer.

DO-059
Neuropilin interacting proteins and their impact on radiosensitivity of prostate cancer

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Aims. We have shown that Neuropilin-2 plays an important role in autophagy induction and Docetaxel resistance of prostate cancer. GIPC1/Synectin is an essential adaptor protein that interacts with both Neuropilins. This studies explores the role of GIPC1/Synectin in radioresistance of prostate cancer and as a possible predictive marker for outcome of primary radiation therapy.

Methods. The effect of RNA interference mediated GIPC1/Synectin depletion on clonogenic cell survival after irradiation with 0, 2, 4 or 6 Gy was assayed in two different, GIPC1/Synectin expressing human prostate cancer cell lines. Subsequently, retrospective analysis of clinical outcome data of 358 men who underwent radiotherapy of prostate cancer in curative intention was performed. Uni- and multivariate analysis of prostate specific antigen recurrence-free survival and overall survival in correlation with protein expression in pretreatment biopsy specimen was performed. GIPC1/Synectin was stained by standard immunohistochemistry methods. The evaluation of immunohistochemistry was executed by two surgical pathologists independently and in a blinded fashion.

Results. In cell culture experiments, no change in radiosensitivity after depletion of GIPC1/Synectin in GIPC1/Synectin expressing prostate cancer cell lines could be detected. Furthermore, there was no correlation between GIPC1/Synectin expression in human pretreatment biopsy samples and overall or biochemical recurrence-free survival after radiotherapy in a retrospective analysis of the study cohort.

Conclusions. Our in vitro results do not support a role of GIPC1/Synectin in the cellular radiation response. Still, the role of GIPC1/Synectin in the progression of prostate cancer and its precursors should be subject to further research.

DO-060
MED12 p.L1224F mutation analysis in prostate cancer

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Aims. MED12 plays a role in both, transcription activity and repression, and is involved in many developmental processes. MED12 is part of the mediator complex that regulates RNA Pol II activity. MED12 mutations were recently described in uterine leiomyomas with a high frequency, and all found mutations clustered in exon 2 and intron 1. These mutations seem to be unique for uterine leiomyomas and could not be detected in other tumors. Most recently mutations in the MED12 gene have been reported in 6% of prostate tumors analysed by exome sequencing (Barbieri et al., 2012). In >70% of prostate tumours with MED12 mutation, a recurrent p.L1224F mutation was found. The aim of this study is the validation of the MED12 p.L1224F mutation in an independent cohort of prostate tumours.

Methods. Sections from formalin-fixed, paraffin-embedded and cryopreserved prostate tumours were used for DNA isolation. After precise microdissection the mutation hotspots in exon 26 of the MED12 gene

were analysed by direct Sanger sequencing. Overall, a cohort of 162 un-selected prostate tumours was analysed so far.

Results. All cases could be analysed successfully. The MED12 p.L1224F mutation could not be detected in any of the cases. All tumours showed wildtype sequence.

Conclusions. So far, the recently reported MED12 p.L1224F mutation could not be detected in our unselected cohort of prostate tumours. These data indicate that MED12 gene mutation might either play no role in prostate carcinogenesis or this alteration might be relevant in only a small subgroup of tumours.

DO-061
Significance of Neuropilin-2 and VEGF-C expression in human prostate cancer as predictive markers in a retrospective cohort study

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Aims. We have already shown that a Neuropilin-2/VEGF-C axis controls autophagy and impacts Docetaxel resistance in prostate cancer cells in vitro (Cancer Res, in press). In this study we evaluated the significance of Neuropilin-2 and VEGF-C as predictive markers in a cohort of 353 patients with locally advanced pT3 and pT4 prostate cancer who are treated with prostatectomy alone, with prostatectomy and adjuvant radiation therapy and with prostatectomy and adjuvant hormonal therapy between 1990 and 1997 at Mayo Clinic, USA.

Methods. An Olmsted County patient cohort was developed by selecting 353 patients who underwent radical prostatectomy for locally advanced prostate cancer (pT3 or pT4) without any regional or distant metastasis at Mayo Clinic between 1990 and 1997. One group of patients received adjuvant radiation therapy, one group of patients received hormonal therapy and one control group received no adjuvant therapy. Immuno-histochemical staining for Neuropilin-2 and VEGF-C protein expression was executed in FFPE samples of prostatectomy specimen using a standard protocol at the Institute of Pathology, University Hospital Carl Gustav Carus, TU Dresden. Three surgical pathologists in Dresden and at Mayo Clinic evaluated the staining independently in a blinded fashion. Staining results were correlated with time to progression in both groups—with and without adjuvant treatment.

Results. There was no correlation between time to progression and protein expression of Neuropilin-2 and VEGF-C in the whole cohort in Kaplan Meier analysis. Interestingly, when stratifying for adjuvant treatment, Neuropilin-2 expression influences treatment outcome significantly in Kaplan Meier analysis. In contrast, VEGF-C has no significant impact on treatment outcome. Further statistical analyses including multivariate Cox analysis are ongoing.

Conclusions. Neuropilin-2 might be a viable predictive marker for treatment outcome in patients with locally advanced prostate cancer who receive adjuvant radiation or hormonal ablation therapy.

DO-062
Switch of cadherin expression as a diagnostic tool for Leydig cell tumours

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Aims. Leydig cell tumours belong to the sex cord stromal tumours and comprise approximately 3% of all testicular tumours. The pathogenesis of Leydig cell tumours is still poorly understood. Cadherins (calcium-dependent adhesion molecules) are structural components belonging to the group of adherens junctions. N-Cadherin, the neuronal cadherin, is expressed in different tissues including nervous system and is involved in neurobiological processes and invasion of cancer cells. P-cadherin was first identified in mouse placenta and is expressed in various tumours.

Methods. We investigated 36 testis with Leydig cell hyperplasia or Leydig cell tumours for their expression pattern of P- and N-cadherin. Samples included normal testis (n=20), leydig cell hyperplasia (n=13) and benign Leydig cell tumours (n=23) and were investigated by immunohistochemistry.

Results. P-cadherin proved to be a specific marker for Leydig cells. We could show a switch of expression of P- into N-cadherin in Leydig cell hyperplasia and Leydig cell tumours in comparison to normal Leydig cells.

Conclusions. Cadherin switching could be established as a new immunohistochemical marker for this testicular tumour entity; the switch in Leydig cell tumours could play a role in the differentiation of normal Leydig cells into Leydig cell hyperplasia/tumours.

AG Informatik, digitale Bildanalytik, Biobanking I

DO-063
HL7-Implementation Guide „Pathology Report"

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Aims. Information exchange between pathologists and clinicians, researchers and registries requires a common understanding about the content and the concepts of pathology reports. HL7-CDA as IT-standard enables a structured exchange between all health care sectors in Germany, thus opening the opportunity for pathologists, too, to share automatically their detailed knowledge in pathology reports with other actors in the health care system and in medical research. An Implementation Guide for setting up the interfaces between pathology management systems and hospital information systems, tumor registries, and others according to HL7-CDA is being developed by an interest group of pathologists and informatics specialists.

Methods. Based on the HL7-Implementation Guide "Discharge Letter based on HL7 CDA R2 for German Health System" and coordinated with Austrian ELGA initiative the particularities of pathology reports were defined. Document types have been agreed, taken into account the pathology workflow. The mapping to international code systems and to standard terminologies is in progress.

Results. The HL7-CDA header and body structure of the Discharge Letter is useful for pathology report, too. In only two document types (Pathological-anatomical assessment, Postmortem assessment) and 19 sections (mandatory and optional ones) all aspects of reports in histo- and cytopathology and autopsy can be taken into account. The sophisticated

solution for tumor staging and ICD-O coding from the HL7-Diagnosis Implementation Guide can be used without modifications. The principles of stepwise diagnostics in pathology have been respected. The material section comes out as a key element for organizing and structuring the entire report. It therefore reflects agreements in IHE and DICOM activities. Both general items for describing essential pathological techniques (e.g. for immunohistochemistry evaluation) as well as cancer checklists defined by German Pathology Authorities have been adapted to HL7-CDA requirements. Gaps between genuine German and reference terminologies are being stepwise closed.

Conclusions. A reuse of pathology information from pathology reports depends on a deep semantic understanding of the structure and content of the reports by the possible users. The HL7-Implementation guide will allow the developers and vendors of both pathology management systems and hospital information systems or others to make their software systems really interoperable with each other, across all sector borders.

DO-064

A collaborative approach to medical parameter set acquisition for biobanking projects

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Aims. The successful operation of a biomaterial bank not only requires optimal biospecimen preservation but also well-specified data sets associated with the samples. At the RWTH centralized Biomaterial Bank (RWTH cBMB) data related to a specimen (e.g. associated medical data, research data, sample storage information and personal data of the donor) are managed by an infrastructure of distributed IT-systems facilitating data access for research on the biomaterial while providing highest level of patient data privacy protection.

Methods. At the RWTH cBMB sample storage information is managed by the laboratory information management system StarLIMS. Medical data is acquired and managed exclusively in clinical information systems of the hospital (medico and SwissLab). It is necessary to provide data access for researchers without revealing the personal patient data of the biospecimen donor. Data protection is achieved by pseudonymization and a project-specific restricted access to relevant data (i.e. exactly corresponding to the information need of the research project). The medical parameters required for sample characterization must preferably be defined by the respective researcher in advance. To provide a platform for the collection and specification of these parameter sets, the Biobank WiKi Aachen (BIWIKA) has been deployed and pre-configured with a base set of oncological parameters. BIWIKA also provides collaborative features supporting the communication amongst researchers.

Results. Over the previous time of productive operation of the RWTH cBMB, standard procedures for specimen storage and data administration have been established and proven to be feasible and reliable. Researchers adopted the collaborative tool BIWIKA to collect medical parameter sets. Currently, 139 users are registered in BIWIKA, 33 projects for different studies have been created. Consented parameters have been specified in 15 of them. In total, 606 medical parameters are recorded in BIWIKA.

Conclusions. To successfully run a large biomaterial bank, it is not only necessary to perform quality-controlled biomaterial preservation and tracking thereof, but also to create standard procedures of high-quality data collection, administration, and provision. By using the BIWIKA platform, the feasibility to collaboratively pre-define parameter sets was provided. For involved studies and projects, aggregated data collections based on these parameter sets can be made available to requesting researchers.

DO-065

A content management system for pathology e-learning, postgraduate education and reference

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Aims. Undergraduate and postgraduate teaching in histopathology increasingly depends on the availability of a flexible and expandable internet platform to host teaching cases including whole slide imaging.

Methods. We have developed an internet platform (patholearning.uni-hd.de) that is built according to the needs of the Heidelberg Curriculum Medicinale (HeiCuMed) course system. The platform is divided into general pathology (including neuropathology and dental students), systemic pathology, and a slide reference (digital pathology atlas). All three parts are completely independent, but built upon common routines for the administration of virtual slides, case histories, static gallery images, and literature links. The Textpattern system is used to dynamically generate web pages employing procedural XHTML code. The course content can be extracted into PDF to be used as a course handout by conversion of the Textile markup language into LaTeX employing the Ruby/Redcloth system. Virtual microscopy slides are entirely embedded into the website's categorized content with live preview images.

Results. For the teacher, the platform provides simplified access for the in-class presentation of virtual histology slides and related content. The students make use of the internet site both for in the classroom and home study. The content has been split up into short case presentations, accompanying explanatory texts, quiz material, image galleries and a virtual slide box. The web usage statistics record between 1000–5000 page views per day. Access to the virtual slide atlas of organ pathology is provided by a build in search engine, or alternatively, through a nosological hierarchical system of organ sites and diagnostic terminology. This allows for an associative and disease-oriented browsing of virtual slides. More than 1000 teaching slides have already been deployed together with metadata.

Conclusions. A well organized content management system for pathology teaching greatly facilitates the presentation of teaching cases and related content for the teachers and is very well accepted by the students.

DO-066

Development of an international web-based pathology training program in disease modeling

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Aims. Experimental disease models are increasingly used from basic science to preclinical drug development. Therefore there is a clear need for experts analyzing pathophysiological changes in animal models representing human diseases. The work of disease model pathologists clearly differs from that of the human or veterinary pathologists. Despite this proven increasing need for well-trained experts in translational pathology there are no European overall training programs available yet. The aim of this project is to develop an international, web-based disease model pathology training program for students with higher university degree.

Methods. The program will consist of versatile e-learning components including online lectures, activating learning exercises and video clips demonstrating hands-on work. Web-microscopy will be the most im-

portant training tool in demonstrating differences and similarities between mouse models and the corresponding human diseases. It will comprise common mouse model strains (e.g. C57BL6N) and their distinct spontaneous morphological phenotypes, basic human and murine lesions as well as tumor models and their corresponding human neoplasms. A pilot course will run in spring 2013.

Results. An international web-based training program for animal disease modeling is being developed merging the already existing national training programs and expertise from the participating countries Finland and Scotland. In detail the partners chose Moodle as an e-learning environment, collected material to digitize and add it to the web-microscope, which has been developed in the University of Helsinki, prepared lectures with corresponding voice over and video clips showing routine work in this field. Images scanned to the web-microscope can be linked to the Moodle, and exercises linked to the images can thus be developed in Moodle.

Conclusions. This training program will help to educate specialists in mouse model pathology, therefore raising the number of trained specialists for the increasing demand in research.

DO-067
MOVO.ch: enhancing lectures with mobile voting

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Aims. In 2005 eVoting has been introduced to support pathology lectures at the university of Basel. Acceptance of students and faculty was equally high. The existing system had been regularly used at the Bachelor and Master level of the medical curriculum. It allows the lecturer to ask comprehension questions and to give further explanations on difficult topics dependent on the answers given by the students. The previous system consisted of an emitter device and a computer with a receiver and a software for the evaluation of the results. The results were presented in a parallel beamer projection. As this survey is anonymous, the students participation was high. Unfortunately, this system could be used only in one lecture hall and by one lecturer at the same time. In addition, the batteries of the 150 emitter devices had to be replaced annually. Therefore we aimed at developing an easy-to-use web application, which can be used independently in any lecture hall by the faculty of all Swiss Universities.

Methods. An SQL-based web application (movo.ch) was developed which allows to easily generate multiple choice questions with multi and single choice answers. As a voting-frontend ("clicker") both notebooks and smart phones can be used. A survey among the students had shown that 90% of the students have a smart phone or a notebook. User authentification is done via the Authentication and Authorization Infrastructure (AAI) of the SWITCH network of Swiss universities. All faculty of Swiss universities have access to the voting software. The user-interface-components of the application are: – Vote Management (desktop/notebook web-application): 1. Create Vote (wizard), 2. Run Vote (wizards), 3. Manage Votes (administration of votes) – Voting (web application as interface for the users, for various types of devices)

Results. MOVO has been successfully introduced in the pathology lecture in September 2012. The system was easier to use for the faculty than the previous system. On average, around a third of the students actively participated in the votings. They expressed a positive attitude towards eVotings in lectures.

Conclusions. Movo.ch is an easy-to-use and cost effective alternative for eVoting applications in lectures or for surveys. As most students have access to a mobile device (laptop, smartphone, iPad) this web-based solution has a great potential to enhance teaching.

DO-068
What does it means, when my pathology report says...

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Aims. Patients often have problems understanding the information given in pathology reports. Inspired by Jonathan I. Epstein's "The FAQ Initiative Explaining Pathology Reports to Patients" (http://www.adasp.org/FAQs/faqs.htm), we have started the development of an internet-based free access platform, answering the most frequently asked question in connection with pathology reports in German.

Methods. We used the existing contents of the website as a guideline and translated the questions and answers into German. To provide a better orientation for patients, we designed a colour-coded layout. A brown background is used for fundamental knowledge, green stands for "healthy", red for "malignant" and yellow for everything in between. Additionally we added new topics for example "cervix", based on often-asked questions by patients in Germany.

Results. We have built a free access internet-based platform which provides useful and easy-to-understand information for uncertain patients, but also for physicians and medical students. Additionally it is planed to connect the pathology-platform to other similar platforms of different medical disciplines such as surgery to provide a broader range of information.

Conclusions. The free access internet platform for uncertain patients, who want to understand their pathology reports or find out more about pathology is a firm start, but it is necessary to update the website's content regular and add new topics. We believe that our website offers a helpful and fast assistance for unsettled patients.

DO-069
Open source and public domain molecular modeling software for evaluation of submolecular properties after mutation analysis with emphasis on EGFR in non-small cell lung cancer

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Aims. Recently targeted cancer therapy is becoming more and more widely distributed and mutation analyses are performed on a routine basis among pathology laboratories. Several methods have been established to perform mutation analysis and due to wide spread investigations new mutations are described nearly on a daily basis. Thorough sequence analysis reveals an incidence of approximately 1.6% of uncommon mutations in the EGFR gene of non-small cell lung cancer patients, alone. Thus, for a molecular pathologist it is necessary to provide some insights into submolecular changes of mutations to provide best patient care. We therefore investigated the use of priceless tools for molecular modeling.

Methods. The principle of homology modeling usually is alignment of the new aminoacid sequence to a known protein. Prerequisites are ideally more than 80% sequence homology and data from x-ray crystallography. These structural data are collected in open access protein databanks. Modeling of protein structures is offered by several internet based services and some open source software suites. Modeling results can be visualized by open source programs, some of which are also able to characterize surface properties such as electrostatic potential or hydrophilic and hydrophobic domains. In our study focus was on EGFR analysis of 17 uncommon mutations. Protein templates were retrieved from the ProteinDataBank. We used the ExPASY SWISS-MODEL server with FASTA modeling algorithms for homology modeling, Rasmol software suite and SWISSProt PDB-Viewer for visualization and electrostatic force field calculations.

Results. Retrieval of protein sequences as well as protein structure files used as templates requires some knowledge upon protein structure. Since SWISS-MODEL requires specific input data files some formatting

changes need to be made prior to modeling submission. Calculation times varied but notification about results was usually sent within 3 hours. Resulting pdb-files are downloadable from the SWISS-MODEL website and are ready for visualization using pdb-viewers. Structural changes can be recognized by comparison with the template. Structure relaxation, hydrogenbond calculation and forcefield characterization are possible using the SWISSProt PDB-Viewer.

Conclusions. Open source and public domain services and software suites enable diagnostic molecular pathologists insights to submolecular changes resulting from mutations at a cost-effective basis.

DO-070
Statistics of validation in immunohistochemistry

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Aims. With the in-house immunohistochemical procedures covered by the Medical Device legislation some key figures of their performance have to be demonstrated before a diagnostic use is allowed. For the validation of the procedures the inter- and intraassay precision, the specificity and sensitivity, and the accuracy have to be determined. Statistical approaches are necessary for getting reproducible results. A field study was designed as to develop and test a validation process for class-I-immunohistochemical procedures.

Methods. For two antibodies (pan-CK and Calcitonin) positive and negative tissue controls were selected, and with up to 10 different immunohistochemical protocols studied. In each protocol the reactions were evaluated qualitatively as positive or negative in the positive as well as in the negative tissue parts. The specificity and sensitivity and their appropriate p-values in each protocol were computed. Finally, the robustness, the precision, and the accuracy were determined.

Results. Depending on the tissue controls chosen the performance figures vary in a considerable range. For getting p-values below 0.05 a higher number of tissue controls as well as of protocols might become necessary, especially in those settings which have ambiguous positive or negative tissue controls (so-called cross reactions). The results show the relation between accuracy and number of tissue controls as well as number of protocols to be tested.

Conclusions. Validation immunohistochemistry is a progress in pathohistological diagnostics from subjective observation to objective measurement. It requires considerably higher efforts for the lab. So far the results of the validation have to be reported only. So far not any limits in terms of statistics have been agreed, which could exclude a validated procedure from diagnostic use. For a single institute it will be difficult but necessary to establish those positive tissue controls which are appropriate for the diagnostic problems. This problem will be multiplied for the validation of class-II-immunohistochemical procedures.

DO-071
TMA data analysis by combinatorial means—application examples and quality control

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Aims. After a long incubating phase with only some few publications per year based on TMA data analysis, the publication numbers are now increasing. We have over the years refined a TMA data analysis approach, which focus on unrevealing the dependency between the measured markers, and is exploiting the high number of replicates of TMA data. The algorithm is now mature, and while be optimized for computer clusters, it will be applied on many different real world data sets, to define the characteristics and quality limits of the procedure even more precisely. Some of these results will be critically reviewed here.

Methods. Additionally to the basic invasive breast cancer collectives of 366, 589 and 938 cases, 132 male breast cancers, some smaller collections of 183 squamous cell carcinoma of the oral floor, 144 giant cell tumours of the bone, 390 oropharynx cancers and 243 salivary duct carcinomas, we tried to extend the approach to scenarios with less balanced antagonistic players in e.g. lung cancers with 271 respective 80 cases. The combinatorial algorithm, driven by small, non independent, variances in the collected entities, is defining the dependency structure. These, non random, variances are mirroring some aspects of the underlying biological network.

Results. The algorithm is sensitive for a too homogeneous and uniform shape of expression profiles. If this is the case the mean of all contributing correlations tend to be noticeable different from zero. The resulting dependency becomes fuzzy and instable. The difference between random controls and result is disappearing. Looking on the number of replicates of each analysed cancer entity, it can be observed, that a certain number of replicates is mandatory to generate non random results. This parameter is influenced by several effectors of the system and it seems to be wise, to always calculate the sample global sum of square range along with the specific global sum of square value, to test, if the size of a certain collection is sufficient.

Conclusions. The algorithm with all its test procedures reveals to be stable, as long as certain constraints will be considered. The algorithmic procedure is conceptionally superior to the conventional correlation analysis and other multivariate methods, in that the procedure is deciphering dependencies.

AG Informatik, digitale Bildanalytik, Biobanking II—Bildanalytik, Biobanking

DO-072
A survey of the Bundesverband Deutscher Pathologen about digital pathology in Germany

G. Haroske im Namen des Vorstandes des Bundesverbandes Deutscher Pathologen

Aims. Digital pathology is the integrated management and interpretation of pathology information by information technologies that enables a collaborative approach to patient care. It contributes to bringing pathology back in the center of patient care. Digital pathology is assumed

to gain momentum and acceptance by reduce time and expense in pathology workflow, including the handling of pathology images. A survey was held by the Bundesverband Deutscher Pathologen as to learn to what extent Digital pathology meets the needs and expectations of pathologists in Germany.

Methods. A questionnaire with 10 simple as well as multidimensional questions covering preferably aspects of digital imaging, image management, and usage of digital images was sent out to all members of the Bundesverband. During a period of two and a half weeks the answers were collected.

Results. By the end of the regular deadline sixty-seven pathologists responded. Most of them are older than 50 years and are working as residents. All have a set of imaging devices at their hands; more than 20% are equipped with slide scanners. About 50% of the imaging devices are directly linked with pathology management systems, but more than 30% have no connection to pathology software at all. In only 17% there is an automatic image transfer into the PMS. Almost 30% of the answering pathologists are using their imaging facilities for consultation and/or second opinion. Most of them do it between one and five times a month. Their proportion of all consultations is below 5%. Slide scanners are used in about two third of all applications for consultation and e-learning, in about 15% for tumor conferences, and in less than 5% for routine diagnostics and archiving.

Conclusions. Digital imaging is a technology routinely used in German pathology. However, in the majority of use cases this technology is not closely linked to the routine workflow of the institutes. Although used for consultation regularly, its share in all such applications is rather low. A surprisingly high number of pathologists are already equipped with slide scanners, however suffering from the same limitations as the usage of other digital devices, and hardly used for routine workload. So far e-learning and education is the main field of the application of virtual slides.

DO-073
Cancer diagnostics using machine learning-based image analysis

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Aims. Reflecting their genomic alterations cancer cells display a large variety of morphological phenotypes, whose classification is the basis of modern imaging-based tumor diagnostics and research. The increasing demand for reliable histological feature quantification in tissue specimens, such as grading or (immunohistological) biomarker evaluation calls for standardized, automated analysis approaches. Here, we present a machine learning- based approach for cancer cell detection and classification in histological samples.

Methods. We adopt state-of-the-art machine learning technology using bag-of-words features and present the currently largest available gold-standard data base (the Berlin Pathological Cancer Database) containing over 100,000 manually annotated breast cancer and normal cells. These data are used for training and method evaluation both for cancer cell detection and survival prognosis.

Results. Our approach avoids both the limitations of conventional image analysis, because no segmentation is required, and those of classical machine-learning by allowing for a precise localization of the classification results to individual cells within the respective image regions. The method achieves >95% accuracy in cancer/normal tissue classification and is capable of detecting even individual cancer cells in complex tissues. Moreover, the used image features may also serve as prognostic markers.

Conclusions. In summary, combining machine learning-based methods with the currently largest available cancer image database we introduce a novel computational image analysis approach that connects the ad-

vantages of direct classification and segmentation methods to robustly detect breast cancer cells and predict clinical outcome in histological samples.

DO-074
Adapting computer-based evaluation of immunohistochemically detected tumor-infiltrating immune cells to target cell and tumor morphology: the breast cancer example

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Aims. The spectrum of digital image analysis of immune cells ranges from simple quantification of immunohistochemically stained areas based on intensity per pixel to complex object-based detection of comprehensive patterns of infiltration. The goals of this study were to identify appropriate analysis approaches for the evaluation of lymphoid effector cells and macrophages, to specifically adjust them to applications in breast cancer, and to validate them comparing between manually and automatically quantified tumor infiltrating macrophages and lymphoid cells.

Methods. Digital image analysis was performed in a modular workflow comprised of commercially and publicly available software components, followed by statistical assessment of concordance between pathologist and computer. More than 1200 high power fields of single and duplex immunohistochemistry detecting macrophage and lymphoid effector cell subpopulations were assessed manually und by automated analysis.

Results. The morphology of the target cell population as well as the tumor architecture had significant impact on the accuracy of the automated analysis: while the approximately round to oval-shaped lymphoid cells were accurately reconstructed as distinct image objects, the detection of macrophages was less robust due to variable morphology with different sizes, variable cell processes and -protrusions, and inhomogenous partially cytoplasmic/granular, partially membranous staining patterns. As a consequence, the concordance between conventional and automated evaluation was clearly reduced for macrophages as compared to lymphoid cells (concordance correlation coefficient >0.9 for lymphoid cells and <0.5 for macrophages). A particular challenge were heavily infiltrated areas of intratumoral, desmoplastic breast cancer stroma with densely packed immune cells. Here, automated quantification was limited, while pathologists could provide reasonable approximations to cell numbers based on experience and knowledge of immune cell morphology.

Conclusions. In conclusion, our results confirm that robust evaluation of tumor-infiltrating immune cells requires careful adaptation of computer-based algorithms both to the morphology of the target cell population as well as to the specific architecture of the respective tumor entity. For breast cancer, we show that two fundamentally different analysis strategies, pixel/area-based for macrophages and object-oriented for lymphoid cells can be the optimal solution for robust immune cell assessment.

Abstracts

DO-075

Comparison of image analysis algorithms for the detection of nuclear, membranous and cytoplasmatic antigens in a large cohort of breast cancer patients

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Aims. The differentiation of prognostic subgroups in breast cancer is facilitated by quantitative methods for the evaluation of tumor markers. Image analysis of virtual microscopy images is the preferred method for the quantification of immuhohistochemistry (IHC). We have compared various algorithms for automatic tumor-stroma segmentation and detection of nuclear and cytoplasmatic antigens with manual and semiautomatic techniques.

Methods. TMA arrays with duplicate cores of more than 1500 breast cancer samples were used for IHC staining with 24 conventional and experimental biomarkers for breast cancer, resulting in >500 TMA slides and >70,000 tissue spots to be analyzed. All TMA slides were scanned at 20× (CS Scanner, Aperio Inc., Vista CA), and TMA cores were segmented using the TMA LAB II software and eSlide manager. Image analysis software included nuclear, membrane and cytoplasmatic algorithms, as well as self-learning algorithms for tumor-stroma segmentation (all software by Aperio Inc., Vista CA). Conventional, visual analysis for classic antigens was carried out without knowledge of the image analysis results. All results were compared with clinical data for the respective antigens.

Results. For nuclear antigens, a good correlation between manual evaluation and image analysis was obtained for Ki-67 (r=0.8911), ER (r=0.8431), and PgR (r=0.8652). Results were impaired by technical artifacts and dependent on high quality, low background IHC staining. For the detection of membranous antigens, all HER2 3+ cases were correctly identified by image analysis. However, another 16% of cases were overcalled as 3+, and 27% were overcalled as 2+, indicating that HER2 image analysis may be too sensitive. The measurements for intensity and area of staining for cytoplasmic antigens was generally good, but depending on tumor grade, with high grade tumors most reliably detected.

Conclusions. Image analysis of nuclear, membranous and cytoplasmic antigens is a reliable tool for the quantification of immunohistochemistry in breast cancer. However, results have to be checked visually, and corrected where needed to account for staining artefacts. Further improvements of image analysis algorithms are required to increase the rebustness of this tool.

DO-076

TMARKER: a robust and free software toolkit for histopathological cell counting and immunohistochemical staining estimation

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Aims. Assessment of immunhistochemical staining intensity and percentage of positive cells generally suffers from high inter- and intra-observer variability. Besides, estimation of the intensity and percentage of immunostained cells often involves manual cell counting and is therefore time consuming.

Methods. A novel, free, and open-source software toolkit was developed, connecting already available work flows for computational pathology and immunohistochemical tissue assessment with modern active learning algorithms from machine learning and computer vision.

Results. A new and platform independent software program, called TMARKER, was developed and introduced. The validity and robust-ness of the used algorithms has been shown on a test dataset of human renal clear cell carcinomas and prostate carcinomas.

Conclusions. This new software program together with a user-friendly Java-based graphical user interface enabled comprehensive computational assistance in pathological tissue rating. Routine use of this software toolkit may provide more reliable and robust biomarkers for treatment decision.

DO-077

A simple and reliable method to classify cellular shapes from digitized histological images

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Aims. Quantitative histology is becoming an increasingly important tool in modern pathology. While quantitative information of visualized elements (e.g. staining intensity) has become part of the standard repertoire, quantification of cellular shapes remains currently unexplored in routine diagnostics. Here we established and tested a method to classify cellular shapes in histological images.

Methods. Histological sections were digitized using a whole-slide scanning system (.slide; Olympus systems) and images were selected as regions of interest (ROI) in various formats (Olyvia 2.4; Olympus). Images were processed in ImageJ (Version 1.47F) using the ROI-color coder plugin (v.5; 2012). Images were segmented by color and pixel intensity threshold, analyzed by particles and outlines were color-coded according to the shape measures circularity (0=line; 1=circle), aspect ratio, solidity and roundness. Feasibility was determined using circularity measures in images of prototypic round-cell- and spindle cell lesions whereas diagnostic applicability was tested in challenging images of desmoplastic- and spindle cell melanoma. Variability between cells was ascertained visually and quantitative shape measures were extracted for statistical analysis that included routine statistical measures and t-tests; significance was defined as $P<0.05$.

Results. When automated for 0.7 megapixel images, the conversion time including extraction of quantitative information can be performed in less than 1 minute. Comparison of circularity in prototypic round cell lesions (0.6±0.045; n=131 cells) vs. spindle-cell lesions (average 0.5±0.023; n=677 cells) revealed a significant difference (P=2.3E-8). Comparison of circularity of tumor cells in desmoplastic vs. spindle cell melanoma also revealed a significant difference (P=0.0005) whereby desmoplastic tumor cells were more round (0.6±0.036; n=297 cells) than spindle cell melanoma nuclei (0.6±0.033; n=350 cells). These data demonstrate that quantitative cell shape information can be used to derive a classification cut-off.

Conclusions. We provide proof of principle that the developed algorithms represent a quick and reliable way to characterize cellular shapes.

DO-079

Tissue bank for inflammatory diseases Heidelberg. An innovative platform for translational immunology

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Aims. The tissue bank for inflammatory diseases (GEZEH) in Heidelberg, was founded in 2011 by the Institute of Pathology and the Department of Dermatology as a section of the Sonderforschungsbereich 938. It is a nonprofit organization with a completely evaluated legal and ethical framework and is embedded in the Biomaterial Bank Heidelberg (BMBH) concept.

Methods. Its main aim is the acquisition and characterization of fresh-frozen and paraffin-embedded non-neoplastic human tissues according to the standards of good scientific practice and the promotion of interdisciplinary translational immunology research of the Sonderforschungsbereich and its cooperating partners. It also offers expert project assistance: a project leader can submit a short proposal, and the tissue collecting/preparing process will be performed in cooperation with a specialised pathologist and, if applicable, an experienced clinical researcher.

Results. The tissue bank, in cooperation with the tissue bank of the National Centre for Tumour Diseases (NCT) in Heidelberg is also a central platform for further developing of innovative technologies for tissue handling, e.g. multi-tissue-array and virtual microscopy, with links to digital image analysis and bioinformatics.

Conclusions. Thus, the GEZEH tissue bank represents a model for innovative biobanking and for institutions with active interdisciplinary translational immunology research.

DO-080

Collection of tumor tissue samples exposed to different ischemic conditions before preservation for quality control and biomarker stability studies

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Aims. Accurate detection and quantification of biomolecules in tissue is fundamental to the development of personalized medicine and improvement in the quality of human health care. In particular, molecular profiles in tissue samples should represent the in vivo state rather than a modified or artifactual state induced by pre-analytical variables. Unfortunately, the pre-analytical phase of clinical tissue sample processing and its effect on biomolecules is ill defined. One of the pivotal aims of the Munich Biobank (www.m4.de/) is to establish a unique tumor tissue sample collection for quality control and biomarker development programs to fill this gap.

Methods. The distinctive feature of this tissue collection is that the samples have been exposed in a controlled fashion to different ischemic conditions before freezing or formalin fixation, and that the samples are extensively annotated. Parameters recorded for each sample include: TNM staging, medication before surgery, anesthesia, start of surgery, time of vessel ligation (begin of warm ischemia), time of hand-over to pathologist (start of cold ischemia), time of preservation (liquid nitrogen or formalin). Importantly, a subset of samples was exposed to five experimental cold ischemia time points up to 180 min.

Results. So far, we have collected 1065 samples from 95 patients, composed of 378 and 331 frozen tumor and reference samples, respectively. In addition, we collected from several patients formalin-fixed and paraffin-embedded (FFPE) samples as well (192 tumor, 164 reference samples). The tissue types represented in our collection include 23 colon cancers, 13 gastric cancers, 14 liver cancers, 10 pancreatic cancers, 7 rectum cancers, 7 esophagus cancers, and 21 others. The main characteristics of this unique tissue collection are:
– extensive documentation (clinical data, warm and cold ischemia times),
– malignant and matched normal reference tissue samples,
– additional experimentally delayed cold ischemia times up to 180 min,
– impact of different preservation methods (liquid nitrogen, FFPE).

Conclusions. We envisage studies based on our tissue collection for improving accurate quantitative measurements of the activation state of signalling pathways and for analysing the stability of biomarkers during the pre-analytical phase. These studies will be essential for the discovery of reliable biomarkers for targeted therapies aiming to inhibit deregulated signalling pathways in tumors.

DO-081

Mono- or polychromatically bar coded filter-ribbons of celluloseacetate to individualize tissue specimens from the biopsy needle to the stained section

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Aims. Specimen mix-up in the histology laboratory is a fortunately rare, but potentially devastating source of medical error concerning especially biopsy specimens of different organs (e.g. breast). The main reason why it is difficult to detect the accidental switching of tissue specimens is the fact that normally not the tissue itself but the sample container (e.g. transportation containers, embedding cassettes, slides) is marked. One method to minimize the specimen mix-up is the inking of the specimens in the histology laboratory with six different colors as described by Renshaw et al. To achieve a real individualization of the specimens, I propose the marking of the biopsies with a bar coded filter-ribbon of celluloseacetate directly after the removal of the tissue from the biopsy needle or the endoscopic forceps in the clinic.

Methods. Filter-ribbons of celluloseacetate like those distributed as Endokit by Bio-Optica, Milano, Italy, were bar coded mono- or polychromatically. These filter-ribbons were attached to breast biopsy specimens directly after the removal from the body, fixed in buffered 4% formaline, paraffin embedded, cut and stained with hematoxylin-eosin. Additionally, adhesive labels were printed with the same bar codes and the correspondent alphanumeric information and fixed on the requisition form.

Results. Mostly, the filter-ribbons adhered to the breast biopsy specimens until the tissue was poured into a paraffin block. The bar codes could be simply identified by eye even in the paraffin block and by light microscopy on the hematoxylin-eosin stained histological sections. The comparison between the bar code on the tissue and that on the requisition form was easily possible.

Conclusions. By using mono- or polychromatically bar coded filter-ribbons of celluloseacetate it is possible to individualize biopsy specimens directly after the removal from the body. Combining this technique with a scan system for these bar codes (e.g. CheckMate, Thermo Scientific, USA) and with the paraform sectionable cassette system (Sakura, Japan) may enhance the value of this technique and reduce the specimen mix-up to zero.

AG Hämatopathologie

DO-082

HIF-1 α is a protective factor in conditional PHD2 deficient mice suffering from severe HIF-2 α -induced excessive erythropoiesis

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Aims. Erythropoiesis must be tightly balanced in order to guarantee adequate oxygen delivery to all tissues in the body. This process relies predominantly on the hormone erythropoietin (EPO) and its transcription factor hypoxia inducible factor (HIF). Accumulating evidence suggests that oxygen-sensitive prolyl hydroxylases (PHDs) are important regulators of this entire system.

Results. Here, we describe a novel mouse line with conditional PHD2 inactivation (cKO) in renal EPO producing cells, neurons and astrocytes that displayed excessive erythrocytosis due to severe over-production of EPO, exclusively driven by HIF-2 α. In contrast, HIF-1 α served as a protective factor, ensuring survival of cKO mice with hematocrit values up to 86%. Using different genetic approaches, we show that simultaneous inactivation of PHD2 and HIF-1 α resulted in a drastic PHD3 reduction with consequent overexpression of HIF-2 α -related genes, neurodegeneration and lethality.

Conclusions. Taken together, our results demonstrate for the first time that conditional loss of PHD2 in mice leads to HIF-2 α-dependent erythrocytosis, whereas HIF-1 α protects these mice, providing a platform for developing new treatments of EPO-related disorders like anemia.

DO-083
A novel murine model of myeloproliferative disorders generated by overexpression of the transcription factor NF-E2

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Aims. The molecular pathophysiology of myeloproliferative neoplasms (MPNs) remains poorly understood. Based on the observation that the transcription factor NF-E2 is often over-expressed in MPN patients, independent of the presence of other molecular aberrations, we generated mice expressing an NF-E2 transgene in hematopoietic cells and characterized their phenotype.

Methods. After an autopsy with gross evaluation of the sacrificed mice we performed histological stainings of formalin fixed and paraffin embedded murine bone marrow and spleen tissue as well as cytological staining of bone marrow smears. In addition to standard stainings (H&E, May-Grunwald Giemsa) we established histochemical stainings (NACE, PAS), fiber stainings (EvG, Gomorri), and immunofluorescence stainings (CD3, B220, Ter119). We evaluated the stainings and the results were interpreted according to the "Bethesda proposals for classification of nonlymphoid hematopoietic neoplasms in mice".

Results. These transgenic mice exhibited many laboratory features of MPNs, including thrombocytosis, leukocytosis, Epo-independent colony formation, and expansion of the stem and progenitor compartments in the bone marrow. Moreover, they showed proliferation of myeloid cells in bone marrow and spleen, including erythropoietic, granulopoietic, and megakaryopoietic cells, the latter also presenting morphological abnormalities like cell atypia and clustering. After 18–20 months 10–15% of the mice showed spontaneous transformation to acute myeloid leukemia with leukocytosis, thrombopenia, and anemia as well as increased blasts in bone marrow, spleen, and peripheral blood. The MPN phenotype was transplantable to secondary recipient mice.

Conclusions. According to the Bethesda proposals the NF-E2 transgenic mice exhibited a myeloproliferative disease and spontaneously transformed into an acute myeloid leukemia without maturation.

DO-084
Comprehensive mutation profiling of archival bone marrow trephines using next generation sequencing methodology reveals new features of clonal evolution in myeloproliferative neoplasia

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Aims. This project addresses the question whether comprehensive mutation analysis of fixed, decalcified and embedded bone marrow trephines using high throughput sequencing technologies is possible and whether this approach reveals new features of clonal evolution in myeloproliferative neoplasia (MPN).

Methods. Genomic DNA was isolated from 10 pairs of bone marrow aspirates and bone marrow trephines obtained on the very same day. 60 genes described to be mutated in myeloid malignancies with a frequency higher than 1% were selected from the literature. For target enrichment we designed a solution-based system utilizing 120 nucleotide long biotinylated cRNA baits. The fragment libraries were sequenced on Illumina's GA IIx and MiSeq system according to the manufacture's protocols for paired end sequencing of 76 and 150 bp, respectively. Sequence analysis was performed after BWA short read alignment to the human reference sequence (NCBI hg19) using the GATK analysis tool kit for mutation calling. The mean coverage of each targeted position was more than 100-fold.

Results. More than 10 million base pairs were sequenced per sample, providing a minimum coverage of greater than 30 times for at least 80% of the loci under study. With 100 million base pairs per sample sequenced a minimum coverage of greater than 30 times was achieved for 100% of the loci under study. Individual loci displayed a mean coverage below these values indicating differences in capturing efficiency and the representativeness of the library construction. The concordance rate between aspirate and trephine for all sequence variants was in all sample pairs greater than 92% (mean: 97%). The concordance rate for known pathogenic mutations (0–3 per sample pair) in all sample pairs was 100%. The subsequent analysis of bone marrow trephines from patients who developed a MPN later on identified mutations thought to be MDS-specific, which might represent new risk markers for the development and fibrotic progression of MPN.

Conclusions. Comprehensive mutation profiling of archival bone marrow trephines using high throughput sequencing technologies is possible. The analysis of sequential bone marrow trephines representing disease progression in MPN will lead to the identification of new risk and progression markers.

DO-085
Detection of SRSF2 mutations in chronic myelomonocytic leukemia in decalcified, paraffin-embedded bone marrow trephine biopsies

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Aims. Chronic myelomonocytic leukemia (CMML) belongs to the group of myelodysplastic/myeloproliferative neoplasms. A diagnosis of CMML as outlined in the WHO classification is based on a combination of somehow arbitrary clinical, laboratory and morphological parameters, since CMML lacks specific phenotypical or molecular features. Recently, mutations of SRSF2, encoding for a component of the splicing machinery, were found at high frequency in CMML, and in few cases of MDS and MPN. Aim of the study was to establish an assay for the detection of SRSF2 mutations in decalcified, paraffin-embedded bone

marrow (BM) trephine biopsies and to correlate the findings with clinical and morphological parameters.

Methods. Bone marrow biopsies of CMML as well as cases of MPN and reactive controls were selected from the files of the Institute of Pathology, Tübingen University. All slides were reviewed, and clinical data including PB counts and cytogenetics were reviewed. DNA was isolated from five 5 μm sections. The region containing the mutational hotspot P95 in exon 1 of SRSF2 was amplified by PCR and directly sequenced in both directions. In addition, restriction fragment length polymorphism (RFLP) analysis using the restriction enzyme BsaJI was established.

Results. 31 patients showed the histopathological features of CMML in the BM biopsy and fulfilled the diagnostic WHO criteria. In 15/31 (48%) patients, SRSF2 mutations were identified by Sanger sequencing, including 7 P95H, 5 P95L, 2 P95R and 1 P95A. With the exception of the single P95A case, all SRSF2 mutations were also identified by RFLP analysis. In 7 cases with repeat biopsies, SRSF2 mutational status remained the same in all samples. One of three cases clinically classified as CMML, but not fulfilling the WHO criteria of absolute monocytosis >1×109 showed a P95L mutation, whereas the remaining two cases, as well as cases of MPN and 10 normal controls showed wild type SRSF2.

Conclusions. SRSF2 mutations can be reliably detected in paraffin-embedded BM biopsies. RFLP analysis identifies the vast majority of mutated cases and may serve as screening tool. The frequency and distribution of SRSF2 P95 mutations in CMML identified in our study is comparable to published data, with approximately half of our cases showing a mutation. Further studies will focus on the morphological and clinical features of mutated vs. unmutated CMML and the utility of mutational analysis for the differential diagnosis of MDS, MDS/MPN and MPN.

DO-086

Comparative analysis of hematopoiesis supporting capacity and matrix remodeling of bone marrow stromal cells isolated from patients with myeloproliferative neoplasms

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Aims. Myeloproliferative neoplasms (MPN) are clonal disorders characterized by excessive production of mature blood cells and secondary stromal changes in the bone marrow leading to myelofibrosis. The aim of this study is the comparative analysis of BMSC from MPN patients and non-MPN donors in regard to hematopoiesis supporting capacity, extracellular matrix (ECM) remodeling as well as their in situ localization in the bone marrow.

Methods. BMSC from bone marrow routine aspirates were obtained from patients diagnosed with chronic myeloid leukemia (CML; n=5), essential thrombocythemia (ET; n=5) and polycythemia vera (PV; n=5) and compared to non MPN (control)-BMSC.

Results. Myeloid CFU activity was highest in the supernatant of control BMSC suggesting a decrease of hematopoiesis supporting capacity in BMSC from MPN patients. In the collagen assay, only BMSC from ET (3/5) or CML (1/5) patients extensively remodelled and significantly contracted the collagenous matrix. A significant up-regulation of ECM proteins was detected by qtRT-PCR and immunohistochemistry. In bone marrow biopsies, co-stainings with the recently identified BMSC markers CD271 and CD146 revealed strong co-expression with fibronectin in BMSC from ET patients and showed that BMSC were mobilized from their perivascular and endosteal niche—supposedly attracted by dysplastic megakaryocytes. Remarkably, reticulin staining was absent in these patients suggesting a predictive role for fibronectin in myelofibrosis. As reticulin staining does not correlate with disease progression/prognosis, other ECM proteins including fibronectin were analyzed in bone marrow core biopsies on tissue microarrays including CML (n=16), ET (n=15) and PV (n=16) in comparison to primary myelofibrosis (MF, n=12) and non-Hodgkin's lymphoma (n=19). Correlation with clinical data revealed a positive correlation for higher fibronectin and CD271 expression with lower haemoglobin levels (p=0.001 [both]) and higher blast counts (p=0.006 [fibronectin]; p=0.027 [CD271]) at diagnosis, but no correlation with JAK2 V617F or BCR/ABL expression or spleen size.

Conclusions. In conclusion, our data indicate that MSC from MPN patients actively participate in the process of myelofibrosis. Further analysis will elucidate if fibronectin and CD271 are suitable routine markers for disease prognosis and potential targets for therapeutic strategies aimed at the prevention of disease progression towards secondary MF.

DO-087

Subcellular mislocalization of the transcription factor NF-E2 in erythroid cells discriminates pre-fibrotic primary myelofibrosis from essential thrombocythemia

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Aims. The WHO classification of myeloproliferative neoplasms (MPN) comprises several entities including essential thrombocythemia (ET), primary myelofibrosis (PMF), and MPN-unclassifiable (MPN,U). Differential diagnosis between ET and the pre-fibrotic stage of PMF can be challenging. However, accurate classification is critical as clinical course, therapy and outcome vary considerably between ET and PMF. We have previously shown that the transcription factor NF-E2 is aberrantly expressed in MPN patients. We hypothesized that immunohistochemical staining of NF-E2 can distinguish between ET and PMF.

Methods. We analyzed NF-E2 immunohistochemical staining in two sets of bone marrow biopsies (n=108 total). The first set consisted of 14 healthy controls (HC), 10 patients with reactive thrombocytosis (RT), 25 with ET, 23 with PMF, and 10 with polycythemia vera. The second set consisted of 19 patients with MPN,U. By follow-up, these were subsequently diagnosed as either ET (n=10; MPN,U-ET) or PMF (n=9; MPN,U-PMF). In addition, we examined 7 patients (ET-PMF) initially interpreted as ET, who were, however, found to satisfy the criteria for PMF in the follow-up biopsy. Per case 300 erythroid precursor cells were scored as nuclear or cytoplasmic NF-E2 positive. The results were statistically analyzed by Wilcoxon-test, by the ".632+ bootstrap" method of cross-validation, and by Spearman's rank correlation.

Results. While ET patients, similar to RT patients and HCs showed low levels of nuclear NF-E2 staining in erythroid cells (mean 15%), PMF patients displayed a marked increase in nuclear NF-E2 staining (mean 32%; p<0.0001). Moreover, NF-E2 staining separated MPN,U-ET from MPN,U-PMF and ET-PMF patients statistically highly significantly (p<0.0001 and p<0.01). A cut-off of 20% nuclear NF-E2 staining in erythroid cells correctly classified 18 of the 19 MPN,U samples (95%). The threshold of 20% was cross-validated and revealed an expected error rate of 8.2%. The inter-observer concordance between the two independently scoring pathologists was high (Spearman's coefficient: 0.826).

Conclusions. Here we demonstrate that quantitative immunohistochemistry of bone marrow biopsies for NF-E2 reveals highly significant differences in subcellular localization of NF-E2 between patients with ET and PMF in erythroid cells. We therefore propose that quantitative NF-E2 immunohistochemistry represents a diagnostic tool which can reliably be used to support a differential diagnosis between ET and pre-fibrotic PMF.

DO-088
Complex phosphorylation dynamics control the composition of the Syk interactome in B cells

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Aims. Spleen tyrosine kinase Syk provides catalytic activity for a variety of ITAM-based immune cell receptors and is essential for the development and activation of B cells. Moreover, Syk has been identified as a tumor suppressor an as an oncogene in diverse tissues. Its catalytic activity and the ability to interact with other signaling elements depend critical on dynamic phosphorylation of Syk. Thus comprehensive understanding of the phosphorylation and the interaction of Syk with specific effector proteins is important and purpose of this study.

Methods. The analysis of Syk phosphorylation and its interactome was performed in a three-step experiment. Firstly we used stable isotope labeling with amino acids in cell culture (SILAC) to distinguish between specific Syk interaction proteins an unspecific background and furthermore to compare the phosphorylation state of Syk at different time points of B cell antigen receptor (BCR) stimulation. Secondly we analyzed purified Syk in a global and unbiased manner by high-resolution mass spectrometry. Thirdly we investigated the impact of selected phosphorylation sites and bindig partnes on Syk regulation by mutational analysis.

Results. We have identified the full spectrum of phosphoacceptor sites in human Syk and quantified its complex dynamics in response to BCR stimulation. While the majority of inducible phosphorylations occurred on tyrosine residues, one of the most frequently detected phosphosites encompassed serine 297 located within the linker insert distinguishing the long and short isoforms of Syk. Furthermore we could elucidate the Syk interactome in resting and activated B cells, consisting of more than 25 interacting proteins. One newly discovered group of interacting partners is the 14-3-3 family of adapter proteins, which bind directly to phosphoserine 297 after BCR stimulation. The latter complex attenuates inducible plasma membrane recruitment of Syk, thereby limiting antigen receptor-proximal signaling pathways. Part of this work was published as frontline article in the "European Journal of immunology".

Conclusions. Collectively, the established ligand library provides a basis to understand the complexity of the Syk signaling network. The described regulation of Syk by 14-3-3 represents the first reported serine dependent inhibition of Syk.

DO-089
Heterogeneity of defective germinal center reactions in CVID patients

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Aims. In most CVID patients defective antibody production is accompanied by normal numbers of naïve but low or even absent numbers of circulating class switched memory (cs-mem) B cells and plasmablasts. As differentiation into high affinity antibody secreting plasma cells and cs-mem B cells depends on adequate germinal center (GC) reactions, GC reaction is expected to be disturbed in CVID. The objective was to study defects in GC reactions of CVID patients by combined immune-histomorphologic and flow cytometric examination.

Methods. Immunohistochemical studies were performed on lymph node biopsies from ten CVID patients with benign lymphoproliferation. GC shape, size and polarization were evaluated immunohistologically as well as plasma cell distribution, T-cell zones and granulomatous disease. Lymph nodes of three patients were further investigated by flow cytometry. Peripheral blood samples had been taken into account for all CVID patients.

Results. Most tissue samples showed structurally and immunohistochemically abnormal GC formation with hyperplastic, ill-shaped GC and absent GC in one case. Five cases showed granulomata, two cases among them had giant cells. Two cases showed atypical organization of B- and T-cell compartments. The flow cytometric examination of three CVID lymph node samples showed normal CD10+CD77+CD44lowBCRlow GC-B cells in two patients with GC formation, but not in the patient with absent GC. Interestingly, GC-B cells exhibited an impaired up-regulation of CD86. Irrespective of the presence of GCs however very few IgD-IgM-27+ cs-mem B cells and plasma cells were detectable. In addition, T cell analysis revealed an expansion of PD-1+ CD4 and CD8 T cells. CXCR5hiPD-1hi T follicular helper cells were present in all three examined lymph nodes.

Conclusions. We describe several distinct patterns of disrupted GC formation in CVID patients with benign lymphoproliferation. While centroblasts and centrocytes are still present in most patients, memory B cells and plasma blasts are clearly diminished suggesting rather a failure of the output than the formation of GC in CVID.

DO-090
Development of an allele-specific PCR for the detection of the MYD88 L265P mutation in paraffin-embedded bone marrow biopsies of lymphoplasmacytic lymphoma and other small B-cell NHL

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Aims. The MYD88 L265P mutation has been shown to promote NF-κB signaling, thus mediating cell survival in B-cell neoplasms. In addition to a subset of DLBCL of activated B-cell type, this mutation is present in 67% to 91% of cases of lymphoplasmacytic lymphoma (LPL) and a subset of marginal zone lymphomas. Since LPL is usually diagnosed in the bone marrow, we aimed to design a sensitive molecular assay for the MYD88 L265P mutation in decalcified, paraffin-embedded BM biopsies.

Methods. Bone marrow biopsies of LPL and other small B-NHL as well as reactive controls were selected from the files of the Institute of Pathology, Tübingen University. In total, 40 cases of LPL, 10 cases of marginal zone lymphoma (MZL) and 11 cases of B-cell chronic lymphocytic leukemia (CLL) were analysed, with infiltration ranging from 10 to 100% of marrow volume. DNA was isolated from whole BM sections without microdissection. As positive control, we used the TMD-8 DLBCL cell line known to contain the MYD88 L265P mutation. For identification of the MYD88 L265P mutation, we developed an LNA-clamped PCR with subsequent melting curve analysis.

Results. Using a dilution series with the TMD-8 cell line, the mutation was reliably identified when present in 10% of analyzed cells. In the BM samples, MYD88 L265P was found in 30/40 (75%) LPL and 3/10 (30%) MZL cases, whereas all CLL samples and reactive controls remained negative.

Conclusions. LNA-clamped PCR with subsequent melting curve analysis is a fast and sensitive method for analyzing MYD88 L265P mutations in FFPE bone marrow samples. As previously reported, this mutation is common in LPL (75%), but not entirely specific in the setting of small B-NHL in the bone marrow, since it can also occur in cases of MZL. Detection of the MYD88 L265P mutation is a valuable tool for the diagnosis of small B-cell proliferations in the bone marrow biopsy.

DO-091
Spindle shaped CD163+ rosetting macrophages replace CD4+ T cells in HIV-related classical Hodgkin lymphoma

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Aims. Combination antiretroviral therapy is highly effective in HIV-infection, leading to decreased incidences of AIDS-defining neoplasms. However, HIV-patients still have a 10-fold increased risk of developing classical Hodgkin lymphoma compared with the general population. As Hodgkin- and Reed-Sternberg cells represent only a minority in the tumor infiltrate, the aim of the present study was to characterize the microenvironment of HIV-related classical Hodgkin lymphoma and compare it with classical Hodgkin lymphoma cases of immunocompetent individuals.

Methods. CD4+ and CD8+ T cells, CD163+ macrophages as well as CD30+ Hodgkin- and Reed-Sternberg cells were quantified in the immunohistological stainings of the lymph nodes using a light microscope equipped with a camera system in 24 HIV+ Hodgkin patients and 15 HIV− Hodgkin patients.

Results. The major morphologic differences were the presence of necrotic foci and the absence of epithelioid cell formation in HIV-related Hodgkin lymphoma. We observed a significantly decreased number of CD4+ T cells and a significantly increased number of CD163+ macrophages in HIV-related Hodgkin lymphoma. Cases exhibiting a "sarcomatoid" pattern of the reactive infiltrate exhibited significantly greater numbers of macrophages, associating the "sarcomatoid" pattern to the presence of spindle shaped macrophages. Whereas rosetting of CD4+ T cells around Hodgkin- and Reed-Sternberg cells was frequently observed in classical Hodgkin lymphoma in immunocompetent persons, rosetting in a subset of HIV-related Hodgkin lymphoma cases appeared to involve cytoplasmic protrusions of spindle shaped macrophages.

Conclusions. HIV-related Hodgkin lymphoma, therefore, is characterized by unique morphologic features, which should be recognized by pathologists.

DO-092
Sequential Hodgkin's and non-Hodgkin lymphomas in non-immunocompromised patients

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Aims. Rarely, two different varieties of Hodgkin lymphoma (HL) or non-Hodgkin lymphoma (NHL) can occur in the same patient either sequentially or simultaneously. Two distinct lymphoid malignancies occurring simultaneously in the same anatomic site are defined as composite lymphomas and are often clonally related. In this study, we assessed the frequency of HLs and NHLs diagnosed at our institution in order to identify sequential or simultaneous lymphomas.

Methods. Upon review of our institutional database for nodal and extranodal lymphomas diagnosed either by excisional lymph node biopsy or organ biopsy (1993–2011), we retrieved 445 cases of B-NHL, T-cell or NK/T cell neoplasms and 99 cases of HL. Four of 540 cases (0.9%) occurred in the same patient. All cases were re-classified according to the current WHO classification system (2008) and additional immunhistochemical and molecular analyses were performed.

Results. Interestingly, all four cases consisted of a combination of HL and NHL with a latency of occurrence between 4 and 11 years. Not all HL were EBV associated and none of these patients were known to suffer from autoimmune disorders or had a history of immunosuppression.

Conclusions. When progression of disease was excluded, sequential lymphomas were exceedingly rare in our collective (4/540) and represented HL followed by NHL or vice versa. Sequential lymphomas can occur with a long latency indicating the need for long term follow up and are not always associated with EBV infection, autoimmune disease or immunosuppression.

DO-093
CD10 is expressed by a subset of TFH cells: further evidence that CD10 positivity in angioimmunoblastic T-cell lymphoma (AITL) reflects ontogeny

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Aims. CD10 is a well-known marker of germinal center (GC) B-cells. It is also variably expressed by neoplastic T cells in angioimmunoblastic T-cell lymphoma (AITL), which is known to derive from the follicular helper T cell (TFH) subset located within the GC. However, CD10 expression is regarded as aberrant in AITL and CD10 has not yet been reported in normal TFH. We aimed to identify CD10-expressing T cells in reactive conditions and B-cell lymphomas and to characterize this population as a subset of TFH.

Methods. Fifteen reactive lymph nodes and 6 tonsils, 24 follicular lymphoma (FL), 9 marginal zone lymphoma (MZL), 5 mantle cell lymphoma (MCL) and 6 nodular lymphocyte predominant Hodgkin lymphoma (NLPHL) were analyzed for the presence of CD10-expressing T cells by immunohistochemistry.

Results. 17/21 (81%) reactive lymph nodes or tonsils showed a variable number of strong (stronger than in GCB cells) CD10-positive lymphocytes. These lymphocytes were distributed throughout the GC, with tendency to accumulate at their periphery and paralleling the distribution of PD1+, CXCL13+ and/or ICOS+ cells. Strong CD10+ cells were also observed in FL (11/24, 46%) and MZL (9/9, 100%), but neither in MCL nor NLPHL. These cells did not co-express PAX5, but stained for CD3, ICOS, PD1 and/or CXCL13. Multicolor flow cytometry confirmed a stable proportion of CD10-expressing CD4posCXCR5hiICOShi TFH cells within reactive tonsils (19±8%, n=9). Interestingly, the percentage of CD10-positive TFH is also strongly correlated with the percentage of CD19posCD10pos GCB cells.

Conclusions. Expression of CD10 in mature T-cells is physiological and can be attributed to a subset of TFH in reactive GC and in the microenvironment of FLs and MZL. Therefore, CD10 in AITL appears to reflect ontogeny and likely is not aberrant. Cell interactions in reactive and neoplastic GC and in AITL might play a role in the regulation of CD10.

AG Paidopathologie

DO-095
MYC-N and its regulation in nephroblastomas

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Aims. Amplification and deregulation of the cellular protooncogene MYC-N has been described in different solid neoplasms of childhood. The number of copies of the MYC-N gene in nephroblastomas did not correlate with expression levels in all the samples described so far. In

our study we therefore investigated possible copy number variations, MYC-N expression levels and the expression levels of microRNA 34a, which has already been identified to target MYC-N.

Methods. Total RNA and genomic DNA were extracted from formalin-fixed, paraffin-embedded samples. After generation of cDNA, a quantitative REAL time (qRT) PCR was performed to quantify mRNA expression levels of MYC-N. We also investigated the regulation of this oncogene in nephroblastomas. A possible amplification was examined by PCR-based genomic copy number assay, the results were also verified by FISH analysis. The expression levels of miR-34a were examined by qRT PCR.

Results. So far we investigated several different subtypes of nephroblastomas and in part of the samples an overexpression of MYC-N was identified. Gain of chromosomal material in the chromosomal region of MYC-N was found in part of the tumors analysed. However, not in all samples a correlation between MYC-N expression levels and copy numbers of this gene was found.

Conclusions. Deregulation of MYC-N can be seen in a subset of nephroblastomas. Different and complex regulatory mechanisms appear to play a role in the deregulation of MYC-N.

DO-096
Stratification of chromosomal translocation in malignant solid tumors of childhood

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Aims. Solid tumors of childhood are relatively rare entities, but many of them are highly malignant. In most cases a multimodal treatment including surgical resection, chemotherapy and radiotherapy is necessary. Many of the young patients suffer from side-effects their whole life. The identification of patients that need only reduced therapy is therefore of utmost importance. We introduced a new method to screen all solid tumors of childhood for the presence of the most frequent and best characterized chromosomal translocations, which have also been shown to contribute to the malignant transformation or progression of these tumors.

Methods. Total RNA is extracted from formalin-fixed, paraffin-embedded tissue and cDNA is prepared. qPCR reactions are set up with primer pools amplifying the most common translocations and appropriate control genes. The emergence of a PCR product indicates the presence of at least one of the translocations tested by the primers. PCR products are then purified and sequenced by next generation sequencing using ion torrent PGM technology to determine the exact identity of the translocation.

Results. In our samples analysed so far, we have been able to demonstrate the presence of the typical chromosomal translocations in the most relevant tumor entities of early childhood. Our primer pool currently includes multiple sets of primers for EWSR-ERG, EWSR-FLI1, PAX3-FKHR, PAX7-FKHR, SYT-SSX and is well suited for further expansion. In further steps we will investigate a larger number of chromosomal breakpoints in different malignant entities of childhood thus increasing the statistical power and clinical relevance of our investigations.

Conclusions. Exact characterization of chromosomal translocations enhances the quality of diagnostic preciseness in combination with morphological features. Additionally we are gaining insight into a larger number of transcriptional pathways involved in the pathogenesis of these malignancies contributing to an optimized and personalized treatment for the patients.

DO-097
Histiocytic disorders involving the bone marrow in children

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Aims. An increase in histiocytes in pediatric bone marrow biopsies or aspirates can be reactive or neoplastic.

Methods. The differential diagnosis in children includes hemophagocytic syndromes, storage diseases, Langerhans cell histiocytosis and other histiocytic and dendritic cell neoplasms.

Results. Here, we present two cases, a hemophagocytic syndrome and the rare case of a systemic juvenile xanthogranulomatosis (JXG) in order to illustrate the pathomorphologic, immunhistochemical and clinical findings as well as the differential diagnosis.

Conclusions. The diagnosis of histiocytic disorders involving the bone marrow in children is frequently challenging, as clinical, biochemical and sometimes, genetic findings need to be integrated in the final interpretation.

DO-098
Dermatoses in foreskins removed for juvenile phimosis

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Aims. Phimoses in children and juvenile patients are commonly induced by lichen sclerosus (LS; outdated synonymes: balanitis or balanoposthitis xerotica obliterans; Lichen sclerosus et atrophicus) and occasionally by lichen planus (LP; synonymously lichen ruber). LS affects the inner side of the foreskin, LP can occur in hair bearing skin, modified mucosa and mucosal surfaces. Particular advanced disease stages and erosive LP have a high morbidity. Furthermore, LS and LP have been implicated etiologically in HPV-negative penile cancers with a low risk for LS and LP of keratinized epithelium, but a cancer risk of up to 5% in mucosal LP.

Methods. Archival foreskin specimens removed for phimosis of 120 patients (2–18 years) were examined. The inflammatory infiltrate was analyzed immunohistochemically with antibodies against CD 3,4,8,20 and for monoclonal rearrangement of the T-cell-receptor gamma locus.

Results. 60% of foreskins revealed LS. Early stages showed a continuous, band-like, CD8>CD4 lymphocytic infiltrate along a mildly thickened basement membrane. The typical band-like sclerosis with hyalinized blood vessels was diagnosed most commonly and as early as in 2-year-old boys. Late stages with atrophy, cystic degeneration and loss of epithelium were observed beginning at age 4 years. In 14% a LP with a patchy lichenoid interphase dermatitis, saw tooth-like epithelial acanthosis, keratinocyte apoptoses and wedge shaped hypergranulosis was diagnosed. Highly active dermatoses, in particular LP, featured a lymphohistiocytic vasculitis and erosions. About 20% LS and LP revealed a monoclonal rearrangement of the T-cell-receptor gamma locus. Advanced LS and LP were difficult to distinguish based on histological criteria alone and needed macroscopic and/or clinical correlation. An incidentally discovered HPV-HR-induced intraepithelial neoplasia in a 17-year-old, but no invasive cancers, and non-specific inflammation and fibrosis were diagnosed in the remaining foreskins.

Conclusions. LS and LP are manifestations of an exuberant local immune reaction to an (unknown) antigen, which is evidenced by accumulation of monoclonal T-lymphocytes in affected tissues. Detection of the subtle changes of early LS/LP, but also small foci of precancerous lesions requires examination of the entire specimen for establishing a diagnosis. Due to the cancer risk of longstanding LS and LP, patients with a penile LS/LP should be offered regular clinical examinations and appropriate treatment for residual disease.

DO-099

A proposed new placental pathology classification—pioneer on old terrain

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Aims. At present there is no internationally accepted, clinically easy understandable, morphological placental classification. This hampers international benchmarking and comparisons, and clinical research.
Methods. We have constructed a new, clinically oriented simplified classification system with one main diagnosis related to major pathological processes, based on partly modified internationally published and accepted criteria of macroscopic and microscopic findings. In addition to the strict standardized line of the main diagnosis, all additional findings or clinical relevant discussions are posted in a separate, commentary paragraph. The new classification system has been used in routine placental diagnostics in our hospital for more than two years and has been tested on sets of placental specimens to evaluate reproducibility and user-friendliness. In an international workshop the criteria of this classification scheme will be tested on routine specimens by perinatal and placental pathologists.
Results. The results of this international workshop will be presented.
Conclusions. Conclusion will be presented.

DO-100

Pectus carinatum—a metabolic lesion? First ultrastructural findings

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Aims. Pectus carinatum (PC) represents the most common congenital chest wall deformity, but the pathogenesis is still unknown. The two existing theories include a biomechanical weakness of the sternocostal cartilage due to disturbed cartilage metabolism and overgrowth of the sternocostal cartilage resulting into an elevation of the sternum.
Methods. Sternocostal cartilage specimens from a 15-year-old boy suffering from PC were gained during an open surgical correction procedure. The specimens were fixed in buffered formaldehyde and prepared according to standardized methods for histological and transmission electron microscopic (TEM) evaluation, the latter after isolation from the paraffin-block and embedding in epoxid-polymer.
Results. In the histological examination hyaline cartilage was present and the chondrocytes were characteristically distributed in the extracellular matrix (ECM). Nevertheless, a moderate degradation of the ECM with demasking fibres and fibrillations could be detected. In the TEM analyses, single chondrocytes with tubular, crystalline intracellular inclusions were found. Also, a loss of proteoglycan granules and a thickening of collagen fibres could be demonstrated. Furthermore, the fibrillar architecture of collagen fibres with the characteristic striation was lost in many fibres.
Conclusions. These interesting findings in pectus carinatum might help to clarify the pathomechanisms of this chest wall deformity. The crystalline inclusions are of special interest, since these correlate with disturbances in basic enzymatic activity and hence in cellular metabolism as shown e.g. in diabetes mellitus or lead intoxication. Also, low zinc levels were revealed in PC cartilage tissue, which might play a major role in the pathomechanism leading to PC, since zinc-dependent enzymes and zinc finger proteins are involved in cartilage formation. Our results support the metabolic hypothesis. Further studies should clarify whether the inclusions result from a lack of zinc or if they are related to cartilage degradation. Additionally, it should be clarified what metabolic disturbance leads to crystalline inclusions and why these are not found in all chondrocytes. For this purpose, further ultrastructural analyses need to be performed on PC and other chest wall deformity specimens

to clarify if these inclusions can be found in other chest wall deformities as well or exclusively in PC.

DO-101

Expression of collagen IV, V, IX, and XVII and corneal thickness measurements in different developmental stages of the human fetal cornea

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Aims. This study aims to investigate the expression of different collagens that are related to corneal development in the human fetal cornea. In addition, corneal thickness was measured to correlate the gestational age with morphology.
Methods. 19 formalin-fixed fetal eyes [age range: 11 to 38 weeks of gestation (WoG)] and a newborn's eye were investigated. The eyes (including the corneal thickness) were measured microscopically and, in addition to routine haematoxylin and eosin (H&E) and periodic acid-Schiff (PAS) stains, immunohistochemical labeling with antibodies to collagen IV, V, IX, and XVII was performed.
Results. Measurements of corneal thickness correlated well with corneal development as a basic indicator for maturation. Expression of collagen IV was found up to 23 WoG in the corneal basement membranes (BM). In older eyes, staining was confined to the limbal area. Collagen V expression was found in the corneal stroma and epithelial BM. Bowman's layer was not labeled. Collagen IX expression was predominantly found in the conjunctival and corneal epithelium. Collagen XVII was expressed in the corneal and conjunctival BM [1].
Conclusions. Our results indicate maturation-associated variations of collagen expression in the human cornea. Measurements of the corneal thickness may serve as an additional parameter to narrow down the developmental age with possible implications for pediatric pathology and forensic issues.

1. Herwig MC, Müller AM, Holz FG, Loeffler KU. Immunolocalization of different collagens in the cornea of human fetal eyes. Curr Eye Res (in press)

DO-102

Are there lymphatics in the human fetal eye?

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Aims. This study would like to contribute to the still debatable question if human fetal eyes harbour lymphatics. Thus, we examined the distribution of the lymphangiogenic marker podoplanin (D2-40) in human fetal eyes with regard to a maturation-dependent expression and possible findings of lymphatic structures in the choroid.
Methods. 40 formalin-fixed paraffin-embedded eyes from 40 human fetuses [age range: 10 to 32 weeks of gestation (WoG)] were investigated. Immunohistochemical stains were performed for D2-40. A semiquantitative analysis of immunoreactivity in different segments of the eye was performed by light microscopy. The intensity of staining was graded with a score ranging from 0 to 3.
Results. Podoplanin was expressed in 39 human fetal eyes. It was seen in vascular structures of the conjunctiva as well as in conjunctival epithelium, chamber angle, and the optic nerve sheaths. In some specimens a slight, equivocal staining reaction was noted in the choroid. However, no definite intraocular lymphatic vessels resp. their progenitors were found.
Conclusions. Podoplanin (D2-40) is, even at an early gestational age, expressed in several structures of the human fetal eye and the ocular

adnexae. D2-40 reactive structures were found in the choroid and the chamber angle but no lymphatic vessels or their progenitors could be unequivocally identified at these sites.

DO-103

Smith-Lemli-Opitz syndrome—delineation of fetal phenotype in a series of 8 fetuses with DHCR7 mutations, including one anatomical preparation from the "Madtower Collection" in Vienna

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Aims. The Smith-Lemli-Opitz syndrome (SLOS—OMIM #270400) is an autosomal-recessive MCA/MR-syndrome with an estimated incidence in Europe of at least 1 in 22.700. It is caused by a defect of the enzyme 7-Dehydrocholesterol reductase (DHCR7), thus affecting the last step in the synthesis pathway of cholesterol. Major features are microcephaly with bitemporal narrowing, characteristic facial dysmorphisms, polydactyly, syndactyly 2 to 3 of toes, and various degrees of mental retardation (MR) and of hypospadia in affected males. Other structural defects may concern the brain, heart and kidneys. The diagnosis is based on clinical status, biochemical and/or mutation analysis.

Methods. We present our morphological and molecular findings in eight SLOS-fetuses of 12 to 36 gestational weeks.

Results. Induced abortion followed prenatal ultrasound diagnosis of multiple congenital anomalies (MCA) in five male cases. They presented with a variety of different but mostly typical features allowing SLOS-diagnosis at autopsy and retrospective SLOS-diagnosis in two similarly affected undiagnosed sibs. In two cases prenatal biochemical analysis of 7-dehydrocholesterol, performed because of a SLOS affected sib, revealed elevated 7-dehydrocholesterol concentration in amniotic fluid. In these two male and female fetuses SLOS-typical features were almost missing. One additional case with "hexadaktylia manus" was a specimen from a patho-anatomical museum that had been archived in formalin for more than 80 years. This female fetus was diagnosed as having SLOS by us because of additional syndactyly and characteristic facial features. SLOS-diagnoses were confirmed by mutations in the DHCR7-gene.

Conclusions. Our series demonstrates that the phenotypic impact of SLOS varies in extent leaving many cases undiagnosed. Thus we can assume a higher than originally anticipated incidence of SLOS.

Hepatopankreatobiliäre Pathologie – experimentell

FR-001

The functional relevance of tenascin-C (TNC) in pancreatic cancer development

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Aims. Our main focus in this study was to investigate the function of the extracellular matrix protein tenascin-C (TNC), produced by pancreatic stellate cells, in pancreatic ductal adenocarcinoma (PDAC) progression in a genetically modified mouse model.

Methods. For generating the model, the well-established LSL-KrasG12D/+;Ptf1a+/Cre(ex1) (KC) model was crossbred with TNC knock-out mice to achieve the triple mutant LSL-KrasG12D/+;Ptf1a+/Cre(ex1);TNC+/− (KC-TNChet) and LSL-KrasG12D/+;Ptf1a+/Cre(ex1);TNC−/− (KC-

TNCko) mice. A cohort of 106 mutant mice was generated and mice belonging to the age groups 15-12, 9, 6, 3, 2 and 1 months and their respective littermate controls were subjected to detailed histopathological characterization, focusing on the degree of architectural distortion of the pancreas, the type and number of precursor lesions, as well as the frequency, histological subtype and grading of invasive adenocarcinomas. H&E, Pas-Alcian and Masson-Goldner stainings and immunohistochemistry (Ki67, Caspase-3, CK19, Claudin 18, p16) were performed. For a quantitative assessment of histochemical stainings and immunohistochemistry, computer-based morphometric analysis was applied on digitalized slides.

Results. One to three months old KC-TNChet and KC-TNCko mice developed an earlier architectural distortion with fibrosis, inflammation and acinar-ductal metaplasia when compared to KC mice. In all three groups precursor lesions (murine pancreatic epithelial neoplasia, mPanIN) of PDAC could be observed by the age of one month, though high-grade lesions (mPanIN3) and PDAC were exclusively present in KC-TNChet and KC-TNCko mice.

Conclusions. Absence of the extracellular matrix protein TNC affects pancreatic carcinogenesis by influencing tissue regeneration and stromal homeostasis.

FR-002

The role of the T cell alternative p38 activation pathway in pancreatic cancer

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Aims. Evaluation of the role of T cells and the production of proinflammatory cytokines by tumor infiltrating T cells (TIL) in pancreatic cancer.

Methods. Mice in which p38 α and β where replaced with isoforms lacking Tyr323 (double knockin, or DKI mice) and therefore unable to activate p38 downstream of the T cell receptor (TCR) were inoculated with PANC02 pancreatic cancer cells. Tumor volume and weight were followed, and TIL infiltration was determined by flow cytometry and immunohistochemistry. TIL were isolated from pancreatic tumors and stained for activation markers (CD25, CD44, CD62L, CD69) and proinflammatory cytokines (TNF-α, IFN-γ, IL-10, IL-17) with or without restimulation. CD4+ T cells were isolated from tumors and T-cell relevant transcription factors (Irf4, NFATc1, T-bet, GATA3, FoxP3, Rorc) were quantitated with qRT-PCR. In some experiments, TNFR1-/- mice, and in others TCR α -/- mice that had received CD4+ T cells from DKI or WT mice were inoculated with tumor cells.

Results. PANC02 tumors developed more slowly in TCR α -/- mice that lack T cells than in WT animals, demonstrating that T cells have a pro-tumor effect. This was due at least in part to the production of TNF-α, because tumors also grew more slowly in TNFR1-deficient animals. CD4+ TIL expressed high levels of activation markers such as CD25, CD44 and CD69, and low levels of CD62L. The role of TCR-activated p38 was investigated in mice with DKI T cells. Pancreatic tumor growth was markedly delayed in the presence of DKI compared to WT T cells. Proinflammatory cytokines such as TNF-α, IFN-γ, IL-10, and IL-17 were markedly reduced in both unstimulated and restimulated DKI compared to WT TIL. Furthermore, expression of Irf4, NFATc1, and Rorc was substantially lower in CD4+ DKI TIL. TCR α -/- mice with CD4+ T cells from DKI mice also had smaller tumors than mice with CD4+ T cells from WT mice.

Conclusions. CD4+ T cells infiltrate pancreatic tumors and are highly activated. Their p38-dependent transcription factors and proinflammatory cytokines play a pivotal role in the progression of pancreatic cancer in an in vivo model.

FR-003

Akt/Notch activation drives rapid cholangiocarcinoma development from mature hepatocytes in the mouse

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Aims. Intrahepatic cholangiocarcinomas (ICCs) are primary liver tumors with a dismal prognosis. Activated AKT/mTOR pathway promotes both HCC and ICC development in the mouse liver. Notch signaling is known to play a critical role during bile duct development. Here we hypothesized that activated Notch signaling converted AKT-overexpressing preneoplastic hepatocytes into ICC in vivo.

Methods. Activated Notch (NICD) alone or together with the activated form of AKT (myr-AKT) were stably transfected into the mouse liver via hydrodynamic injection. Dominant negative form of RBP-J (dnRBP-J) was co-injected with AKT to block canonical Notch pathway in the mice. Notch and AKT pathway status was determined in human ICC samples by Western blotting and immunohistochemical staining.

Results. Overexpression of NICD alone induced cystic cholangiocellular tumors, including cystic ICCs, over long latency. Concomitant activation of Notch and AKT signaling synergized to promote rapid cholangiocarcinogenesis in mice. Intriguingly, we found that the ICC tumor cells originated from mature hepatocytes. Using a mouse model of hepatocyte fate tracing in combination with morphological and ultrastrutural evaluation by light and electron microscopy, we demonstrated that Notch and AKT coexpression transformed normal hepatocytes into ICCs. The hepatocyte-cholangiocyte conversion occurred at the earliest stages of the tumorigenic process, supporting that it is the critical step leading to the malignant initiation and subsequent progression. Mechanistic studies revealed the increase in both cell proliferation and glycolysis in AKT/NICD induced ICC lesions. Furthermore, blocking canonical Notch signaling with dnRBP-J in AKT-injected mice prevented ICC, but not HCC development in these mice, supporting that AKT-induced ICC development was dependent on Notch signaling. Finally, coordinated activation of AKT and Notch signaling was detected in a subset of human ICC samples.

Conclusions. Our studies suggest that hepatocytes can be the cellular origin of ICC in mice and possibly also in humans. Activated AKT and Notch signaling pathways have critical roles during ICC development, and targeted therapy against these pathways may be useful in treating human ICC.

FR-004

Complete inhibition of mTORC1 cascade is required to suppress hepatocarcinogenesis induced by AKT and Ras co-expression

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Aims. Co-expression of constitutively active forms of AKT (Myr-AKT) and activated Ras (N-RasV12) rapidly induces liver tumors in mice. mTORC1 is the main downstream effector of AKT, acting mainly by phosphorylation of RPS-6 and 4E-BP1, and thus regulating multiple cellular processes. However, in which manner mTORC1, RPS-6 and 4E-BP1/eIF4E do contribute to hepatocarcinogenesis remains unknown.

Methods. Myr-AKT, RasV12, 4EBP1A4 (dominant negative form of 4E-BP1), or 4EBP1WT (wild type) were stably transfected into the mouse liver by hydrodynamic injection. The mTORC1 inhibitor Rapamycin was intraperitoneally administrated. Western blotting and immunohistochemistry were performed to analyze the expression levels of proteins in the tissues.

Results. AKT/Ras mice were treated with rapamycin (AKT/RAS/Rapa) or vehicle (AKT/RAS/Veh) daily for 7 weeks. All of the AKT/RAS/Veh mice developed large HCCs and were required to be euthanized. By contrast, none of the AKT/RAS/Rapa mice showed HCC, but microscopically small regressive tumor-like nodules and preneoplastic hepatocellular foci were still visible. Withdrawal of rapamycin treatment was followed by a relapse in HCC development. Biochemically, Rapamycin inhibited the phosphorylation of RPS6, but had no effect on phosphorylated/inactivated levels of 4E-BP1. To define the role of 4EBP1/eIF4E, we overexpressed 4EBP1A4 or 4EBP1WT along with AKT and Ras into the mouse liver. Seven weeks later, AKT/Ras/4EBP1WT mice developed large HCC, equivalent to those developed in AKT/RAS/Veh mice. However, only few very small hepatocellular adenomas (HCA) developed in the livers of AKT/Ras/4EBP1A4 mice. These lesions, together with preneoplastic hepatocytes, occupied 40–60% of the liver parenchyma. 20 weeks post injection, the AKT/Ras/4EBP1A4 mice eventually developed large liver tumors, including HCC, indicating that inhibition of the 4E-BP1/eIF4E axis efficiently delayed AKT/RAS-induced liver tumor development, yet was unable to completely block it. Finally, we co-injected AKT/Ras mice with 4EBP1A4 and treated the mice with Rapamycin. Only this simultaneous blockade of p-RPS6 and p-4EBP1 completely inhibited AKT/Ras induced hepatic carcinogenesis.

Conclusions. Our experiments demonstrate the critical role of mTORC1 in mediating activated AKT and Ras induced liver tumor development in vivo. The two major downstream effectors of mTORC1, RPS6 and 4E-BP1/eIF4E, are both required for tumorigenesis. Yet they are likely to have distinct roles within the neoplastic process.

FR-005

miR-198 acts as a tumor suppressor in hepatocellular carcinogenesis by regulating expression of cellular adhesion proteins

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Aims. Hepatocellular carcinoma (HCC) is one of the leading causes of cancer deaths, worldwide. MicroRNAs, inhibiting gene expression by targeting various transcripts, are involved in genomic dysregulation during hepatocellular tumorigenesis. In previous studies, microRNA-198 (miR-198) was shown to be significantly downregulated in HCV-positive hepatocellular carcinoma (HCC). Herein, the function of miR-198 in hepatocellular carcinoma cell growth and gene expression was studied.

Methods. First, transcription levels of miR-198 as well as of the liver specific transcription factors HNF1 α and HNF4 α were determined in different hepatoma cell lines. Putative regulation of HNF1 α and HNF4 α by miR-198 was investigated by miR-198 transgenic expression and RNA interference experiments. Consecutively, gene expression profiling in response to miR-198 overexpression in the hepatoma cell line Pop10 was performed by Affymetrix microarray hybridisation. After data interpretation by different spotfire based software, real-time PCR and western blotting analysis was used for evaluation of miR-198 affected transcript and protein expression.

Results. Both, transcription factors HNF1 α and HNF4 α as well as miR-198 were down-regulated in different hepatoma cells. Importantly, we could show that miR-198 expression is induced by these two liver-specific transcription factors. Gene expression profiling after miR-198 overexpression revealed a prominent dysregulation of several signal transduction pathways such as insulin and TGF-β signaling. In particular, bioinformatic analysis and subsequent comprehensive transcript and protein analyses demonstrated that the expression of the adhesion proteins E-cadherin and claudin-1, is highly affected by miR-198 overexpression. This is of particular interest because these proteins, involved

in adherence, are decreased during HCC progression. Furthermore, RNA interference silencing and miR-198 overexpression revealed that the miR-198/claudin-1 and E-cadherin axis affects hepatoma cell migration.

Conclusions. In conclusion, miR-198 acts as a tumor suppressor by repression of motogenic pathways, diminishing cell growth and migration.

FR-006

Loss of imprinting and allelic switching at the DLK1-MEG3 locus in human hepatocellular carcinoma

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Aims. This project aims at the identification of imprinted genes deregulated in human HCC, because our knowledge about imprinting defects in human hepatocellular carcinoma (HCC), the third leading cause of cancer death, is still limited.

Methods. The publicly accessible database Oncomine was screened for the expression of 223 imprinted loci in human HCC specimens. Quantitative mRNA expression analysis was performed employing real-time RT-PCR as well as allele-specific pyrosequencing. Quantitative allele-specific methylation analyses were performed using pyrosequencing and bisulfite sequencing of cloned PCR products. Global methylation levels in patient samples were measured using LINE-1 pyrosequencing. Inhibition of DNA methylation in cancer cells was accomplished by siRNA targeting DNA methyltransferases.

Results. Thirty eight imprinted genes were identified as differentially expressed in HCC. The DLK1-MEG3 locus turned out to be the most frequently deregulated. Using a series of 40 HCC cases, DNA methylation analysis of differentially methylated regions (DMRs) in the DLK1-MEG3 locus revealed extensive aberrations in more than 80% cases, accompanied by dysregulation of DLK1 and MEG3 expression. Hypermethylation correlates inversely with expression of DLK1 and MEG3 expression ($p<0.05$) while loss of methylation correlates linearly with global loss of DNA methylation in HCC ($r2=0.63$, $p<0.0001$). Inhibition of DNMT1 using siRNA in HCC cells led to decreased methylation at the MEG3-DLK1 locus and a concomitant increase in MEG3 RNA expression. Allele specific expression analysis demonstrated loss of imprinting in 10 of 31 informative samples (2 with biallelic expression and 8 with allelic switching, 32%). In 2 cases with gain of bi-allelic expression, loss of methylation at the MEG3-DMR could be demonstrated while in 8 cases displaying allelic switching, gain or loss of DNA methylation was shown primarily at IG-DMR1. Analysis in hepatocellular adenoma (HCA, n=10), focal nodular hyperplasia (FNH, n=5), and healthy liver (n=5) confirmed that this epigenetic instability is specific for the process of malignant transformation.

Conclusions. Deregulation of proper imprint control turned out to be a very frequent phenomenon in human HCC. This widespread epigenetic deregulation in imprinted loci might serve in the future as prognostic or predictive biomarker and for monitoring response to epigenetic therapy in HCC.

FR-007

The protumorigenic role of the nuclear transport factor cellular apoptosis susceptibility (CAS) in hepatocarcinogenesis is linked to the anti-apoptotic function of X-linked inhibitor of apoptosis (XIAP)

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Aims. Aberrant expression of nuclear transport factors has been reported to play a role in cancer development. As an important member of the nucleo-cytoplasmic transport machinery the export factor Cellular Apoptosis Susceptibility (CAS) re-shuttles importin-α from the nucleus to the cytoplasm. In different scenarios (e.g. TNF-α- or p53-induced apoptosis) CAS was shown to have pro-apoptotic properties. Here, we analyzed the role of CAS under unstressed conditions in hepatocarcinogenesis.

Methods. The impact of siRNA-mediated CAS knockdown on tumor cell viability, proliferation, apoptosis, cell migration and invasion was determined in different HCC cell lines by using FACS, MTT-, BrdU-, scratch-, and invasion assays as well as life cell imaging. CAS expression in 191 HCCs, 9 dysplastic nodules and 19 normal liver samples was analyzed immunohistochemically and in a subset of samples by quantitative real-time PCR (qRT-PCR). Gene expression arrays were used to identify "downstream" targets of CAS that may contribute to the observed phenotype.

Results. CAS depletion by RNAi in hepatocellular carcinoma (HCC) cell lines significantly decreased cell viability, proliferation, migration, invasion, and led to an increase in cell death. Consistent with these findings overexpression of CAS was observed on mRNA- and protein-level in up to ~70% of HCC tissue samples analyzed. Gene expression arrays upon CAS knockdown revealed downregulation of the X-linked Inhibitor of Apoptosis (XIAP) and direct ablation of XIAP by RNAi partially recapitulated the effects observed after CAS knockdown. Finally, we observed a correlation of CAS and XIAP expression in HCC patient samples.

Conclusions. Our data suggest a protumorigenic role of CAS in hepatocarcinogenesis under unstressed conditions that is partially mediated by XIAP. Thus, considering previous reports pro- and anti-apoptotic functions of CAS seem to be context-dependent.

AG Molekulare Pathologie

FR-013

Comparison of new next generation sequencing methods for diagnostics in molecular pathology

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Aims. The determination of the mutation status of certain genes has become increasingly important within the last years, most notably because of the rapid development of high throughput sequencing methods. The treatment of non-small cell lung cancer (NSCLC) patients with tyrosine kinase inhibitors in case of an activating mutation in the EGFR gene shows the urgent need for individual therapies. The great potential of the next generation sequencing (NGS) systems opens up the way to a personalized therapy not only because mutations present in a small entity can be detected, but also patients might be divided according to their mutational status. Accordingly, the detection of certain specific gene

loci, that are mutated and therefore promising targets for therapy, is of major interest. Our aim was the qualitative assessment of different current NGS technologies namely MiSeq (Illumina) and IonTorrent (Life Technologies) sequencing method, for the use in the clinical research and diagnostics. Furthermore we aimed to validate two different gene panels for lung cancer as well as chronic lymphatic leukaemia (CLL).

Methods. DNA from macrodissected FFPE tumor tissue from five patients with bronchial carcinoma (NSCLC and SCLC) as well as B-Cell DNA from peripheral blood from five CLL patients was used for PCR multiplex library preparation. Afterwards, system specific bar code adapter were ligated and adapter library pools of each case were applied to MiSeq respectively IonTorrent sequencing platform. Conventional Sanger sequencing served as a reference technology.

Results. Tumor relevant loci of 16 genes representing 189 loci were detected in five lung tumors with semiconductor and Illumina technology. In the five CLL samples 15 targets genes representing 73 amplicons were detected with both technologies as well. Mutation hits in genes, previously characterized as hotspot locations, such as p53, ATM, DDX3X and many others could be clearly detected in lung tumors as well as CLL samples by both technologies.

Conclusions. Both, FFPE as well as native tumor cells showed a good performance with both sequencing methods. Sample multiplexing by the semiconductor or Illumina sequencing accelerate the sample processing, resulting in a powerful and efficient tool for wide mutation analysis in future molecular pathology.

FR-014

Evaluation of amplicon-based semiconductor sequencing for diagnostic screening of formalin-fixed paraffin embedded tumor material

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Aims. In view of a personalized medicine, the screening of somatic mutations in an individual tumor that predict therapeutic outcome has become an increasing need in the clinical and molecular diagnostics. In most laboratories, Sanger sequencing has become an accepted standard, but due to the limited screening throughput and the increasing demand for targeted sequencing, the application of next-generation sequencing in the molecular diagnostic has turned into focus. Whole-genome or whole-exome sequencing approaches provide a comprehensive view of an individual tumor mutation load, but the current high costs and the excess of information without a direct clinical implication limit the routine use of this technology. In this study, we evaluated a targeted re-sequencing approach that focuses on somatic hotspot cancer mutations based on semiconductor sequencing. We especially took attention on the reliability of the method concerning sample quality, mutation type and reproducibility.

Methods. We used DNA extracted from formalin-fixed, paraffin embedded (FFPE) tumor tissues (biopsy and resection) of different tumor degree and overall quality that were previously screened for the presence of EGFR mutations (exon 18–21) by Sanger sequencing. 190 amplicons covering hotspot mutations in 46 genes were generated in a multiplex PCR reaction (AmpliSeqTM), bar coded and 8 samples were run on a single IonTorrent 318 chip.

Results. We were able to successfully sequence all samples with a mean coverage rate of 670.000 reads (AQ20:560.000) and an average read depth of 2947 AQ20 reads/amplicon. In all but one sample, variant calling identified the EGFR mutation, missing only one low level 9bp insertion in exon 20. Moreover, we identified additional 43 mutations in 17 genes and uncovered three previously unknown EGFR amplifications.

Conclusions. Taken together, amplicon-based semiconductor sequencing is a powerful and cost-effective method working with low-quality DNA material, enabling routine diagnostic next-generation sequencing.

FR-015

In silico design of a gene panel for targeted re-sequencing of recurrent, potentially targetable mutations in pancreatic ductal adenocarcinoma

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Aims. Pancreatic ductal adenocarcinoma (PDAC) is one of the deadliest cancers and for patients with metastatic disease, chemo-radiation remains the most effective therapy. Genomic approaches have revealed recurrent mutations in potentially targetable pathways and several trials indicate that molecular-genetic characterization may be an efficient way to stratify patients for optimized therapy. Currently genome-wide approaches are not practical and targeted resequencing has been suggested as a practical bridging technology. To determine whether targeted resequencing can detect a clinically relevant number of patients, we designed a gene panel for PDAC.

Methods. Datasets from recent studies (pubmed), including whole-exome sequencing data, were combined with annotated mutation frequencies (COSMIC database). Using the annotated mutational spectrum, the genelist was screened for mutations that are detectable by targeted resequencing (SNaPshot, mycancergenome.com) and the resulting list was assessed for targets implicated in ongoing clinical trials (clinicaltrials.gov). Given that combined assessment of selected mutations has not been performed and whole-exome data suggest mutually exclusive mutation patterns among patients for at least a subset of genes, predicted detection rates were calculated assuming both a combined as well as a mutually exclusive distribution of mutations.

Results. Initially, 12 genes were screened (top COSMIC entries) that revealed four prevalent mutations that can be easily analyzed by targeted resequencing: KRAS (71%), CDKN2A (31%), PIK3CA (5%) and BRAF (2%). These four mutations were selected as driving oncogenes with therapeutic relevance. Although not accessible through targeted therapy, in addition we selected TP53 (52%) and SMAD4 (25%) as two indicators of poor prognosis in PDAC. Using this 6-gene panel for PDAC, the predicted detection range is at least 38% (e.g. mutually exclusive combination of CDKN2A/PIK3CA/BRAF) up to 71% (e.g. all mutations overlapping with KRAS). These predicted numbers may overestimate the actual detection frequency in patient samples and do not take sample variability into account.

Conclusions. Despite the limitations of in silico analyses, the presented data indicate that targeted single-nucleotide re-sequencing in PDAC will identify important subsets of patients for targeted therapy. Thus our data emphasize the feasibility of resequencing in PDAC to identify patients for prognostication and/or targeted therapy.

FR-016

New BRAF-V600E-mutation-based methods for the diagnosis and therapy of hairy cell leukemia

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Aims. Hairy-cell leukemia (HCL) is a mature B-cell lymphoma that harbors BRAF V600E mutation in virtually all cases. This feature can be exploited for new diagnostic and therapeutic applications that target the mutated BRAF. The aim of this study was to assess the immunohistochemistry with BRAF V600E mutation-specific antibody (clone VE1) for differential diagnosis and monitoring HCL during the treatment with BRAF specific inhibitor vemurafenib.

Methods. A total of 52 routinely processed formalin-fixed paraffin-embedded tissue specimens were investigated (bone marrow, n=46; spleen,

n=6) for expression of V600E-mutated BRAF protein. Direct sequencing was used to confirm the mutation on genomic level.

Results. All 32 cases of HCL were scored positive, and all non-HCL cases were scored negative. In 28 of 30 HCL cases the presence of a BRAF V600E mutation could be confirmed by direct sequencing, whereas no BRAF mutations were detected among 20 HCL mimics. One HCL patient with disease refractory for purine analoga received an off-label treatment with BRAF inhibitor vemurafenib. Complete clinical remission was achieved on day 43 and the vemurafenib therapy was discontinued after 56 days. Despite discontinuation of vemurafenib treatment, remission continues to persist 6 months after treatment. The sequential bone marrow biopsies proved marked reduction of HCL infiltration during the first weeks of therapy. No BRAF-V600E positive tumor cells were detectable in bone marrow biopsy at day 36.

Conclusions. In conclusion, we demonstrate that immunohistochemistry with BRAF V600E mutation-specific antibody can be used to reliably differentiate HCL from HCL-mimicking entities. This on-slide technique might be particularly helpful in interpreting challenging biopsies with low tumor content and for assessment of minimal residual disease after HCL therapy.

FR-017
Reliability of pyrosequencing for detection of rare BRAF mutations in melanoma patients

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Aims. The detection of V600E BRAF mutations has fundamental clinical consequences since the treatment option with BRAF inhibitors such as vemurafenib or dabrafenib yields response rates of approximately 50%. Since clinical responses have been seen also in patients with non-V600E BRAF mutations, it is essential to acquire knowledge about these rare mutations and to define reliable methods to detect them. We aimed to validate the pyrosequencing method in comparison to other screening techniques regarding their reliability to detect rare non-V600E mutations.

Methods. DNA was prepared from formalin-fixed paraffin embedded sections of melanoma tissues. A new BRAF assay was designed to determine BRAF mutations by pyrosequencing. Rare mutations were confirmed by capillary sequencing and compared to findings by COBAS test and immunohistochemistry using a novel BRAF antibody. Clinical data with respect to melanoma type, tumor site, and survival were summarized for patients with rare mutations.

Results. Among our study population, a total of 14 patients exhibited rare BRAF mutations. The V600EK601del and V600DK601del mutations (1 case each) have not been described before. Furthermore, V600K (6 cases), V600E2 (GAA; 2 cases), and V600D, V600G, V600R, and L597S mutations (one case each) were detected. Mutations were not found by COBAS test in 7 out of 11 patients. Data were correlated with immunohistochemical findings.

Conclusions. Accurate diagnosis of rare mutations is crucial since vemurafenib is approved for treatment of melanoma with V600 mutant BRAF and not limited to V600E mutations. Patients with rare BRAF V600 mutations could respond to treatment with BRAF inhibitors. We show that pyrosequencing is a reliable and time-saving alternative to detect rare BRAF mutations.

FR-018
HR23b as a predictive biomarker for HDAC inhibitor based therapy in soft tissue sarcomas?

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Aims. Histone modifications have been shown to be key players in epigenetic alterations and are often dysregulated in cancer. Therefore, histone deacetylases (HDAC) seem to be promising therapeutic targets for HDAC inhibitor based therapy in cancer. HR23b, the UV excision repair protein Rad23 homolog B, was revealed as a potential biomarker for the sensitivity of HDAC inhibitor based therapy in cutaneous T-cell lymphoma. Furthermore, in hepatocellular carcinoma HR23b expression was associated with stable disease under HDAC inhibitor therapy. Therefore, we asked whether HR23b could be a biomarker for HDAC inhibitor based therapy in sarcomas.

Methods. Using tissue microarrays (TMAs), the expression of HR23b was retrospectively analysed in 571 sarcoma samples including 211 gastrointestinal stromal tumours (GISTs) and 360 sarcomas of other entities. Immunohistochemistry was performed with a HR23b specific primary antibody. Overall intensity and frequency of cytoplasmic and nuclear staining were determined. Results of both localisations were added and staining was classified as negative/intermediate positive and positive. Statistical analysis was done by IBM SSPS version 20.0. Immunohistochemistry, immunoblots and MTT assays were performed to evaluate the sensitivity of sarcomas for HDAC inhibitor based therapy in relation to the measured HR23b expression.

Results. Immunohistochemistry revealed no differences in the staining pattern of HR23b in the nucleus or cytoplasm. Of the 211 GIST one quarter was strongly positive for HR23b. GIST harbouring strong HR23b staining are characterised by a low mitotic count. Furthermore, GISTs of the small intestine show a significantly stronger HR23b expression than GISTs localised in the stomach. Within the other sarcoma entities only dedifferentiated liposarcomas, leiomyosarcomas and malignant peripheral nerve sheath tumours exhibit strong HR23b expression in over 20% of cases. The specificity of the immunohistochemical staining was confirmed by immunoblots. Treatment with four HDAC inhibitors in different sarcoma cell lines caused dose dependent reduction of viability in vitro in correlation to the immunohistochemical staining.

Conclusions. HR23b expression is not a general feature in mesenchymal tumours but is found in subgroups of GIST and certain sarcoma entities which are characterised by an aggressive clinical behavior. Thus, the expression of HR23b might serve as a biomarker for a HDAC inhibitor based therapy. Further studies are, however, needed.

FR-019
Changes in miRNA expression during primary systemic therapy of breast cancer and prediction of treatment response

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Aims. Specific miRNA signatures have been identified in malignant tumors, including breast cancer, and may have diagnostic, prognostic and predictive value. The aim of this study was to determine I) changes in miRNA expression during primary systemic (neoadjuvant) chemotherapy of breast cancer, and II) to assess whether miRNA expression in pretherapeutic biopsies may predict treatment response.

Methods. Seventy-one patients with large (≥3 cm) or locally advanced primary invasive breast cancers undergoing anthracyclin- and taxane-based primary systemic therapy were studied. Total RNA was extracted from microdissected paraffin-embedded biopsies before chemotherapy (n=68) and post-chemotherapy surgical specimens (n=48). The relative

expression of 10 miRNAs, including miR-7, -21, -34a, -29a, -29b, -125b, -155, -200c, -340, and miR-451 was determined before and after chemotherapy by qRT-PCR using RNU48 as reference gene. Histopathological response including complete pathological response and minimal residual disease, as well as progression free and overall survival served as endpoints.

Results. Following primary systemic therapy, 13 (18%) patients showed histopathological response, including 10 patients with complete pathological response and 3 patients with minimal residual disease. The majority of miRNAs (including miR-7, -21, -200c, and -340) showed a decrease in expression from before until after chemotherapy (mean 16%, range 11–27%). Two miRNAs (miR-29a, -29b) showed no considerable change and one miRNA, miR-451, showed an increase (mean 90%) during chemotherapy. The pre-chemotherapeutic expression of miR-7 was significantly (3-fold) higher in histopathologic responders compared to non-responders (p=0.01). However, individual patients showed a considerable variation in miRNA changes during chemotherapy.

Conclusions. We observed a significant correlation between miR-7 expression and treatment response. Patients with high intratumoral pretherapeutic levels of miR-7 achieved significantly more often a complete pathologic response compared to patients with low expression. Our results demonstrate that miRNA expression profiles are profoundly altered during neoadjuvant chemotherapy of breast cancer, and provide a basis for future assessment of miRNAs as molecular biomarkers in the prediction of treatment response and prognosis.

FR-020
Novel clinically relevant genes in gastrointestinal stromal tumors identified by high-throughput methods

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Aims. Apart from mutations in KIT and PDGFRA, recurrent molecular mechanisms in gastrointestinal stromal tumors (GIST) are largely unknown. Chromosomal gains and losses resulting in altered gene dosage are known to be recurrent in GIST and believed to play a role in their molecular pathogenesis. Nevertheless, the target genes within these regions remain to be identified. Aim of our study was the identification of clinical relevant genes in the candidate regions.

Methods. Fresh frozen tissue of 29 GIST was studied for the delineation of the minimal region of interests using Affymetrix SNP 6.0 array. Tissue micro arrays from formalin fixed, paraffin embedded tissue of 145 GIST were constructed, status of chromosomes 1p36, 1q25, 11q13, 13q14, 14q32, 15q11 and 22q11.2 was investigated using fluorescence in situ hybridization (FISH). Thirteen GIST were subjected to exome sequencing using a HiSeq2000 (Illumina), reference DNA was obtained from peripheral blood lymphocytes or normal tissue. Analysis was restricted to regions of interest, comprising the minimal region of overlap if at least 4 samples showed copy number variations in this region at array analysis. Candidate genes were evaluated immunohistochemically.

Results. Array analysis of 29 GIST revealed recurrent rearrangements and included losses of chromosomal arms 1p (n=13), 3p (n=4), 13q (n=5), 14q (n=17), 15q (n=7), and 22q (n=11) as well as gains of chromosomal arms 1q (n=3), 4q (n=6), 5q (n=8), 7p (n=3), 11q (n=4), and 12p (n=3). FISH studies of these rearrangements in 145 GIST demonstrated that relative loss of 1p was associated with shorter disease free survival (DFS). Exome sequencing revealed 15,882 variants in 350 genes, variants in at least 3 samples were observed in 37 genes. After data analysis using Cancer Genes and Gene Databases, number of target genes was reduced to 10 in addition to KIT and PDGFRA, which were also identified as target genes using our approach. At immunohistochemical investigation of target genes Nesprin was associated with DFS and overall survival

(OS), RAD45L2 with shorter and KIT with longer OS, and DIAPH1 with shorter DFS.

Conclusions. Using a novel approach combining DNA arrays, exome sequencing and immunohistochemistry, we were able to identify three novel clinically relevant genes in GIST. As next steps, we will investigate the clinical relevance of mutation status in addition to expression of genes of interest. Furthermore, we will also investigate DNA mutations outside regions of interest.

FR-021
Sensitive methods for detection of secondary KIT mutations in gastrointestinal stromal tumours

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Aims. Gastrointestinal stromal tumours (GISTs) are the most common mesenchymal tumours of the digestive tract and are characterised by activating mutations of the KIT or the PDGFRA gene. Advanced GISTs harbouring a primary KIT mutation are treated with the tyrosine kinase inhibitor imatinib. During the course of treatment most patients experience disease progression and acquire resistance to imatinib. The most common resistance mechanism is the development of secondary KIT mutations in addition to the primary mutation. Until now it is not proven if secondary KIT mutations develop during imatinib treatment or if rare cells with secondary KIT mutations are already present in the primary GISTs, called minor clones. This study aims to establish more sensitive methods than the conventional Sanger sequencing for detection of minor clones with secondary KIT mutations in primary GISTs with known secondary resistance.

Methods. A cohort of well characterised primary and secondary GISTs, diagnosed by experienced pathologists and with previously determined mutational status of KIT, was enclosed in this study. DNA was extracted from FFPE tissues by the BioRobot M48 (Qiagen) and quantified. For analysis the Roche 454 GS Junior FLX Titanium System was used. KIT exon 9, 11, 13, 14, and 17 target and sample specific primers were designed. A multiplex amplicon library was generated and used for emulsion PCR and parallel 454 sequencing. All steps were performed according to the manufacturer's instructions. Additionally, a sensitive allele-specific PCR was established. Primers were designed for KIT secondary mutations. Amplification products were analysed by gel electrophoresis and the LightCycler® 480 System (Roche).

Results. The conditions for parallel 454 sequencing of KIT could be established in over 40 GISTs. In most samples KIT mutations previously determined by Sanger sequencing could be verified with 100–1600 reads per exon and sample. By this approach, we did not detect minor clones in primary GISTs. Therefore, we changed the experimental set-up to be able to gain more reads per exon and sample and thus to increase the sensitivity.

Conclusions. Parallel 454 sequencing is an adequate platform to perform sensitive mutational analysis and is helpful for minor clone detection of secondary KIT mutations in GISTs. Whether it will be possible to detect minor clones prior to treatment still has to be determined. Allele-specific PCRs might be a good alternative for minor clone detection and is currently evaluated.

FR-022

Comparative analysis of detection methods of EML4-ALK translocation in NSCLC

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Aims. Rearrangement of the ALK gene has been described in 3–5% of non-small cell lung cancers (NSCLC), primarily adenocarcinomas in younger patients without a smoking history. Recently the ALK inhibitor crizotinib has been shown to achieve an objective response rate in up to 57% of NSCLC patients demonstrating an ALK translocation. Therefore accurate detection of EML4-ALK+ NSCLC is essential, which is hampered by the variety of EML4-ALK fusion variants. Different methodologies including RT-PCR, FISH and immunohistochemistry have been described with rather heterogeneous results regarding sensitivity. Here we compare these methodologies in a large sample of resected NSCLC cases to analyze respective sensitivities and specificities.

Methods. 200 resected NSCLC cases were retrieved from the archives and clinical follow up data acquired. All cases were reevaluated morphologically, by immunohistochemistry and typed according to modified ATS/IASLC criteria. DNA and RNA were extracted and prepared from selected tumor areas. From corresponding areas punch biopsies were retrieved and multi-tissue arrays constructed. For immunohistochemical analysis of ALK expression three different antibody clones (ALK1, 5A4 and D5F3) were used. For FISH analysis three different probes (Abbott, Kreatech, Zytomed) were chosen. Furthermore, a quantitative RT-PCR (qPCR) assay and a qualitative RT-PCR assay covering the most frequent translocations of ALK were established.

Results. Immunohistochemistry and qPCR show sensitivity above 90% with similarly high specificity for AML4-ALK translocations. There are significant differences regarding the antibody clones. Using stringent criteria (>20% break apart signals) for the interpretation of FISH, the sensitivity is below 80%. RT-PCR shows high sensitivity, however size of transcripts leads to a rather high drop out rate of cases in formalin fixed tissue.

Conclusions. Immunohistochemistry and qPCR appear as sensitive and specific screening tests for EML4-ALK translocation in NSCLC.

FR-023

Anaplastic lymphoma kinase (ALK) gene rearrangement in non-small cell lung cancer (NSCLC): a glance at "borderline" cases by means of IHC and FISH

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Aims. Lung cancer is the leading cause of cancer related mortality in men and women. In ~4% [0.9–13%] of NSCLC a genomic EML4-ALK fusion has been described, showing therapeutically sensitivity for ALK-inhibitors as crizotinib. As therapy (XALKORI) is available in Europe since October/November 2012 for ALK-altered NSCLC, reliable diagnosis is of urgent priority. Never the less the exact parameters of FISH-based (threshold of positive cells, signal distance) diagnosis vary among the different studies.

Methods. We performed retrospective screening (tissue microarray based) of 551 NSCLC [384 adenocarcinomas (ADC), 167 squamous cell carcinomas SCC)] for ALK-expression (IHC-intensity: 0–3) using immunohistochemistry (Dako, Novocastra, Cell Signaling) and ALK-breaks using FISH (Abbott; ALK break-apart). The evaluation of the IHC and FISH data was performed independent from each other. As ALK split-signals can be found in up to 11% of tumor-free lung tissue, special attention was given to cases with ALK-breaks in the ranges of 12–14% and 15–20%.

Results. 95.1% (524/551) of the cases were ALK-break-negative (<15% split signals), 4.9% (27/551) ALK-break positive (≥15%). Concordant absence of ALK-expression (0) and ALK-breaks (<15%) was found in 72.1% (397/551), concordant ALK-expression (1–3) and ALK-breaks (≥15%) in 3.1% (17/551). Apart from the most commonly applied threshold for the percentage (15%) of ALK-break positive cells, we extended our view to the range between 12–14% [45 cases (35×ADC, 10×SCC) with ALK-expression in 28.8% (13/45)] and 15–20% [22 cases (17×ADC, 5×SCC) with ALK-expression in 54.5% (12/22)]. Only 5 cases (5×ADC) showed more than 20% ALK-break positive cells all with ALK-expression.

Conclusions. The definition of ALK-positive cases eligible for therapy with ALK-inhibitors is—beyond clear-cut negative and positive cases—a diagnostic challenge. A considerable number of cases with 12–20% ALK-break positive cells might be potentially misclassified. Clinicians and pathologists should be aware of this "borderline group" whose incidence, biological behavior and response on TKIs needs further investigation, as well as its reliable detection e.g. by PCR and improved IHC-systems, the latter might by possible with a new ALK-IHC antibody system (Ventana). Its evaluation in our sample of 551 NSCLC is under examination and will be presented in Heidelberg.

FR-024

MET expression status before TKI-Start is a predictive biomarker for TKI response in EGFR-mutant lung adenocarcinoma

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Aims. EGFR mutations in non-small cell lung cancer (NSCLC) have been tightly linked to tyrosine-kinase inhibitor (TKI) responses. Ultimately, TKI-resistance limits responsiveness and among several oncogenic drivers, MET causes resistance in at least 30% of patients. MET-positive lung cancer is clinically more aggressive; however, it is unclear whether this is the case in TKI-treated EGFR-mutant NSCLC. We tested the MET-status as a predictive biomarker for TKI response in EGFR-mutant lung adenocarcinoma (ADC).

Methods. Our retrospective study used the following inclusion criteria: EGFR-mutation within exon 18–21, histologically confirmed ADC (stage III/IV), TKI-treatment for at least 1 mo., clinical follow-up and sufficient tissue for MET assessment by immunohistochemistry. Primary outcome measure was overall response to TKI (OR) at end of observation. Secondary outcome measures were best radiological response (by CT, RECIST 1.1) and progression free and overall survival; $p < 0.05$ denotes statistical significance.

Results. Of 812 genotyped NSCLC patients, 74 (9%) had EGFR mutations and 18 fulfilled the stringent inclusion criteria. MET-positivity was present in 8 tumors (44%) and OR was progressive disease (PD) in 4 patients (MET: 2+; 2−), stable disease in 9 patients (MET: 6+; 3−), all 5 patients who had a partial response (PR) were MET− (p=0.05). Patients with MET− tumors had more evident tumor shrinkage to TKI (50.5% MET− vs. 30% MET+; p=0.02, t-test). Tumors of patients with PR had significantly lower MET- expression (p=0.04; t-test) and MET status correlated significantly with best radiological response (R2: 0.3; p=0.04). Patients with MET+ tumors had earlier responses whereas patients with MET− tumors achieved their best response later (MET+ 2.5 months vs. MET− 7 months; p=0.03, log-rank). There was no association of MET with progression free- or overall survival; however, patients with PD had the shortest progression free and overall survival (both p<0.0001; log-rank).

Conclusions. MET status before TKI-start predicts TKI response. Specifically, patients with MET– tumors show better responses than patients with MET+ tumors. While the stringent selection criteria are necessary to test the biomarker value in this specific therapeutic setting, the resulting cohort size limits the effect. Nonetheless, the predictive effect of MET reached statistical significance. Given the simplicity of MET assessment before TKI-start, our findings warrant validation in an independent cohort.

FR-025
Selected reaction monitoring mass spectrometry (SRM-MS): targeted proteomics for prostate cancer protein network analysis

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Aims. A key barrier to the realization of personalized medicine for cancer is the identification of novel biomarkers. We have recently used proteomics-based technologies and computational modeling to characterize the PTEN-dependent glycoproteome of prostate cancer that can be used as a serum-based screening test for patients with prostate cancer. Despite large efforts in prostate cancer research, the mechanism of prostate cancer cell survival after androgen-ablation therapy and the definition of significant versus insignificant prostate cancer disease are unsolved problems. We will address these important questions using protein network analysis and targeted proteomics.
Methods. A type of targeted proteomics is selected reaction monitoring mass spectrometry (SRM-MS), a reliable and robust mass spectrometry methodology. In numerous multi-lab cross validation studies, aliquots of the same sample were analyzed by SRM-MS and the results showed a CV of only 20%. Contrary to conventional shotgun mass spectrometry, SRM-MS monitors a predefined set of proteotypic peptides allowing for targeted surveillance of many proteins over numerous samples. A prerequisite of SRM-MS is the knowledge of chromatographic and mass spectrometric properties of peptides to be monitored by SRM-MS, the knowledge of which is summarized in spectral libraries.
Results. We established a spectral library for SRM-MS using whole cell lysates of representative prostate cancer cell lines as well as prostate tissue samples using extensive two-dimensional fractionation of peptides derived from whole cell lysate and analyzed each individual fraction using shotgun proteomics. Further, we have established a network of proteins important for prostate cancer, which includes a detailed analysis of the androgen receptor complex and general oncogenes.
Conclusions. SRM-MS is a robust, antibody independent mass spectrometry methodology which allows for robust discovery research and will hopefully allow pathologists to monitor complex protein signatures in patient samples for the advent of personalized precision medicine in the future.

Molekulare Pathologie

FR-034
Delayed tissue preservation does not induce a systematic phosphoprotein response

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Aims. The aim of this study was to characterize the potential effects of delayed preservation ("cold ischemia") on the stability of phosphoproteins using a non-targeted proteomic approach.
Methods. Murine and rat liver samples were exposed to different cold ischemic conditions before cryopreservation. The phosphoproteome was analyzed using quantitative tandem mass spectrometry (LC-MS/MS). The workflow included the following steps: extraction and aliquotation of mouse and rat liver tissue in triplicates, immediate preservation of reference samples (time point 0), collection of four to five ischemia time points up to 360 min, lysis of fresh frozen liver samples, application of a global, quantitative phosphoproteomics workflow using strong cation exchange chromatography (SCX) and immobilized metal ion affinity chromatography (IMAC) followed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis.
Results. The phosphoproteomic analysis of ischemic mouse liver tissue samples by LC-MS/MS indicated no significant alterations of 791 phosphorylation sites in all three replicate experiments of all five time points (0, 10, 20, 30, and 60 min) by global rank test statistics. Similarly, for the rat liver tissues, no significant alterations of any of 1,692 phosphorylation sites identified in all three replicate experiments of all four time points (0, 15, 60, and 360 min) could be detected by global rank test statistics. For both data sets grouping of similar time-course profiles of ischemia-induced changes of phosphorylation sites shows an equal distribution over all 8 profile types identified. None of which was found to be overrepresented.
Conclusions. The data from our comprehensive LC-MS/MS approach indicates that the phosphoproteome globally undergoes undirected and unspecific changes with increased ischemia time without reaching statistical significance for single phosphorylation sites. We conclude that tissue samples exposed to prolonged and moreover different delays before preservation are not ideally suited for accurate quantitative measurements of the activation state of signalling pathways. Our study has immediate impact for the discovery of biomarkers for targeted therapies involving kinase inhibitors which have recently been a focus in the field of personalized medicine.

FR-035
Detection of the activating GNAS gene mutation in fibrous dysplasia by polymerase chain reaction using a locked nucleic acid probe (LNA)

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Aims. The diagnosis of fibrous dysplasia (FD) with conventional histological methods may be sometimes difficult. The detection of the activating GNAS gene mutation by polymerase chain reaction (PCR) using a peptide nucleic acid (PNA) probe (clamping technique) in formalin fixed paraffin embedded tissue (FFPE) proved to be of great diagnostic help. The PNA probe is designed to bind preferentially to the target wild-type genomic DNA, thus preventing the PCR DNA primer from

annealing to wild type DNA and consecutively enhancing the amplification of mutated DNA. Because of the restricted availability of PNA probes due to patent issues this study should demonstrate whether PNA probes could be replaced by locked nucleic acid (LNA) probes.

Methods. The DNA of FFPE control tissue and FFPE, EDTA decalcified tissue of eight cases of confirmed FD were examined for a mutant GNAS gene by PCR using one LNA probe and two DNA primers [Forward: 5-GTTTCAGGACCTGCTTCGC-3; Reverse: 5-GCAAAGCCAAGAG-CGTGAG-3; LNA: CgCTgCCgTgTC--NH (LNA monomers are indicated in upper case letters)]. The mutation was confirmed by Sanger sequencing or light cycler analysis.

Results. Only by using the LNA probe in the PCR reaction it was possible to suppress the amplification of wild type GNAS and to get enough amplified mutated GNAS DNA to perform a consecutive Sanger sequencing or light cycler analysis for final proof of the GNAS mutation.

Conclusions. LNA probes are suited to inhibit the amplification of the wild type GNAS gene and therefore constitute a convenient method to detect the mutated GNAS gene in FD.

FR-036
Melting peak analysis by SMART-PCR: at the peak of epigenetics

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Aims. Cancer cells show hypermethylation of thousands of genes whereas mutations affect only tens of genes. Changes in the methylation status of DNA are an early phenomenon in tumorigenesis. Therefore DNA methylation has gained attention as auspicious biomarker in tumorigenesis. Hypermethylation of a promoter region decreases gene expression of the adjacent gene and can lead to loss of function. In this way tumor suppressor genes and other important regulatory genes become inactivated contributing to cancer development.

Methods. For analysis of such epigenetic biomarkers isolated DNA was treated by sodium bisulfite to convert unmethylated cytosines to uracils but leaving methylated cytosines unaffected. Afterwards converted DNA was assessed by sensitive melting analysis after quantitative real-time PCR (SMART-PCR) using DNA-intercalating fluorescent dyes or HybProbes for imaging. SMART-PCR uses primers that do not differentiate between methylated and unmethylated sequences, and Hyb-Probes that target CpG-dinucleotides with the highest discrimination power between wild type and mutant DNA.

Results. By high-resolution melting (HRM) curve analysis each tumor entity exhibits methylation-pattern specific melting peaks. The method was found to identify low levels of methylated DNA (approx. 1%) in a background of differentially methylated DNA. Preliminary results of our investigations showed distinct melting peaks between different tumor types and wild type DNA making the method a favourable tool for molecular diagnostics.

Conclusions. As result of the study methylation patterns were found that can be potential markers for bijective identification of tumor subgroups and to improve personalized treatment by early detection, identification of the primary tumor and clarify metastatic status (medical importance).

FR-037
An in silico approach to delineate a gene panel for targeted re-sequencing of recurrent, potentially targetable mutations in diffuse large B-cell lymphoma

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Aims. Targeted single-nucleotide re-sequencing (TRS) of drugable mutations is a valid approach for the molecular characterization of solid tumors. Recently, data from whole-exome studies of diffuse-large B-cell lymphoma (DLBCL) have suggested a set of recurrent gene mutations in potentially targetable pathways. Currently, the fraction of DLBCL patients harboring mutations that can be identified using targeted re-sequencing has not been specifically addressed. Here we determined a gene-panel for TRS in DLBCL and assessed whether this approach identifies a clinically relevant number of DLBCL patients.

Methods. Datasets from recent studies (pubmed), including whole-exome sequencing data, were combined with annotated mutation frequencies (COSMIC database). Using the annotated mutational spectrum, the genelist was screened for mutations that are detectable by TRS-approaches (SNaPshot, mycancergenome). The resulting list was assessed for targets implicated in ongoing clinical trials (clinicaltrials.gov). Given that combined assessment of selected mutations has not been performed but whole-exome data suggest mutually exclusive mutation patterns among patients for at least a subset of genes, predicted detection rates were calculated assuming both a combined as well as a mutually exclusive distribution of mutations.

Results. An initial 33 gene-list (20 top entries from COSMIC and 13 genes from the literature) were screened and we selected KIT, EZH2, MYD88, and BRAF as actionable mutations that can be easily assessed by TRS. The predicted detection range was between ~8–18%. These predicted numbers might overestimate the actual detection frequency in patient samples and does not take biological or sample-related variability into account. Aberrations in BCL2, BCL6, BCL10, SOCS1 and MLL2 are more frequently encountered (8–26%); however, reliable detection cannot be achieved by TRS alone and would require combination of at least three methods (e.g. TRS, FISH, sequencing).

Conclusions. Despite the limitations of in silico analyses, the presented data indicate that targeted single-nucleotide re-sequencing in DLBCL will only identify a small but important subset of patients. At the same time, our analysis revealed that combination of complementary methods increases the predicted detection rate into more clinically relevant levels. Thus, collectively our data emphasize combined detection strategies as a valid approach to identify DLBCL-patients for targeted therapy.

FR-038
Validation of chromogenic in situ hybridization for the diagnosis of ALK rearrangements in non small cell lung cancer

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Aims. The determination of anaplastic lymphoma kinase (ALK) rearrangement is necessary for choosing the appropriate treatment for non-small cell lung cancer (NSCLC) patients. Currently, fluorescence in-situ hybridization (FISH) is considered as the primary method for assessing formalin-fixed, paraffin-embedded tissue for ALK translocation. However, FISH requires a specialized and expensive equipment, the signals fade rapidly and it is difficult to detect overall morphology and tumor heterogeneity. Chromogenic in situ hybridization (CISH) has been successfully introduced for detection of several genetic aberrations like HER2 amplification. This study evaluates the concordance between

ALK gene rearrangement in adenocarcinomas of the lung as subgroup of the NSCLCs assessed by FISH and a newly developed chromogenic in situ hybridization (CISH) assay.

Methods. We examined 101 adenocarcinomas of the lung by FISH and CISH and compared the number of aberrant tumor cells between both methods.

Results. ALK gene rearrangement was identified by FISH in 16 patients (15.8%). In the ALK-positive cohort positive rearrangement signals were identified in 23–98% (mean, SD, 49.5%, 23.9%) of tumor cells using FISH and in 16–90% (mean, SD, 49.5%, 23.4%) using CISH. We named cases with 7 to 14% positive cells borderline group. In total we had ten borderline cases with a mean of 6.9±3.1% detected by FISH and a mean of 4.3±4.0% detected by CISH. The cut-off value for positivity was 15%. The results of CISH were in complete concordance with FISH.

Conclusions. CISH is a highly reproducible and feasible method for the detection of ALK gene rearrangements in adenocarcinomas of the lung. Our results suggest that CISH is a suitable alternative to the current gold standard FISH. Furthermore it is able to complement genetic analysis by FISH in difficult cases.

FR-039

Simultaneous mutation analysis of EGFR, HER2, KRAS, HRAS, NRAS, BRAF, PIK3CA and MAP2K1 in NSCLC samples using a 454 multiplex sequencing assay

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Aims. Non-small cell lung cancer (NSCLC) is the most common cause of cancer-related deaths in western countries. Recently, genome-wide sequencing studies have identified several oncogenic driver mutations with possible therapeutic consequences. However, most of these mutations occur only in small fractions of ~1–8% of NSCLC cases. Thus, molecular pathology labs are in the need to establish novel analysis methods covering all these rare genetic aberrations at the same time, to provide the clinician with full genetic background information for optimal treatment stratification of the individual patient.

Methods. We developed a multiplex protocol to cover the most frequent driver mutations with potential therapeutical impact in NSCLC, including EGFR exons 18–21, KRAS exons 1–2, NRAS exons 1–2, HRAS exons 1–2, BRAF exon 15, PIK3CA exons 9 and 20, HER2 exon 20 and MAP2K1 exon 4. DNA extracts from lung cancer cell lines with known mutations in these genes were employed to evaluate the sensitivity, specificity and reproducibility of this assay. Clinical samples from FFPE tissue were evaluated by the 454 multiplex sequencing assay, and the results were compared to Sanger sequencing.

Results. The sensitivity of this novel multiplex 454 sequencing assay was superior to classical Sanger sequencing, in that the novel assay reliably detected mutations down to 1% allele frequency, compared to only 20–30% allele frequency by Sanger sequencing. Notably, after exclusion of homopolymer stretches, background variants occurred with a frequency of only <1%. The detection of mutated variants was highly reproducible, with a standard deviation of <2%. In clinical samples, mutations were much better detected compared to Sanger sequencing, especially in samples with low tumor cell contents (<30%).

Conclusions. We hereby present a novel multiplex 454 sequencing assay, which simultaneously detects a broad panel of potentially targetable driver mutations in NSCLC samples. This assay is superior to classical Sanger sequencing in terms of cost, sensitivity, turnover time and material consumption.

Hepatopankreatobiliäre Pathologie – klinisch

FR-040

Precursor lesions of familial pancreatic ductal adenocarcinoma: characterization of atypical flat lesions, PanIN and IPMN

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Aims. The well characterized precursors of pancreatic ductal adenocarcinoma (PDAC), PanIN, IPMN and MCN, show a ductal phenotype. Since genetically engineered mouse models have revealed that murine PDAC may arise from atypical flat lesions (AFL) developing through a process of acinar-ductal metaplasia, an alternative site of origin of human PDAC in the centroacinar-acinar compartment through a metaplasia-dysplasia sequence can be hypothesized. Recently, lesions comparable to murine AFL were observed in areas of acinar-ductal metaplasia in resected pancreatic tissue from individuals with a history of familial pancreatic cancer (FPC). In this study, we analyzed pancreas resection specimens from FPC individuals in order to search for PDAC precursor lesions, focusing on the role of AFL as possible alternative precursors of PDAC.

Methods. Pancreatic resection specimens from eight individuals with a FPC history were screened for lesions such as tubular complexes, AFL, PanIN and IPMN. Immunostaining for MUC1, MUC2, MUC5, MUC6, p53, smad4, Her2neu, MIB1 and Claudin18, as well as K-RAS mutation analysis were performed on all identified lesions.

Results. In addition to classical precursor lesions (PanIN, IPMN), AFL were found in all 8 cases in areas of lobulocentric atrophy with acinar-ductal metaplasia. AFL showed predominantly a MUC1/MUC5+, MUC2– immunophenotype with a variable positivity for MUC6 and a focally elevated Ki-67 proliferation index. Low-grade PanIN were partially MUC5/MUC6+ and MUC1/2–, high-grade PanIN showed additionally a partial MUC1-positivity. Claudin18 displayed a strong membranous expression pattern in low-grade as well as in high-grade PanIN, whereas AFL were moderately Claudin18-positive with a cytoplasmatic expression pattern. Smad4 did not show a loss of expression in any of the precursor lesions. Her2neu was moderately expressed in low-grade PanIN and IPMN. AFL were Her2neu-negative.

Conclusions. Pancreatic tissues from FPC individuals show AFL in addition to PanIN and IPMN. AFL display a different immunophenotype compared to classical PDAC precursors, particularly concerning the expression of mucin core proteins and of claudin18. The identification of AFL in all specimens suggests a potentially alternative pathway of carcinogenesis in FPC that starts in acinar-ductal metaplasia areas. Whether AFL also play a role in the development of sporadic PDAC has to be further investigated.

FR-041

Identification of three novel subtypes of pancreatic ductal adeno-carcinoma with distinct clinical outcomes and drug sensitivities

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Aims. Pancreatic ductal adenocarcinoma (PDAC) ranks fourth among cancer related deaths in the U.S. with an overall survival rate of <5%. Despite treatment the median survival after diagnosis is about 6 months. Less than 20% of pancreatic cancer patients present with localized disease that allows for a potentially curative Ro resection. Hence, novel therapeutic strategies and markers that allow for risk stratification are urgently needed.

Methods. Xenografts of primary tumors and of cell lines generated from primary tumors were implanted in immune-deficient NOD.Cg-Prkdcscid Il2rgtm1Wjl (NSG) mice. Compound screening for drug sensitivity was conducted with a cell viability assay (cellTiterBlue). The tissue microarray was constructed from patients that received partial pancreatoduodenectomy for PDAC between 1991 and 2006 at the Charité University Hospital Berlin.

Results. We identified patient-based models of three PDAC subtypes, i.e. classical, quasi-mesenchymal and exocrine-like which allow for 1) patient stratification according to biomarkers and 2) the prediction and pre-clinical verification of distinct drug sensitivities. The three subtypes differ in frequency and were associated with dramatic differences in survival times of pancreatic cancer patients (16–43 months). Further, we present evidence for subtype specific response to Gemcitabine and Erlotinib. Strikingly, our data also uncovered sensitivity of classical PDAC to Dasatinib.

Conclusions. The study introduces novel patient-specific models and suggests strategies to stratify and target human PDAC.

FR-042

The expression of markers of epithelial-mesenchymal transformation is distinctive and prognostically significant in periampullary carcinomas

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Aims. Periampullary carcinomas can be divided into prognostically meaningful subtypes by their histomorphologic growth pattern und their immunhistochemical profile independent of their anatomical localization. Thereby, the pancreatobiliary subtype, resembling the classical ductal adenocarcinoma of the pancreas, features one of the worst survival data, comparable to the pancreatic carcinoma. Whereas the importance of the epithelial-mesenchymal transformation (EMT) has been shown in pancreatic carcinoma, only sparse investigations analyse EMT in larger cohorts of periampullary carcinomas or in relation to the histologic subtype.

Methods. We classified our collective of 170 patients with periampullary carcinomas in histomorphologic subtypes, analysed immunohistochemically the expression of the EMT-marker vimentin, e-cadherin and β-catenin in the center of the carcinomas, the periphery and the tumor buds and correlated the results with the survival data.

Results. 107 carcinomas were subclassified as pancreatobiliary, 30 as intestinal, 13 as mixed, 19 as poorly differentiated and one as mucinous subtype. E-cadherin was expressed mostly incomplete membranous in tumor cells. β-catenin showed a switch from membranous expression in the center of the tumor to more cytoplasmatic staining in the periphery of the tumor in the pancreatobiliary and the poorly differentiated subtypes. Especially in the tumor buds, the dislocation of β-catenin became obvious in all subtypes. Vimentin was expressed in 37 carcinomas, in five even in >50% of the tumor cells. Only two of these carcinomas were of intestinal and three of mixed type. Eight of the 19 carcinomas of poorly differentiated subtype were positive for vimentin. The remaining 24 carcinomas showed pancreatobiliary morphology. Vimentin and membranous β-catenin in the periphery of the tumors demonstrated as independent prognostic factors, i.e. Vimentin indicated worse survival, whereas better survival was observed along with regular expression of β-catenin in peripheral tumor parts or even tumor buds. Loss of β-catenin in tumor buds was conspicuous in patients with worse prognosis.

Conclusions. Beyond the prognostic significance of the histomorphologic subtyping, EMT markers are differently expressed in the certain groups and are independent prognostic factors in periampullary carcinomas.

FR-043

α1-antitrypsin PiMZ-mutation and alcoholic steatohepatitis are bi-directionally aggravating amplifiers in chronic liver disease

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Aims. Heterozygous α1-antitrypsin (AAT) deficiency type PiZ (PiMZ) results in chronic liver injury and predisposes to hepatocellular carcinoma. This retrospective case control study focuses on the impact of PiMZ genotype and chronic alcoholic liver disease (ALD).

Methods. 6886 consecutive liver specimens were immunohistochemically tested for PiZ-deposits. On the basis of 254 PiZ-positive patients, 59 PiMZ adults without concomitant liver disease other than ALD were extracted and matched PiMM (wild type) patients by age gender and life-time alcohol consume for a retrospective case-control study. Histomorphological changes and routine laboratory parameters were examined.

Results. Analysis of PiMZ and PiMM patients indicates that PIMZ genotype significantly aggravates alcohol-toxic liver injury: the extent of liver fibrosis and necroinflammatory activity as assessed by Ishak score were significantly higher in PiMZ than in PiMM patients (p=0.001, and p=0.007 respectively). Vice versa, alcohol consumption exacerbates hepatocellular PiZ-deposition in PiMZ patients (r=0.45, p=0.0003) as a factor of disease progression.

Conclusions. Since regional prevalences of PiMZ heterozygosity range between 1 and 5% in Western populations, there is a substantial risk of coincidence with chronic alcohol abuse. PiMZ genotype and ALD are mutually reinforcing pathogenetic components contributing to chronic liver injury. We therefore recommend advising patients with heterozygous PiMZ mutation and concurrent alcohol of their increased risk for disease progression.

FR-044

Revitalized morphological criteria of primary sclerosing cholangitis (PSC) in liver biopsies

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Aims. Primary sclerosing cholangitis (PSC) is a rare, chronic, progressive cholestatic liver disease of yet unresolved etiology. The majority of publications describe few subtle, relatively common and therefore

non-specific as well as stage-related changes and a set of more specific changes. Liver biopsies are believed to rarely contribute to clarification in suspected PSC patients, though, as only 5–40% of liver biopsies show specific changes in keeping with PSC. Therefore performance of a liver biopsy is not routinely recommended in the confirmation of a PSC diagnosis. The aim of this study is a compilation of histomorphological changes observed in liver biopsies from PSC patients verified by "gold standard" ERCP/MRCP.

Methods. We chose liver biopsies of patients with initially unexplained abnormal liver function tests and final diagnoses of non-alcoholicsteatohepatitis (NASH) or drug-induced liver injury (DILI) as control group. We investigated pre-assigned morphological features (e.g. basal membrane thickening, CK7-positivity and copper deposits of periportal hepatocytes, concentric periductal fibrosis, bile duct loss, fibrosis) in liver biopsies of 63 verified PSC patients and 56 control-group patients in this retrospective, blinded study. Each biopsy was stained with common histological stainings (including rhodanine, diastase-PAS and by immunohistochemistry against cytokeratin 7 (CK7).

Results. About 90% of liver biopsies of PSC patients showed distinct morphological alterations of bile ducts and/or periportal hepatocytes. Using all features mentioned above the statistical evaluation showed a sensitivity of 82.5% and a specificity of 96.6% for the histopathological suspicion of PSC. Important and frequent features in PSC are periductal basal membrane thickening and CK7-positivity of periportal hepatocytes ($p<0.0001$).

Conclusions. Key features of evaluation include basal membrane thickening, CK7-positivity and copper deposits in periportal hepatocytes, concentric periductal fibrosis and bile duct loss. The first-mentioned criteria are the most consistent findings in PSC biopsies and good predictors of bile duct disease. The inclusion of all mentioned criteria in the evaluation of liver biopsies renders this diagnostic tool of higher value in the clarification of unexplained abnormal liver function tests in view of suspected PSC, in addition to the exclusion of differential diagnoses (such as NASH and DILI), the detection of inflammatory activity (e.g. overlap-syndrome) and fibrosis.

FR-045

Hepatitis E virus (HEV) infection after liver transplantation: molecular detection and clinico-pathological features of a recently emerging liver disease

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Aims. Hepatitis E virus (HEV) infection is increasingly observed in developed countries especially after solid organ transplantation and other immunocompromised conditions. This retrospective two-center study was performed to investigate HEV infections in patients after liver transplantation (OLT) and evaluate histological features and clinical course of HEV hepatitis.

Methods. 683 liver biopsies of 282 liver transplant recipients were tested. A semi-nested reverse transcription-polymerase chain reaction (RT-PCR) assay targeting the HEV open reading frame (ORF) 2/3 gene region was designed for application on formalin-fixed, paraffin-embedded (FFPE) liver tissues. Histological features, i.e. necrosis, apoptosis, inflammation activity, cholestasis and fibrosis were evaluated in cases tested positive for HEV.

Results. HEV-RNA was detected in ten liver biopsies taken from four different patients (4/282; detection rate 1.4%). Testing on paraffin embedded liver biopsies proved reliable with a similar detection rate of HEV infection as reported for serum-based testing. In all four patients, liver biopsies taken prior to HEV diagnose and liver explants were tested negative indicating de novo infection after OLT. 3 patients had signs of hepatitis for 6 month or longer. All patients, except one who developed chronic hepatitis, showed viral clearance and initially elevated transaminase levels normalized after an average time of 25±11 month. Direct sequencing of amplicons confirmed HEV genotype 3 in all four cases, with inter- but not intra-individual sequence variability. Histologic findings in the early phase of infection were variable and comprised cases with few hepatocyte apoptosis and lacking or only little inflammation sometimes superimposed by features of acute cellular rejection. Cholestasis and interface hepatitis, typical findings in acute autochthonous HEV infection in immunocompetent individuals, was not observed. Fibrosis was present in follow up biopsies of cases with persisting signs of hepatitis.

Conclusions. HEV infection is a relevant cause of liver disease after OLT. Molecular testing for HEV in routinely processed liver biopsies is a powerful supplemental approach for the evaluation of patients with elevated transaminases of unknown origin after OLT. Liver histology of HEV infection under immunosuppression is unspecific, variable and distinct from acute autochthonous HEV infection in immunocompetent individuals.

Workshop Apoptose

FR-048

Basonuclin-1 influences TGF-β1-induced epithelial dedifferentiation and cell death

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Aims. The aim of this work was to elucidate a potential interaction of the transcription factor Basonuclin-1 and TGF-β1-signaling in a cell model of TGF-β1-dependent epithelial dedifferentiation.

Methods. Mammary epithelial cells were treated with TGF-β1 to induce epithelial dedifferentiation. Gene-expression profiling was used to identify differentially expressed transcription factors. For functional analyses the expression of Basonuclin-1 was repressed via RNAi in conjunction with several cellular assays (e.g. marker expression analyses, cell-adhesion, cell death). Downstream targets of Bnc1 during active TGF-β1-signaling were identified via genome-wide expression profiling.

Results. Our results show that Bnc1 is induced upn TGF-β1-stimulation of mammary epithelial cells. Bnc1 affects the outcome of TGF-β1-signaling with respect to epithelial dedifferentiation and cell death. Results from gene-expression profiling revealed that Bnc1 is an important mediator of TGF-β1-dependent gene expression changes and has identified several downstream targets that are putatively involved in Bnc1-dependent phenotypes in response to TGF-β1.

Conclusions. In summary, our work revealed for the first time that Bnc1 is an important downstream mediator of TGF-β1-signaling.

FR-049

Neuropilin-2 and its impact on autophagy in colorectal cancer

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Aims. Neuropilins (NRP) are receptors in neurons and epithelial as well as endothelial cells that bind Class 3 Semaphorins and members of the VEGF family. Two subtypes are known, NRP1 and NRP2, which influence neural guidance, vasculogenesis and angiogenesis. Furthermore, NRPs are expressed in several tumor cells and might have an important function during carcinogenesis. Our group has already shown an important function of NRP2 in autophagy control of advanced prostate and pancreatic cancer cells (Cancer Res, in press). Therefore, we investigated the expression of NRP2 in colorectal cancer and its role in autophagy of colorectal cancer cells.

Methods. We investigated the expression of NRP2 in an already established cohort of rectal cancer patients by immunohistochemistry of formalin fixed paraffin embedded tissue. In cell culture models we used COLO320DM, HCT116 and RKO colorectal cancer cells to detect Neuropilin-2 expression by RT-PCR and Western blot. The role of NRP-2 in autophagy control was tested under 5-FU stress and detection of the autophagic flux using a Light-Chain-3 II immunoblot. Electron microscopy was used to confirm these results. To reduce NRP2 protein levels we executed RNA interference. The sensitivity of cancer cells for 5-FU treatment was measured by cell death assays.

Results. NRP2 was overexpressed in a majority of colorectal cancer patients. All cell lines show a high expression of Neuropilin-2 messenger and protein. RNA interference against NRP2 under 5-FU stress reduced autophagic flux significantly. In turn, the sensitivity to 5-FU therapy was increased in a cell culture model.

Conclusions. In colorectal cancer NRP-2 is an important regulator of autophagy and 5-FU resistance in specific circumstances. Thus, NRP2 might be an interesting target for future therapeutic approaches.

Aktuelle Habilitationen

SA-007

In situ analysis of pathomechanisms of the human intervertebral disc degeneration

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Aims. Low back pain is one of the major causes of pain and disability in the western world, with a constantly rising life-time prevalence of approximately 60–85%. Degeneration of the intervertebral disc is believed to be a major cause of low back pain. In general, disc degeneration is characterized as a multifactorial disease with a hereditary background. The present work deals with pathomechanisms of human intervertebral disc degeneration. Especially with respect to the anatomical complexity of the disc the in-situ analysis allows important insights into the mechanisms which underly cell and matrix changes.

Methods. Semiquantitative macroscopic and microscopic changes of the intervertebral disc were assessed and classified. Furthermore additional methods like immunohistochemistry, in situ hybridization and in situ zymography were used to analyze phenotypic cellular and matrix changes.

Results. I. We have developed and tested a practicable, valid, and reliable histologic classification system for lumbar discs, which can serve as a morphologic reference framework to allow for more sophisticated molecular biologic studies on the pathogenesis of ageing and degeneration of the disc. II. Secondly, we were able to demonstrate that intrinsic (genetic) and extrinsic (overweight) factors have a profound effect on the process of disc degeneration. III. Cells with a notochord-like phenotype are present in a considerable fraction of adult lumbar intervertebral discs. The presence of these cells is associated with distinct features of (early) age related disc degeneration. IV. During the process of disc degeneration, the intervertebral disc shows a progressive and significant reduction in height due to tissue resorption. This matrix loss is related to an imbalance between matrix synthesis and degradation. During this process an inflammatory reaction takes place and resident disc cells are causative involved.

Conclusions. In summary, disc degeneration is a multifactorial disease with a strong intrinsic (hereditary) and extrinsic (mechanical factors) background. The process starts as early as in the second life time decade and shows a high inter-individual difference. The loss of regenerative capacity in the intervertebral disc is probably related to the loss of stem cells e.g. notochord like cells. Resident disc cells are involved in the inflammatory reaction with increased matrix degradation, resorption and reduced matrix synthesis.

SA-008

Molecular characterization of osteosarcomas

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Aims. Osteosarcomas are rare with an estimated incidence of 5–6 cases per million population per year. Since patients are generally treated by (neo-)adjuvant chemotherapy, only biopsy samples obtained for diagnostic purposes can be used for molecular analyses. Since the prognosis of patients has not improved over the last 30 years, more insight in the molecular tumorigenesis is urgently needed to provide new biomarkers and potentially also therapeutic targets.

Methods. We analyzed a set of clinically well characterized pretherapeutic osteosarcoma samples using genome wide SNP chip analyses. Additionally, established osteosarcoma cell lines were investigated for genome wide miRNA expression using cultured osteoblasts and mesenchymal stem cells as normal references.

Results. The most frequent genomic alterations detected using SNP chip analyses included amplifications of chromosome 6p21, 8q24 (harboring MYC) and 12q14 (harboring CDK4) as well as LOH of 10q21.1. All these aberrations and the total degree of heterozygosity of each tumor were significantly associated with an unfavourable clinical outcome of patients. We defined a new chromosomal alteration staging (CAS) system with a superior predictive potential compared to the established histologic regression grading. MicroRNA profiling showed several members of the well known oncogenic miR-17-92 cluster to be upregulated in the majority of osteosarcoma cell lines.

Conclusions. The proposed CAS staging could be a powerful tool in predicting the prognosis of osteosarcoma patients already at the time of diagnosis and consequently before initiating neoadjuvant treatment. Therefore, more individualized treatment protocols according to distinct genetic alterations might be conceivable for osteosarcoma patients. The miR-17-92 cluster is known to interfer with MYC and TP53 expression, two genes that seem to be frequently dysregulated in osteosarcoma. Additional studies are needed to clarify the pathways and mechanisms involved in this particularly altered expression of miRNAs.

SA-009

Renal cell carcinoma: analysis of resistance mechanisms towards apoptosis in order to develop experimental therapeutic strategies

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Aims. Understanding the mechanisms responsible for the resistance of RCCs towards radio- and chemotherapies is mandatory for the development of new, more efficient therapeutic strategies. The currently available "targeted therapies" with Multikinase- and mTOR-Inhibitors, however, have shown only limited success in the treatment of the metastatic disease and, therefore, more efficient therapeutic approaches are still needed. One of these new therapeutic strategies could be the "targeted" induction of proapoptotic pathways, which are known to be strongly disturbed in RCCs. We, therefore, analysed resistance mechanisms towards apoptosis in RCCs in vivo and in vitro to derive experimental therapeutic strategies activating proapoptotic pathways and thus overcoming the broad therapy resistance of RCCs.

Methods. Immunohistochemistry, semiquantitative Real-time PCR, cell culture, Western Blot, Caspase-Assays.

Results. The expression and function of multiple pro- and antiapoptotic factors in RCCs were analysed in vivo und in vitro revealing a shift of the cellular expression context towards the antiapoptotic factors. Based on this more antiapoptotic cellular context, we analysed the exact mechanisms responsible for the inhibition of apoptosis in RCCs. For this purpose, RCC cell lines were treated with TRAIL, Etoposide, Betulinic Acid and the putative BCL-2 antagonist HA14-1 either alone or in combination. These experiments revealed 1) that the apoptotic signalling pathways upstream of the mitochondria were functionally inducible in RCCs 2) that the inhibition of the mitochondrial apoptosis was situated on the level of the mitochondria themselves conducted by the antiapoptototic memebers of the BCL-2 Family and 3) that the signalling pathways downstream of the mitochondria were intact and inducible on release of proapoptotic factors from the mitochondria. Furthermore, both TRAIL- and Topotecan-induced apoptotsis could be synergistically enhanced by HA14-1.

Conclusions. RCCs exhibit a broad spectrum of resistance mechanisms towards the activation of the extrinsic and intrinsic apoptotic pathways. In this context, the disturbed activation of the mitochondrial apoptosis by the antiapoptotic members of the BCL-2 family could be identified as an important level of resistance in RCCs. Thus, overcoming this mitochondrial resistance by new "targeted therapies" like BCL-2 inhibitors could be one new therapeutic strategy to overcome the well known therapy resistance of RCCs.

SA-010

Predictive pathology of prostate cancer

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Ziel. Die einzigen uns derzeit zur Verfügung stehenden Kriterien zur Einschätzung der Prognose des postoperativen Verlaufs eines Patienten mit Prostatakarzinom sind das Tumorstadium (TNM-Stadium), die Beurteilung der Resektionsränder (R-Status) und die Differenzierung des Karzinoms (Gleason-Score). Am Beispiel eines Proteins, einer Histonmodifikation und der Erweiterung einer diagnostischen Methode soll exemplarisch die Verbesserung der Diagnostik und somit auch der Therapie des Prostatakarzinoms beschrieben werden. Die Biologie des Adenokarzinoms der Prostata reicht von lokal begrenzten Tumoren mit geringem Rückfallrisiko bis hin zu Tumoren mit hohem Risiko für die Progression auch nach radikaler Prostatektomie. Derzeit gibt es keine zuverlässigen Biomarker, um ein Tumorrezidiv und einen aggressiven klinischen Verlauf vorherzusagen.

Methoden. In unserer Studie haben wir Expressionsmuster des Androgenrezeptors (AR), der Androgenrezeptor-Koaktivatoren Lysinspezifi-

sche Histon-Demethylase 1 (LSD1) und four and a half LIM-Domain-Protein 2 (FHL2), die Expression des p53 Tumorsuppressorproteins und den Gleason-Score in organbegrenzten Adenokarzinomen der Prostata mit und ohne Rezidiv nach radikaler Prostatektomie untersucht.

Ergebnisse. Unsere Daten zeigen, dass ein hohe Expression von LSD1, eine starke nukleäre Expression von FHL2, ein hoher Gleason-Score und eine sehr starke nukleäre p53-Expression signifikant mit einem hohen Rezidivrisiko korrelieren. Hingegen fand sich keine Korrelation mittels der Expression des Androgenrezeptors und der zytoplasmatischen Expression von FHL2.

Schlussfolgerung. Die vorliegenden Befunde legen nahe, dass LSD1 und nukleäres FHL2 als neue prädiktive Biomarker für Prostatakarzinome mit aggressiver Biologie dienen könnten, des Weiteren spielen LSD1 und FHL2 offenbar eine große Rolle bei der Aktivierung AR-vermittelter Wachstumssignale im Prostatakarzinom und stellen damit mögliche neue Targets für zukünftige Therapien dar.

SA-012

Stem cells and their stromal niche

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Aims. The stem cell niche is the functional and anatomical 'node' that determines the appropriate stem cell behaviour. One important component of every stem cell niche is the stroma with a central role in tissue homeostasis, repair and disease. Mesenchymal stromal cells (MSC) are heterogeneous, dynamic and play a regulatory role in parenchymal and hematopoietic cell function in adult tissues. In recent studies, we analyzed how MSC and extracellular matrix (ECM) components (I) can direct stem- and progenitor cell differentiation for tissue engineering and (II) how stroma is involved in disease progression of ectopic and vascular calcification as well as myelofibrosis.

Methods. 3D-organotypic culture models including stroma cells and ECM components were developed and the directing effect of stroma cells on the differentiation capacities of adult (keratinocytes) and pluripotent stem cells (embryonic stem cells, induced pluripotent stem cells) was analyzed. Further, stroma cells were subjected to osteogenic differentiation and co-cultured with hematopoietic stem cells in an in vitro model of the hematopoietic stem cell niche. In these well-characterized in vitro models, we analyzed the effect of pathophysiological conditions (uremia, myelofibrosis) on both the hematopoietic progenitor cells as well as the stroma components.

Results. The results show that stroma cells significantly direct the maturation of keratinocytes in an organotypic culture model of the skin and can also induce the differentiation of pluripotent stem cells into squamous epithelium. This directing effect of stroma and components of the ECM on differentiation and proliferation was applicable to maintain hematopoietic CD34+ stem- and progenitor cells in the primitive, proliferating state. MSC efficiently differentiate into osteoblasts with a pro-synthetic phenotype providing important components of the bone marrow niche. Further, it was shown that pathophysiological stimuli can significantly disturb the hemostasis of differentiation and matrix remodeling of the stroma leading to a pro-synthetic phenotype with excessive matrix remodeling (e.g. in myelofibrosis), loss of the niche functions and (mal)differentiation going beyond physiological levels (ectopic calcification, vascular calcification).

Conclusions. Concluding, the studies show the impact of stroma in tissue homeostasis. Future studies will determine if the stroma can be therapeutically targeted in the context of myelofibrosis (in MPN) and vascular calcification.

Abstracts

SA-013

Familial GIST—a rare hereditary tumor syndrome with germline mutations in KIT

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Aims. Familial GIST (gastrointestinal stromal tumor) syndrome is a rare autosomal dominant genetic disorder. We report on a kindred in which three family members carry a germline mutation (c.1727T>C, p.L576P) in exon 11 of the KIT gene and review the hitherto reported cases.

Methods. Mutational status was determined by direct Sanger sequencing.

Results. Apart from multiple GISTs in two of the mutation carriers, all of them had multiple hyperpigmented skin macules and a history of achalasia-like stenosis of the esophagus in early childhood. In the index patient more than 100 tumors and a diffuse Cajal cell hyperplasia of the small bowel occurred. Sequencing of DNA extracted from tumor tissue of one of his GISTs revealed the KIT mutation in exon 11 (c.1727T>C).

Conclusions. Together with the family described here, 24 unrelated cases with proven germline mutations in KIT have been reported. Familial GIST are often associated with skin hyperpigmentation, mastocytosis and dysphagia. In these hitherto described families the diagnosis was established from the age of 30 years onwards. Since in one patient reported here the GIST was a coincidental finding at the age of 15 years, the tumors might occur at a very young age and remain unnoticed until they—either due to increasing size, ulceration or malignant progression—become symptomatic. Therefore, we propose to start screening patients with known KIT mutations from a younger age.

SA-014

In-situ analyses of molecular pathways in the carcinogenesis of colon carcinomas

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Aims. Colorectal carcinomas are heterogeneous and include a variety of different subtypes. At least two main pathways have been well characterized, the classical adenoma-carcinoma sequence and the serrated route. It was our aim to further characterize the regulation of molecular mechanism in these pathways.

Methods. A comprehensive immunohistochemical and clinicopathological analysis of a large collection of human colorectal carcinomas was performed applying TMA technology. A mouse model with intestinal epithelial cell specific expression of oncogenic K-rasG12D and a very well documented collection of human serrated polyps were examined and compared in detail. KRAS and BRAF mutational analyses, promoter methylation analysis and MSI analysis were conducted.

Results. TCF4 and LEF1 showed a heterogeneous expression pattern in colorectal tumors. TCF4 expression turned out to be a negative prognostic factor being associated with shorter overall survival (p=0.020). LEF-1 expression correlated with longer overall survival (p=0.015). gamma-Catenin revealed a cytoplasmatic and nuclear upregulation at the invasive fronts which was associated with shortened disease-free survival (p=0.003). Membranous staining of TRAIL-R1 and TRAIL-R2 on cell membranes was an independent predictor of survival (p=0.019 respectively p=0.033). The mouse model reflected the human serrated route with KRAS mutation. In the human collection of serrated polyps/adenomas p16INK4a was upregulated in premalignant lesions and downregulated in invasive carcinomas which was accompanied by hypermethylation of the CDKN2A promoter. Additionally we found an upregulation of c-MYC and SIRT1 in the serrated route to colorectal cancer.

Conclusions. TCF4, LEF1 and gamma-Catenin turned out to be independent prognostic markers considering their subcellular localization. Concerning TRAIL-receptors membrane staining is a common feature which supersedes the prognostic significance of their staining intensity. This finding might be important for selection of patients for clinical trials using TRAIL-receptors targeting compounds. In the serrated route to colorectal cancer we could show that an oncogene induced senescence barrier appears in serrated polyps, which leads to a stop of proliferation. Through the molecular mechanism of hypermethylation of the CDKN2A promoter this barrier is overcome and finally invasive tumors develop. An additional mechanism might be the upregulation of SIRT1 which is known to antagonize apoptosis and senescence.

SA-015

C-MYC mediated regulations in colorectal cancer

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Ziel. c-MYC ist eines der zentralen Onkogene in der Karzinogenese kolorektaler Karzinome mit Wnt-Signalweg-Aktivierung. Das Ziel der Arbeit war c-MYC-regulierte Zielgene und Mediatoren zu identifizieren und funktionell zu analysieren, die zur zellulären Immortalisierung beitragen. Über die Korrelation der Expression von c-MYC und der c-MYC-regulierten Faktoren im Tumor sollte deren pathogenetische Relevanz für das Dickdarmkarzinom ermittelt werden. Ferner sollte geklärt werden, ob die c-MYC-regulierten Signalwege für die kolorektale Karzinogenese im Allgemeinen von Bedeutung sind und bei der Progression der malignen Transformation eine Rolle spielen.

Methoden. Mittels genomweiter Transkriptom-Untersuchungen und durch funktionelle In-vitro-Analysen wurden c-MYC-Zielgene und -Effektoren identifiziert und charakterisiert. Ferner wurde die c-MYC-abhängige Expression der Proteine in Tumor- und Normalgewebe des Dickdarms mittels immunhistochemischer Färbungen untersucht. Die Expression wurde außerdem in einem Progressionskollektiv von prämalignen und malignen Läsionen der serratierten Route des kolorektalen Karzinoms, die durch unterschiedliche molekulare Alterationen charakterisiert sind, bestimmt.

Ergebnisse. SIRT1 wird durch Induktion des metabolischen Enzyms NAMPT, der Zunahme von NAD+ und Sequestrierung von DBC1, einem SIRT1-Inhibitor, c-MYC-abhängig aktiviert. Über Hemmung von p53 trägt dies zur Unterdrückung von Apoptose und Seneszenz in Tumorzellen bei. c-MYC und SIRT1 sind in Form eines positiven Regulationskreises miteinander verbunden, da SIRT1 wiederum die Funktion von c-MYC durch Stabilisierung des Proteins steigert. Sowohl in der klassischen als auch in der serratierten Route des Dickdarmkarzinoms weisen c-MYC und SIRT1 eine korrelierte Hochregulation auf. In der serratierten Route ist dies assoziiert mit Mutationen von K-RAS und B-RRAF d. h. MAPK-Signalwegaktivierung und nimmt mit fortschreitender Progression zu.

Schlussfolgerung. Unsere Untersuchungen zeigen, dass c-MYC ein zentrales Onkogen in der Karzinognese des Dickdarmkarzinoms sowohl der klassischen als auch der serratierten Route ist. Der Regulationskreis, der c-MYC-NAMPT-DBC1 und SIRT1 verbindet, stellt eine essenzielle Schaltstelle für die Entstehung von Tumoren dar, in denen c-MYC als Onkoprotein wirkt. Effektoren innerhalb dieses Regulationskreises repräsentieren potenzielle neue krebstherapeutische Zielstrukturen.

SA-016

The pathogenesis of microsatellite-unstable colorectal cancer and the evaluation of novel diagnostic and therapeutic options

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Aims. Colorectal cancer represents a tumor type with heterogeneous molecular pathogenesis. The majority of colorectal cancers show chromosomal instability. However, about 15% of tumors are characterized by a deficiency of the DNA mismatch repair system. Mismatch repair deficiency leads to the accumulation of numerous mutations in the tumor cell genome. These mutations mainly affect repetitive regions termed microsatellites and lead to the high-level microsatellite instability (MSI-H) phenotype. MSI-H colorectal cancers show particular clinical and histopathology characteristics like a low frequency of distant metastases in spite of an advanced local tumor stage. Patients with MSI-H colorectal cancers have an improved prognosis compared to their microsatellite-stable counterparts. This observation is likely related to a pronounced anti-tumoral immune response of the host, which becomes manifest as dense lymphocyte infiltration at the primary tumor site.
Methods. Topic of the habilitation thesis was the characterization of the molecular MSI-H tumor pathogenesis, with main focus on the clinical consequences of MSI-H-associated mutations.
Results. In frame of the thesis, novel microsatellite mutations were detected, which are of high relevance for tumor progression. The analysis of the immune biology of MSI-H cancers led to the identification of novel molecular mechanisms of immune evasion like defects of HLA class II-mediated antigen presentation. Immune evasion phenomena have a direct consequence of the clinical course of the disease in MSI-H colorectal cancer patients: Mutations of the Beta2-microglobulin (B2M) gene, which occur in more than 30% of MSI-H colorectal carcinomas, are negatively related to the occurrence of distant metastases. B2M mutations represent a promising prognostic marker in colorectal cancer and are therefore evaluated in independent cohorts. The analysis of antigen-specific immune responses in MSI-H colorectal cancer patients demonstrated that DNA mismatch repair deficiency leads to the generation of highly immunogenic antigen structures, termed frameshift peptide (FSP) antigens. These FSP antigens are currently evaluated as vaccination agents in a phase I/IIa clinical immune therapy trial.
Conclusions. In summary, MSI-H tumors paradigmatically illustrate the close interaction between genetic alterations and the clinical appearance of the tumor. Hence, they represent an ideal model tumor for the development of novel tools for tumor diagnostics and therapy.

SA-017

Abelson interactor 1 (Abi1): from synaptic plasticity to colorectal cancer cell migration

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Aims. Colorectal cancer (CRC) is the second leading cause of death from cancer in the western world, and metastatic dissemination of tumor cells is of crucial relevance for patients' prognosis. We have previously shown that Abelson interactor 1 (Abi1), a 65-kD-substrate of the eponymous Abelson tyrosine kinase that is required for synaptic maturation in hippocampal neurons, is overexpressed in tissue samples of colorectal adenomas, invasive CRC and metastases. Furthermore, it has been shown that Abi1 is recruited to a multiprotein complex that regulates actin reorganization leading to cellular movement in a phosphorylation-dependent manner. The aim of this study is to characterize the functional role of Abi1 in CRC cells with regard to migration and metastasis.

Methods. We analyzed the expression patterns of Abi1 and its potential interaction partners, p85 subunit of Phosphatidylinositol-3-kinase (PI3K-p85) and heterogeneous nuclear ribonucleoprotein K (hnRNP K), in colorectal tissue specimens and three different CRC cell lines. Furthermore, we analyzed cellular morphology, protein distribution and actin organization patterns before and after application of the Abelson kinase-inhibitor STI571 (Glivec) by using immunofluorescence microscopy. Finally, we performed co-immunoprecipitation studies to confirm protein interactions and studied the functional role of Abi1 using RNAi knockdown techniques.
Results. We found strong expression of Abi1, PI3K-p85 and hnRNP K in tissue samples of CRC and metastases as well as in the three CRC cell lines. Abi1 and PI3K-p85 were localized in the cytoplasm of the tumor cells, while hnRNP K followed a mostly nuclear distribution pattern. We could show co-localization and co-immunoprecipitation of Abi1 with both hnRNP K and PI3K-p85, the latter could be abolished by treatment with STI571. Treatment with STI571 as well as RNAi knockdown of either Abi1 or hnRNP K led to an increased outgrowth of primitive filopodia and decreased the formation of broad-based lamellipodia from the cells.
Conclusions. In our study, we show that Abi1, PI3K-p85 and hnRNP K are expressed and form multiprotein complexes in a phosphorylation-dependent manner in colorectal carcinoma cells. Taken together, our data provide evidence for an important role of the complex formation between Abi1, PI3K-p85 and hnRNP K in the formation of broad-based lamellopodia as a prerequisite for cellular migration that might be targeted pharmaceutically to prevent the gain of a metastatic phenotype in CRC.

SA-018

Morphology and molecular mechanisms of necroptosis in colorectal carcinoma

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Aims. While cell death research in the last decades has mainly been focused on elucidating apoptosis, the necrosis-like types of cell death are presently obtaining recognition in both physiology and pathology. Necroptosis, a RIP1-dependent form of programmed necrosis occurring under caspase-deficient conditions, has been implicated in chronic inflammation and cancer. The aim of our project is to study the subcellular morphology and the molecular mechanisms of necroptosis in colorectal cancer.
Methods. Cytotoxicity and apoptosis assay, FACS analysis, siRNA knockdown, co-immunopreciptitation experiments, stable and transient transfections, immunohistochemistry, electron microscopy.
Results. We established a cell culture model for chemotherapy-induced necroptosis in colorectal cancer. We found that colorectal carcinoma cell lines were sensitized to chemotherapy-induced cell death when co-treated with a caspase inhibitor, and that this effect was caused by the induction of necroptosis. Mechanistic studies revealed that chemotherapy-induced necroptosis requires the inhibition of caspase activity and occurs in a tumor necrosis factor receptor 1 (TNFR) and receptor interacting protein (RIP) dependent manner. Moreover, some chemotherapeutics augmented necroptosis signaling by up-regulation of TNFR1 surface expression and de-stabilization of inhibitors of apoptosis (IAPs). In order to investigate the biological significance of necroptosis in colon cancer we assessed RIP expression in human colorectal carcinoma samples by immunohistochemistry. RIP was significantly less abundant in colon carcinoma compared to normal tissue. Since the ultrastructural morphological phenotype of necroptosis is unknown, we investigated colon carcinoma cells upon specific induction of necroptosis via electron microscopy. Interestingly, in early phases of necroptosis the cells

exhibited distinct characteristics such as ER dilatation and fusion of enlarged mitochondria.

Conclusions. Necroptosis-inducing agents could re-sensitize colorectal cancer cells to conventional chemotherapy. Necroptosis requires TNFR1 and RIP signaling and is accompanied by characteristic subcellular morphological features. RIP expression is down-regulated in colon carcinoma. Together, our findings suggest that specific induction of necroptosis could represent a novel therapeutic strategy in colorectal cancer.

SA-019
microRNA expression in breast cancer and development

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Aims. The overall goal is to characterize microRNA (miRNA) expression during breast development and assess miRNAs as potential novel prognostic and predictive biomarkers in human breast cancer. Specific aims are I) to characterize miRNA expression during different stages of mammary gland development in a mouse model II) to assess intratumoral heterogeneity of miRNA expression in breast cancer and III) to investigate changes in miRNA expression during and after neoadjuvant chemotherapy of breast cancer.

Methods. In mouse mammary glands, expression of 318 murine miRNAs was analyzed by bead-based flow-cytometric profiling throughout a 16-point developmental time course to derive a comprehensive tissue-specific miRNA expression profile. In human breast cancer, the expression of four candidate miRNAs was assessed by qRT-PCR in 132 paraffin-embedded samples of 16 large primary invasive breast cancers including different tumor zones (peripheral, intermediate, central) as well as axillary lymph node metastases. Expression of miRNAs associated with chemosensitivity or -resistance of breast cancer cells in-vitro was evaluated in breast cancer before and after neoadjuvant chemotherapy.

Results. During mouse mammary gland development, 102 miRNAs were expressed in 7 temporally co-regulated clusters. Breast cancer-associated miRNAs were significantly enriched in these clusters. None of the investigated single miRNAs or miRNA clusters were exclusively associated with a particular development stage. In human breast cancer, miRNA expression showed considerable intratumoral heterogeneity within and between different tumor zones and between different lymph node metastases from the same patient. Candidate miRNAs showed significant changes in expression during neoadjuvant chemotherapy. The pre-therapeutic expression of individual miRNAs was associated with histopathologic tumor response.

Conclusions. MicroRNAs are expressed in temporally co-regulated clusters during breast development, which were significantly enriched for breast cancer-associated miRNAs. In human breast cancer, intratumoral heterogeneity can lead to significant sampling bias, and multiple areas of the primary tumor or several tumor-involved lymph nodes should be sampled when assessing miRNA profiles as prognostic/predictive biomarkers. Individual miRNAs showed significant changes in expression during neoadjuvant chemotherapy of breast cancer and hold promise as novel biomarkers for prediction of treatment response.

Workshop Pankreas

SA-020
Collagen type V influences the tumor microenvironment in pancreatic cancer

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Aims. Pancreatic cancer is a very aggressive cancer that is characterized by a pronounced interaction between cancer cells and stromal cells and the prominent hypoxic microenvironment surrounding the cancer. Aim of this study is to investigate the role that stromal collagen type V (Col V) has on pancreatic stellate cells (PSC) and pancreatic cancer cells (PCC) considering the morphology, activation status and the signaling pathways responsible for the specific behavior of PCC on Col V and its possible role in neoangiogenesis.

Methods. Morphology and activation status of PSC was investigated by immunoblotting and immunofluorescence after Col V knock-down through siRNA. The role of the β1-integrin signaling pathway was analyzed by performing functional analyses (adhesion migration and proliferation assays) after pharmacological and antibody-mediated inhibition. The involvement of Col V in neoangiogenesis was investigated by mRNA expression of different angiogenic and antiangiogenic factors and by tube formation assays in co-culture systems of PSC and HU-VEC.

Results. PSC are the main source of Col V in pancreatic cancer tissues and its knock-down affects their morphology but not their activation status, as shown by αSMA and GFAP staining. The intracellular effects of Col V are at least partially mediated by the β1-integrin signaling pathway, and these effects can be specifically blocked by inhibitors. Conditioned medium of PSC influences tube formation of endothelial cells and Col V affects angiogenesis by modulating the expression of antiangiogenic factors in PSC-HUVEC co-culture systems.

Conclusions. Col V influences PSC and PCC characteristics through activation of the β1-integrin pathway and promotes tumor-stroma interactions in pancreatic cancer. Further experiments are needed to better define the role of Col V in neoangiogenesis in the hypoxic microenvironment of pancreatic cancer.

SA-021
Functional analysis of MicroRNA family MiR-216 and 217 in pancreatic cancer

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Aims. MiRNAs are small noncoding transcripts that regulate gene expression by either repressing the translation or causing degradation of multiple target mRNAs. A growing body of evidence suggests that they orchestrate numerous biological processes including cell growth, proliferation and apoptosis. Consequently, deregulation of miRNAs expression may result in a variety of diseases including cancer. Understanding the biological role and contribution of individual miRNAs to carcinogenesis necessitates identification of their target mRNA molecules. However, identification of miRNA target genes has been challenge, mostly given the fact that each miRNA species may regulate hundreds of target genes. Therefore, accurate identification and validation of miRNA targets is critically important for the functional characterization of individual miRNAs and, subsequently, to understanding the role of miRNA regulation in carcinogenesis. In present study, we aimed at identification and validation of targets of microRNA family miR-216 and miR-217 that are characteristic of pancreatic tissue and have earlier been found

as down-regulated in 94 patient specimens of pancreatic ductal adeno-carcinoma.

Methods. Candidate target genes of interrogated miRNAs were first predicted in silico and further refined by considering most up-regulated potential mRNA targets in cancer samples. A total of 15 genes were then selected for validation in vitro in a panel of 10 pancreatic cancer cell lines.

Results. Ectopic expression of miR-217 reduced mRNA levels of DKK1, E2F3, MSN and RUNX1. These results were fully supported by using precursor miRNA molecules. Concomitantly, reduction of cell viability and induction of apoptosis was detected. This observation was in full agreement with the results of pathway analysis and literature review, which showed involvement of the miRNA-217 target genes in control of cell cycle and apoptosis.

Conclusions. Therefore miR-217 is suggested to operate as a tumour suppressor in by targeting DKK1, E2F3, MSN and RUNX1 and may be of interest in the context miRNA-based therapy of pancreatic cancer.

Workshop Liver Carcinogenesis

SA-042

Integrative genomic analysis of hepatocellular carcinoma reveals a unique genomic aberration and transcription pattern in patients with poor prognosis

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Aims. Hepatocellular carcinoma (HCC) is the third leading cause of cancer death worldwide. Due to high genomic instability, HCC is very heterogenous in its clinical presentation and genetic makeup. The poor survival of HCC patients is partly because of inefficient treatment regimens and the lack of accurate prognostic subgroup stratifications. The discovery of genomic changes in other cancer types has led to the development of novel specific therapies, underscoring the importance of understanding molecular mechanism and tumor biology to provide the best therapy for each cancer patient.

Methods. To this end, we performed high-resolution array-based comparative genomic hybridization of 76 HCC clinical specimens to search for genetically disrupted genes associated with outcome.

Results. In agreement with previous publications, recurrent gains and losses were associated with 1q, 6p, 8q and 4q, 8p, 13q, 16, 17p, respectively. However, only deletions of a few distinct loci on 1p, 4q, 8p and 9p were associated with survival (FDR<0.05). Of the 419 genes which mapped to regions of loss, analysis revealed that gene expression and DNA copy number of 134 genes (31.98%) were significantly correlated (Pearson coefficient r>0.3, p<0.001). Next, we built a 10-gene-survival signature based on the expression of these 134 genes and validated the signature in two independent cohorts. Kaplan-Meier survival analysis revealed that the 10-gene signature was able to predict survival outcome in tumor tissue (log-rank p=0.008) but not in paired non-tumor samples. This gene signature contains six potential tumour suppressor genes (TSGs) on chromosome 8p, the bona fide TSG DLC1 and three novel TSGs. Clonogenicity, cell migration assays and xenograft mouse models confirmed the tumour suppressor function of two of these TSGs, SH2D4A and SORBS3. Functional analysis revealed that SH2D4A and SORBS3 function in a convergent manner to inhibit the IL-6 pathway. Thereby, SORBS3 inhibits the IL-6 pathway via activation of the estrogen receptor alpha (ERalpha and SH2D4A via inhibition of STAT3 signalling).

Conclusions. In this study, we found a 10-gene signature that allows for the stratification of patients with poor prognosis in HCC and breast cancer. Thus, this unbiased approach was effective in identifying a prognostic gene signature and two novel TSGs which functionally cooperate. Future studies may lead to the discovery of HCC subtype-specific drugs that might be used for target specific therapy.

SA-043

The inhibitor of tyrosine kinase Gefitinib reduces tumorigenesis in chemical but not in hormonal induced hepatocarcinogenesis models of the rat

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Aims. Activation of the signaling pathways of the epidermal growth factor receptor (EGFR) by Transforming growth factor alpha (TGFα) promotes the development of hepatocellular carcinoma (HCC). Intervention by use of the selective EGFR-tyrosine-kinase inhibitor Gefitinib prevents hepatocarcinogenesis in rat cirrhotic livers. This potential anti-tumorigen effect could possibly reduce progression of chemical as well as hormonal induced pre-neoplastic liver lesions to HCC.

Methods. In short- (three weeks to six months) and long-term experiments (12 to 24 months) with 776 Lewis rats we performed administration of N-Nitrosomorpholine (NNM; 5 mg/kg body weight daily; n=180, including controls) or an intrahepatic transplantation of isolated pancreatic islets in diabetic (PTx; n=204), thyreoid follicles in thyreoidectomized (TTx; n=176) and ovarian fragments in ovariectomized rats (OTx; n=216) for the induction of hepatocellular pre-neoplastic lesions. Gefitinib was administered daily for two weeks (dosis 20 mg/kg, oral) or three and nine months (10 mg/kg).

Results. After intake of NNM for three months and Gefitinib at the same time pre-neoplastic liver foci reveal a reduced volume portion of liver parenchyma as compared to administration of NNM alone (Gefitinib/control: 1.58±0.28 Vol%/7.25±0.90 Vol%, p=0.001). A decrease of volume portion of hepatocellular adenoma (HCA) could be substantiated after six months and gefitinib therapy of three months (5.43±0.57 Vol%/10.36±0.89 Vol%, p=0.0001). The number of HCC was reduced (each rat: 4.87±0.77/7.93±0.92, p=0.02). Inhibition of proliferation activity of pre-neoplastic liver foci could be shown upon all transplantation models in short-term experiments after administration of high-dosed Gefitinib (PTx six months: 2.27±0.13%/6.06±0.49%, p=0.015; OTx three months: 12.67±2.67%/29.79±2.68%, p=0.02; STx three months: 7.69±1.07%/12.25±1.33%, p=0.02). In long-term investigations HCA, HCC and transplant tumors arose, whose occurrence and quantity could not be inhibited by a Gefitinib dose of 10 mg/kg.

Conclusions. Selective EGFR inhibition by Gefitinib diminishes chemical induced hepatocarcinogenesis on the level of pre-neoplastic liver lesions, HCA and HCC. By contrast, occurrence of hepatocellular tumors whose development is ascribed to a local hormonal surplus (insulin and estrogenes), could not be delayed by EGFR-receptor-inhibiting, indicating only partial relevance of this signaling cascade upon hormonal induced hepatocarcinogenesis.

Abstracts

AG Gynäko- und Mammapathologie

SO-001
Frequency of mutations in vulvovaginal malignant melanoma

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Aims. Malignant melanoma of the skin commonly have amplifications or activating mutations of KIT, resulting in receptor dimerization, autophosphorylation, and activation of several signaling pathways. Genes of the RAF family, which mediate cellular responses to growth signals, encode kinases that are regulated by RAS and participate in the RAS/RAF/MEK/ERK/MAP-kinase pathway. About 50% of melanomas harbors activating mutations in BRAF leading into a targeted therapy in metastastic disease. Chromosome 3 is known to be a predictive factor in uveal melanoma with worse prognosis. Little is known about the role of mutations in rare vulvovaginal malignant melanoma.
Methods. In cooperation with the institutes of pathology in Magdeburg and Augsburg 34 cases of vulvovaginal melanoma were analysed with subsequent macro- or microdissection followed by DNA- extraction of the adequate tissue. To reveal the activating mutation of BRAF p.V600X and KRAS exon 2 codon 12, 13 and 61 we performed pyrosequencing with a sensitivity of at least 5% mutated alleles. Via Sanger sequencing we analysed mutation in KIT exons 9 and 11. Furthermore we designed an assay for SNP-based detection of conversion of chromosome 3.
Results. Currently 3 of 17 cases showed mutations in KIT exon 9 and 11 within vulvovaginal melanoma. In 6 of 28 cases we detected BRAF-p.V600X mutation. Additionally 2 of 10 cases presented a monosomia in chromosome 3. 22 of 34 cases revealed a wildtype for KRAS.
Conclusions. We could demonstrate activating mutations in BRAF and KIT as well as a monosomia in chromosome 3 in vulvovaginal malignant melanoma. At that time activating mutations in KRAS could not be revealed. So, we presume another pattern of distribution of mutations in malignant melanomas of the vulvovaginal region.

SO-002
p53 suppresses type II endometrial carcinomas in mice and governs endometrial tumour aggressiveness in humans

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Aims. Type II endometrial carcinomas are a highly aggressive group of tumour subtypes that are frequently associated with inactivation of the TP53 tumour suppressor gene.
Methods. We generated a conditional p53 knockout mouse model of endometrial type II carcinomas by crossing Trp53fl/fl mice, in which loxP sites are present in intron 1 and intron 10 of the Trp53 gene, the mouse homologue of human TP53, to mice expressing the Ksp1.3-Cre transgene.

Results. We show that mice with endometrium-specific deletion of Trp53 initially exhibited histological changes that are identical to known precursor lesions of type II endometrial carcinomas in humans and later developed carcinomas representing all type II subtypes. The mTORC1 signalling pathway was frequently activated in these precursor lesions and tumours, suggesting a genetic cooperation between this pathway and Trp53 deficiency in tumour initiation. Consistent with this idea, analyses of 521 human endometrial carcinomas identified frequent mTORC1 pathway activation in type I as well as type II endometrial carcinoma subtypes. mTORC1 pathway activation and p53 expression or mutation status each independently predicted poor patient survival.
Conclusions. We suggest that molecular alterations in p53 and the mTORC1 pathway play different roles in the initiation of the different endometrial cancer subtypes, but that combined p53 inactivation and mTORC1 pathway activation are unifying pathogenic features among histologically diverse subtypes of late stage aggressive endometrial tumours.

SO-003
Profiling of stem cell factors reveals different stem cell-like compartments in serous ovarian carcinomas

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Aims. Tumor stem cells and reexpression of stem cell factors are thought to contribute to therapy resistance and recurrent disease in ovarian carcinomas. Therefore, we performed a comparative analysis of stem cell markers in serous ovarian carcinomas, including double immunostains, to identify differential expression and co-expression of stem cell markers in different subpopulations.
Methods. 155 ovarian serous carcinomas (147 high-grade, 8 low-grade) were analyzed by immunohistochemistry in a tissue microarray for stem cell markers SOX2, Nanog, Oct4 and ALDH, as well as c-myc, CD117, and proliferation marker Ki67. A double immunostaining experiment compared the percentage of Ki67-positive cells in the ALDH1-positive and -negative cell fractions case by case. The effect on overall and disease-free survival was analyzed (Kaplan-Meier). Furthermore we used double staining experiments to assess the co-expression of SOX2 and ALDH in subpopulations of individual tumors.
Results. Regarding SOX2 we confirmed the association with poorly differentiated tumors and improved recurrence-free survival (median survival 27 months vs. 21 months, p=0.041). Oct4 or Nanog were not detected, whereas a significant number of tumors displayed positivity for c-myc or CD117. We observed a correlation between ALDH1 and Ki67, indicating a role of ALDH1 in cell proliferation. However, double staining showed, that ALDH1 is mostly expressed in the in the non-proliferating cell fraction. Co-staining experiments with ALDH and SOX2 revealed the existence of four different populations with expression of none of the two markers, either one or coexpression of both markers, sometimes in close proximity.
Conclusions. Stem cell factors show divergent association with tumor characteristics like poor differentiation or survival (SOX2) and resting populations in proliferating tumors (ALDH1). Furthermore, we find both individual and co-expression of both markers within the same tumors, suggesting the existence of stem cell-like cell fractions with transient marker profiles within individual tumors. From these findings we conclude, that stem cell factors in serous ovarian tumors do not reflect a unique stem cell phenotype, but a broad spectrum of stem cell-enriched compartments.

SO-004

Co-expression of aldehyde dehydrogenase 1 (ALDH1)/epidermal growth factor receptor (EGFR) identifies a poor-prognosis subgroup of high grade serous ovarian carcinoma

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Aims. The epidermal growth factor receptor (EGFR) pathway is up-regulated in the aldehyde dehydrogenase 1 (ALDH1) positive cancer stem cell (CSC) fraction in models of triple negative breast cancer (TNBC). As high grade serous ovarian carcinoma (HGSC) reveals strong molecular similarities to TNBC, we investigated the potential link between ALDH1 and EGFR in this entity.
Methods. Expression of ALDH1 was investigated in 131 primary HGSC using quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) and immunohistochemistry. Expression data were correlated with EGFR expression, which had been determined in a previous study, as well as with clinico-pathological parameters and survival.
Results. ALDH1 protein expression was found in 42 carcinomas (32.1%). Positivity was tightly linked to EGFR expression (p<0.0001), and was a significant negative prognostic factor for overall survival both in univariate (p=0.010) and in multivariate analysis (p=0.041). Tumors that were positive for both ALDH1 and EGFR had an exceptionally bad prognosis as compared to carcinomas with one or both markers negative in univariate analysis (p<0.0001) and in the multivariate setting (p=0.004). ALDH1 mRNA expression was not significantly linked to ALDH1 or EGFR protein expression, and no significant prognostic factor.
Conclusions. Similarly to the situation in TNBC, there seems to be a link between ALDH1 and EGFR expression in HGSC. Co-expression of both markers identifies a subgroup of highly aggressive, poor-prognosis carcinomas for which alternative treatment options should be evaluated.

SO-005

Making type-specific ovarian carcinoma research more effective: calculator for ovarian subtype prediction (COSP) is a reliable high-throughput tool for case review

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Aims. There is increasing evidence that ovarian carcinoma is composed of five histologically distinct disease entities reliable identification of histotypes is therefore essential for the success of studies testing novel therapeutics as well as for biomarker discovery research. The aim of this study was to examine the utility of a nine-marker immunohistochemical (IHC) panel designated the calculator for ovarian subtype prediction (COSP) to reliably reproduce the consensus diagnosis of two expert gynaecological pathologists. COSP is an effort to streamline pathology review in a clinical trial setting through the use of a validated, objective diagnostic aid.
Methods. A cohort of 423 cases from the AGO-OVAR11 trial was evaluated using the COSP IHC panel, and compared to both original diagnoses from >100 local contributing pathologists and independent expert gynecopathology review.
Results. Overall concordance between COSP and expert review was 89%, and in cases where a local pathologists' diagnosis was confirmed by COSP, the expert gynecopathologist also agreed in 97.5% of cases.

Conclusions. Given the high degree of agreement between COSP-verified local pathology diagnoses and expert review, COSP has great potential to serve as a high-throughput tool in ovarian carcinoma case review, saving expert time and valuable resources by preselecting the small number of difficult cases that truly require expert review.

SO-006

Comparison of the newly proposed two-tier grading system with the already existing three-tier system in ovarian cancer: testing its reproducibility, prognostic relevance, predictive value and importance for tumorigenesis

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Aims. Until now no universally accepted grading guidelines for ovarian carcinoma exist and the reproducibility among different institutions is low, even though tumor grading is still one of the most important prognostic markers. In particular, the large group of G2 tumors, in between low- and high-grade carcinomas has to be classified better. The aim of this study was to evaluate the two-tier system suggested by the MDACC and analyse its value towards prognostic and predictive relevance, reproducibility as well as respecting the theory of dual tumorigenesis.
Methods. Paraffin embedded tissue of 241 patients (25 grade 1, 107 grade 2, 109 grade 3) was available. Grading, according to the MDACC system, based on nuclear pleomorphism and mitotic activity, was done twice. All new low-grade carcinomas underwent both p53 immunohistochemistry (IHC) as well as KRAS and BRAF mutation analysis. Results of the old and new system were compared with each other, survival analyses were performed.
Results. 48 (20%) cases were reclassified as low-grade, 193 (80%) as high-grade carcinoma. In five (2%) of 241 cases different grading-score was assessed. Formerly G2-carcinomas were divided in 28 (26%) low-grade and 79 (74%) high-grade carcinomas. Six (13%) of 48 low-grade carcinomas were p53 positive in IHC; mutation analysis showed a KRAS mutation in 8/48 (17%) cases and a BRAF mutation in just one 1/48 (2%) case. Correlation between tumor grade and survival was p=0.001 for the two-tier system and p=0.029 for the three-tier system.
Conclusions. The two-tier grading system is user-friendly, easy to learn and of high reproducibility. Survival was much better in the group of low-grade carcinoma; significance of the two-tier system was much better than the three-tier system. 19% of low-grade carcinomas had a KRAS or BRAF mutation, and 13% a p53 overexpression. One knows that ovarian cacinoma tumorigenesis either develops along a stepwise mutation process from borderline tumors to low-grade carcinomas (type I) or involves genetically unstable high-grade "de novo" (serous) carcinomas (type II). Though, our results suggest that there is also a de novo way of developing a low-grade carcinoma. In addition, testing for KRAS and BRAF mutations might be helpful for therapeutic strategy.

SO-007

Heterogeneity of protein and phosphoprotein levels in cancer tissues

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Aims. Accurate detection and quantification of proteins and phosphoproteins in cancer tissues is fundamental to the development of personalized medicine and improvement in the quality of human health care. However, variability of protein and phosphoprotein levels within a single tumor has not been well studied. The aim of this study was to characterize potential intratumoral heterogeneity of protein and phosphoprotein levels in human breast cancer tissues.

Methods. Fifteen large (larger than 3 cm) primary invasive breast cancers were selected. From each case 8–10 samples from different zones of the primary tumor (peripheral, intermediate, central) were collected. After protein extraction from a total of 106 formalin-fixed and paraffin-embedded (FFPE) tissue samples, the levels of 35 proteins, including 15 phosphorylated proteins representing active HER2, EGFR, and uPA/PAI-1 signaling pathways, were quantitatively analyzed by reverse phase protein arrays.

Results. All 35 proteins showed considerable intratumoral heterogeneity in their levels within primary tumors with a mean coefficient of variation (CV) of 30% (range 19–38%). The extent of intratumoral heterogeneity differed among the 35 individual proteins analyzed (p<0.001). Interestingly, there were no significant differences between phosphorylated (mean CV 32%) and non-phosphorylated (mean CV 31%) proteins. There was also no significant difference in the extent of intratumoral heterogeneity within a defined tumor zone (mean CV 28%, range18-38%) or between tumor zones (mean CV 22%, range 12–34%). In comparison, we assessed the variation of protein expression amongst tumors from different patients, which revealed a CV of 51.4% (range 29.3–98.3%).

Conclusions. Our data indicate that assessment of established and novel proteins in breast cancer tissues as diagnostic or prognostic markers may require sampling of the tumor in several distinct locations to avoid sampling bias. Further studies are needed to provide evidence that our findings apply for other cancer types as well. Currently we are analyzing a similar set of 120 FFPE samples from 16 primary ovarian cancers.

SO-008

A temperature-sensitive p53 mutant in IPH-926 lobular breast cancer cells allows for profiling of p53-responsive genes and provides insight into p53-related clonal evolution in lobular breast cancer

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Aims. Profiling of p53-responsive genes has been carried out in different cellular models, most of which involved genetic modifications or cytotoxic stimulation. We report on the utilization of IPH-926 human lobular breast cancer cells for the profiling of p53-responsive genes using a novel approach without such modifications.

Methods. We discovered that IPH-926 cells harbor a homozygous TP53 missense mutation encoding for a rare p53 mutant (E285K) with temperature sensitive (ts) loss of function characteristics. This mutation had evolved as a late, secondary genetic event during the natural clonal evolution of the corresponding lobular carcinoma. In vitro temperature shifts reconstituted endogenous wild type p53 activity in IPH-926, as evidenced by induction of p21Waf1. Transcriptional alterations associated with restored p53 function were profiled using Affymetrix microarrays and a new strategy to gate out non-specific temperature effects.

Results. At the p=0.0005 significance level, 60 genes were differentially expressed following reconstitution of p53 activity. These genes included CDKN1A, MDM2 and PHLDA3, a recently described p53-inducible inhibitor of AKT. Similar transcriptional alterations were observed upon reconstitution of p53 activity in BT-474 cells, which also harbor ts p53 E285K, and in ASPC1 cells transduced with ts-p53 A138V. Consistent with these models, low PHLDA3 expression was associated with nuclear p53 accumulation, indicative of deleterious TP53 mutations, in primary breast cancers.

Conclusions. From a molecular point of view, IPH-926 thus provides a new tool to study transcriptional programs controlled by p53. From a tumor pathology perspective, IPH-926 also provides the first direct evidence of a p53-related clonal evolutionary pathway in lobular breast cancer progression.

SO-009

An epigenomic deep sequencing approach to identify predictive markers for PARP inhibitor response in breast cancer cells

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Aims. Inhibition of poly(ADP-ribose)-polymerase-1 (PARP1) leads to synthetic lethality in BRCA-associated breast cancer cells, and is a promising novel treatment strategy for hereditary BRCA-mutated tumors. Since BRCA1/2 germline mutations are relatively infrequent, our study aims at a systematic identification of epigenetic aberrations that may predict PARP inhibitor (PARib) response in sporadic breast cancer cells.

Methods. Genome-wide DNA methylation was assessed in nine malignant and one non-malignant (MCF10A) breast cell line (BCL) by MBD-isolated genome sequencing (MiGS). Sensitivity to the PARib AG14361 was determined by viability and clonogenicity assays before/after global DNA demethylation in vitro, and was confirmed by the PARib olaparib. Correlation analyses of DNA methylation with PARib sensitivity identified potentially predictive candidate genes and microRNAs. By incubation with increasing concentrations of AG14361 we generated an isogenic PARib resistant BCL originally sensitive to AG14361.

Results. BCL clustered in three sensitivity groups (sensitive, intermediate, resistant) independent of hormone receptor status, but associated with PARP1 level (p=0.042). BRCA1 mutated (MDA-MB-436) and BRCA1 methylated (UACC-3199) BCL were most sensitive to PARibs. After global DNA demethylation sensitive BCL acquired resistance while resistant BCL were modestly sensitized. MCF10A cells did not shift PARib resistance after DNA demethylation. Correlation of MiGS profiles with PARib sensitivity identified 382 genes and seven microRNAs with exclusive promoter/exon1 methylation in sensitive BCL, while 313 genes and one microRNA were affected in resistant BCL. Artificially mediating resistance to BRCA1 methylated cells did neither hypomethylate the BRCA1 promoter nor induce BRCA1 expression, but significantly reduce proliferation. Gene ontology analyses revealed that methylation of WNT, ErbB, SHH, and mTOR signaling genes was significantly enriched in PARib resistant cells, while PARib sensitive cells exhibited methylation in DNA repair genes.

Conclusions. Aberrant DNA methylation seems to be involved in PARib sensitivity/resistance in BCL. By epigenomics we identified several potential candidate genes beyond BRCA1 that might modulate PARib sensitivity or resistance, implying that more patients than currently anticipated may benefit from this drug class. Their expression, DNA methylation and net effect on cancer pathways are currently validated in BCL and explored in primary breast cancer tissues.

SO-010

IR/IGF-1R activation promotes cell survival and growth in PIK3CA-mutated, Tamoxifen resistant breast cancer

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Aims. Therapeutic mTOR inhibition is a treatment option in hormone-receptor positive breast carcinomas which frequently harbour PIK3CA-mutations. In this study, we aimed to analyse the effects of mTOR inhibition and possible interactions with insulin receptor (IR)/insulin like growth factor 1 receptor (IGF-1R) signalling in wildtype and PIK3CA-mutated cell lines as well as in Tamoxifen-resistant breast cancer cells.

Methods. The PIK3CA-mutated cell lines MCF7 (Exon 9 1633G>A), T47D (Exon 20 3140A>G) and PIK3CA wildtype (wt) ZR75.1 were treated with the allosteric mTOR complex 1 (mTORC1) inhibitor Everolimus and the active-site mTORC1/mTORC2 kinase inhibitor PP242. In this setting, the effects of IR/IGF-1R signalling on cell viability and growth were investigated by stimulation and inhibition of the receptors through Linsitinib (OSI 906).

Results. Tamoxifen resistant cells showed both elevated level of IR/IGFR expression and an activated (phosphorylated) ERK1/2 in contrast to untreated controls. Inhibition of mTORC1/2 through PP242 reduced AKT-phosphorylation and led to a complete decrease of cell viability and growth in all cell lines. However, the effect of mTORC1-inhibition alone using Everolimus could be reversed by addition of insulin. Furthermore, mTOR inhibition caused an activation/phosphorylation of IGF-1R as well as AKT in MCF7 and T47D but not in ZR75.1. The addition of OSI 906 reduced this effect and resulted in an additional decrease of cell viability.

Conclusions. Inhibition of mTOR-signalling reduced cell viability and proliferation in wt PIK3CA and mutated breast cancer cells independent of an acquired Tamoxifen resistance. However, our data indicate that activation of IGF-1R-conferred cell growth reduces the effects of therapeutic mTOR inhibition in PIK3CA-mutated breast cancer cells and that additional targeting of the IR/IGF-1R pathway could be useful in this setting.

SO-014

Expression of Mucin-1 in breast cancer as predictive and prognostic marker after neoadjuvant chemotherapy

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Aims. Mucin-1 (MUC1) is frequently overexpressed in breast cancer and its role in triggering an immunologic response is experimentally established. In current clinical trails, cancer vaccines targeting MUC1 expressing cells are being tested. The aim of this study was to evaluate the frequency of MUC1 expression among different breast cancer subtypes and to evaluate its impact on therapy response and survival after neoadjuvant anthracycline/taxane-based chemotherapy (CTX).

Methods. Pretherapeutic core biopsies from patients of the GeparTrio clinical trial (NCT 00544765) were evaluated for MUC1 by immunohistochemistry (IHC; n=691) and quantitative RT-PCR (qRT-PCR; n=286) from formalin-fixed paraffin-embedded (FFPE) tissue.

Results. MUC1 was detectable in the majority (95%) of cases; three orders of magnitude were covered by qRT-PCR. IHC and mRNA data were correlated (p<0.001). Higher levels were found in HR+ tumors and the lowest levels in HR-/HER2- tumors (p<0.001). High MUC1 protein and mRNA expression were associated with lower probability of pathologic complete response (pCR) in the overall population (p<0.001) and the subgroups of HR+ (p=0.004 and <0.001), HER2- (p<0.001) and HR+/HER2- tumors (p<0.001). MUC1 was independently predictive in multivariable logistic regression [high MUC1 protein: odds ratio (OR) 0.464, 95% CI 0.300–0.719, p=0.001; MUC1 mRNA (per 20-dCT unit): OR 0.673, CI 0.546–0.829, p<0.001]. MUC1 protein and mRNA expression were associated with longer survival in the overall population (p=0.03 and <0.001) and in HER2- tumors (p=0.005 and <0.001). MUC1 mRNA was also prognostic in HR+ (p<0.001) and HR+/HER2- tumors (p=0.001). Protein and mRNA expression were independently prognostic in multivariable analysis [hazard ratio (HR) 0.388, CI 0.166–0.907,

p=0.029; MUC1 mRNA per 20-dCT unit: HR 0.763, CI 0.595–0.909, p=0.005].

Conclusions. Expression of MUC1 is frequent in breast cancer and detectable by methods based on FFPE tissue. MUC1 provides independent information on therapy response and survival after neoadjuvant CTX. MUC1 expression may serve as a predictive marker in immunotherapeutic clinical trials.

SO-015

SPARC expression and its predictive value in human breast cancer samples of the neoadjuvant GeparTrio trial

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Aims. The aim of this study was to analyse the frequency of SPARC (secreted protein and rich in cystein) expression in different breast cancer subtypes and to evaluate its predictive value for response to chemotherapy in a large cohort of pre-treatment core biopsy samples from the neoadjuvant GeparTrio trial. As SPARC is an albumin-binding protein and might mediate intratumoral accumulation of nab-Paclitaxel, SPARC expression is currently also analysed in the GeparSepto trial.

Methods. In total 667 pre-treatment core biopsy samples from the neoadjuvant GeparTrio trial were evaluated for stromal and intratumoral SPARC expression by immunohistochemistry. The predictive value of SPARC expression for therapy response was evaluated.

Results. We were able to evaluate stromal SPARC expression in 659 and intratumoral SPARC expression in 667 biopsies. Stromal SPARC expression was higher in patients with ductal compared to lobular breast cancer (p=0.004) and intratumoral SPARC expression was increased in patients with triple-negative breast cancer (TNBC) compared to hormone receptor or HER2 positive subtypes (p=0.039). In the overall study group a positive intratumoral SPARC expression was associated with an increased pCR rate (p<0.001). SPARC positive tumors had a pCR rate of 27%, compared to 15% in SPARC negative tumors. In patients with TNBC the pCR rate was 26% in SPARC negative tumors and 47% in SPARC positive tumors (p=0.032). Furthermore, SPARC was independently predictive for therapy response in the overall study population (p=0.010) as well as the subgroups of patients with TNBC (p=0.036) in multivariate logistic regression analysis adjusted for standard clinicopathological factors.

Conclusions. SPARC is expressed in all histological and biological breast cancer subtypes. Patients with ductal cancer revealed a higher stromal SPARC expression and patients with TNBC revealed a higher intratumoral SPARC expression. Furthermore intratumoral SPARC expression may provide predictive information for response to neoadjuvant chemotherapy.

SO-016

Prognostic value of image analysis of ER, PR and Ki-67 and the IHC4 score in a large cohort of patients with HER-2 negative, luminal type breast cancer

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Aims. In HER2-negative, luminal-type breast cancers, the effectiveness of adjuvant therapy is variable. Improvements are needed to identify which women are at sufficiently low risk in order to safely avoid the use of chemotherapy. In this context, it is unclear if the prognostic value of immunohistology for estrogen and progesterone hormone receptors as well as the Ki-67 proliferation antigen can be improved by image analysis or by immunohistologic scores, such as the newly described IHC4 score.

Methods. Formalin fixed and paraffin embedded tumour tissue was collected from the population-based MARIE study of breast cancer patients. Using a set of tissue microarrays (TMAs) containing duplicates of 1 mm cores, tumours were classified as hormone receptor positive when at least 1% of tumour cells were positive for either estrogen or progesterone receptor. Based on ASCO recommendations, HER2 positivity was assumed for tumours with IHC score of 3+ or amplification of HER2 chromogenic in situ-hybridization with a ration of >2.2 in a dual color assay. 826 tumours with positive hormone receptor status and negative HER2 status were selected for quantitative IHC analysis of nuclear markers using virtual microscopy (technology by Aperio) after the manual selection of tumour areas. A total of 564 cases were evaluable for image analysis and were entered into multivariate analysis.

Results. When comparing quantitative staining results with the visual determination of the ER, PR, and Ki-67 antigens, the image analysis provided better separation of prognostic groups with all 3 antigens analyzed. An additional weighing of nuclear percentage counts by the intensity of staining provided no further improvement. By multivariate analysis using the method of classification and regression trees (RPART), the Ki-67 proliferation antigen was determined as the strongest predictor of survival in the node-negative group, and an optimal Ki-67 cut-off value of 21.4% was calculated (p<0.0001, log-rank test). With the node-positive tumours, the IHC4 score was the strongest predictor of overall survival (p=0.004, log-rank test).

Conclusions. In this cohort of luminal type breast cancers, the quantitative determination of ER, PR, and Ki-67 was superior to the visual estimation of these antigens. In the lymph-node positive group of patients with luminal type breast cancers, the newly described IHC4 score provided the best estimation of survival probability.

SO-017

Influence of mammography screening—analysis of pathological parameters including the associated DCIS components

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Aims. Previous studies demonstrated that screening detected breast carcinomas have a significantly better prognosis, which can be largely be attributed to a shift in tumor size and stage. However, these parameters alone do not explain the full benefit of mammography screening. Therefore, the aim of the study is to compare the pathological and prognostic parameters of screening detected and non-screening detected carcinomas of the same age group.

Methods. In the screening region Neckar-Alb 115,000 mammographies were performed between 01/2008 and 06/2012, thereby 870 carcinomas were detected (80% invasive and 20% non-invasive). The control group contains 569 women aged between 50 and 69 years, diagnosed and treated at the University Hospital Tübingen between 04/2008 and 06/2011 (88% invasive and 12% non-invasive).

Results. In our collective screening detected carcinomas are smaller (pT1 79%, pT2 18% and pT3/4 3%) than non-screening detected (pT1 58%, pT2 31% and pT3/4 10%), are less frequently complicated by nodal metastases (23% vs. 35%) and better differentiated (G1/G2/G3: screening detected: 29%, 57%, 14%; non-screening detected: 15%, 58%, 27%). Non-Screening-detected carcinomas are more frequently Non-Luminal A and triple-negative (screening detected: 87% LumA, 6% LumB, 1% HER2 and 6% triple neg.; non-screening detected: 77% LumA, 8% LumB, 5%, HER2 and 10% triple-neg.) as well as more proliferative (screening detected average 9% Ki67 pos. cells, non-screening detected average 18% Ki67 pos. cells). Women in the control group showed more frequently associated high-grade DCIS and the DCIS component in this group is significantly larger. As expected, there is no significant difference between DCIS grade and size in women with pure DCIS, as both screening and control group consist of mostly asymptomatic patients with DCIS detected in imaging studies.

Conclusions. In summary, screening detected carcinomas are characterized by significant smaller size, less nodal metastasis, lower tumor grade, lower proliferation and more frequent luminal A breast cancer subtype. For the first time our study reports a detailed analysis of associated components of DCIS, which are significantly lower grade and smaller in screening detected carcinomas, thus contributing to a significantly lower frequency of mastectomies. Further prospective studies will have to analyze the impact of these parameters on long term patient prognosis.

Sino-German Cooperation Meeting

SO-018

Development of a nationwide telepathology consultation and quality control program based WSI technology—to improve pathological diagnosis in China

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Aims. Telepathology may play an important role in remote consultation and quality control for pathological diagnosis in China. In order to efficiently use the resource and to improve the quality of pathology diagnosis, the Pathology Quality Control Center of China facilitated and developed a nationwide telepathology consultation and quality control program based on WSI technology in Dec. 2011

Methods. An internet based telepathology platform (www.mpathology.cn) was built as the hub for the program, which connects local hospitals and expert consultants. From 300 expert pathologists with provincial and national reputation, 80 were selected to serve as expert consultants for the program after passing an online test of 30 consultation cases with slide images (WSI). 87 hospitals in 17 provinces were volunteered to participate in phase I of the program, 60 of them were selected after pathologists in those hospitals took an online diagnostic tests of 50 cancer cases.

Results. The nationwide telepathology project consists of 2 parts: second opinion and quality control. Each participating hospital is required to send WSI of cases to the platform for a second opinion. More than 4000 cases have been diagnosed through this platform from Jan. to Oct. 2012. The number of cases for consultation is increase rapidly in last three

months since the pathologists from the local hospitals realize useful-ness of telepathology based WSI technique. The quality of tissue section is to be reviewed, assessed by the experts, and submitted to provincial and national pathology quality control centers. Meanwhile the serial online slide seminars are also open to all pathologists who are interested. The results of phase I of the program are to be used to guide future development of the program across the country.

Conclusions. A nationwide pathology consultation and quality control program can be built through the use of telepathology based WSI technology. Telepathology based WSI technology can be used for second opinion and quality control to improve the quality of pathological diagnosis in China.

SO-019

A web-based virtual microscopy platform for multi-institutional histopathological studies and biobanking working groups

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Aims. Clinical and pharmacological studies demand increasingly on histopathology expertise. Biobanking activities promote huger sample collections from several sites. Thus, digital whole slide microscopy is getting importance. High-resolution images of biological or medical samples scanned with microscopic magnification can be distributed easily to research partners at different locations. However, proprietary file formats and different installable viewer applications are a major drawback while integrating different institutions with various scanning systems. To enable multi-institutional studies, a web-based platform for virtual microscopy has been developed, which is vendor independent and does not require local software installation.

Methods. The platform consists of different modules built on standard web technologies and runs in the most popular web-browsers. A generic framework allows the organization of digital slides and the compilation to cases and projects. A user management enables restriction and grant of project related access. Core of the platform is a virtual microscopy module which enables an efficient and interactive visualization of digital slides. To optimize the transfer rates, only visible areas and details are transferred to the client. This tile generation is either done prior in a conversion step or on-the-fly during client request. Different vendor specific file formats are supported. Clinical partners can provide opinions to slides by an interactive region annotation and textual commenting. A slide or case classification can be submitted using a predefined or open nomenclature. The results can be collected and exported for further analysis by the project administrator.

Results. The developed platform has been evaluated in experimental projects. Thus, the efficiency could be tested prior to routine usage. The transfer rates and the client load are independent of the slide format and of pre- or on-the-fly tile generation. The computation is conducted at the server which has to handle the load. The usability has been approved by multiple test users.

Conclusions. The flexible virtual microscopy platform allows sample assessment procedures and conduction of multicenter agreement studies with different slide providers. The vendor independent implementation allows the integration of different slide scanning systems and efficient transfer and visualization without specific adoptions. A major multi center agreement study, utilizing the platform, is planned for 2013.

SO-020

Mechanisms of cancer stem cells (CSCs) invasion and metastasis

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Aims. Cancer stem cells are a small fraction of cancer cells with potentials of self-renewal, differentiation multipotency, initiation and reconstruction of cancer tissues. They play an important role in tumor invasion and metastasis. However, the mechanisms of CSCs self-renewal and invasion remain to be clarified. Here, we explored the mechanisms mediated CSC self-renewal and invasion in glioblastoma and lung cancer respectively.

Methods. Scanning electron microscope (SEM) and transmission electron microscope (TEM) were used to observe gap junction of GSCs. Malfunctioned gap junctional intercellular communication was evaluated by fluorescence recovery after photobleaching analysis. Western blotting and real-time RT-PCR was preformed to identify protein or gene expression. Coimmunoprecipitation was used to analyze the interaction between $Cx43$ and E-cadherin.

Results. In glioblastoma, all CSCs showed reduced GJIC, and differentiated glioma cells had more gap junction-like structures than CSCs. CSCs expressed very low level of connexins, $Cx43$ in particular, which are key components of gap junction. Reconstitution of $Cx43$ in CSCs inhibited their capacity of self-renewal, invasiveness, and tumorigenicity via influencing E-cadherin and its coding protein, which leads to changes in the expression of Wnt/b-catenin targeting genes. In lung cancer, IGF-1R activation enhances POU5F1 expression on LACSLCs through PI3K/Akt/GSK3b/b-catenin cascade, while the inhibition/knockdown of IGF-1R reduces their capabilities for self-renewal and tumorigenicity.

Conclusions. Our results suggest that glioma stem cells require the low expression of $Cx43$ for maintaining their malignant phenotype, and upregulation of $Cx43$ might be a potential strategy for treatment of malignant glioma. IGF-1R maintains lung cancer self-renewal through PI3K/Akt/GSK3b/b-catenin cascade.

SO-021

Stem cell markers in neuroendocrine neoplasms of the pancreas

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Aims. Neuroendocrine neoplasms of the pancreas reveal a great biological spectrum, reaching from slowly growing to highly aggressive tumors. In the present study, the expression of previously reported tumor stem cell markers was examined in pancreatic neuroendocrine neoplasms to identify possible correlations with pathological parameters and with the patients' clinical outcome.

Methods. The expression of ALDH1, CD24, CD44, CD133, CD166, and EpCAM was immunohistochemically analysed in a series of 173 well-characterized neuroendocrine neoplasms of the pancreas.

Results. Mostly highly significant correlations were found between the Ki67 proliferation index, the resulting tumor grade, and the diagnosis (neuroendocrine tumor G1, G2, neuroendocrine carcinoma G3) with expression of ALDH1, CD133, CD166, CD24, and EpCAM. The expression of CD133 and CD166 significantly varied in tumors of various sizes and T-stages. Based on 128 patients with available follow data, univariate analysis showed a good predictive value of ALDH1, CD24, and CD44. Multivariate analysis revealed that especially CD44 represents a powerful prognostic marker.

Conclusions. Neuroendocrine neoplasms of the pancreas of diverse biological grades show significant differences in the expression of previously elsewhere described tumor stem cell markers. This indicates a role of these markers in the transformation and progression of these tumors.

Furthermore, especially CD44 was found to represent a good prognostic marker for these tumors.

SO-022

IGFBP7 plays a potential tumor suppressor role against colorectal carcinogenesis with its expression associated with DNA hypomethylation of exon 1

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Aims. Insulin-like growth factor binding-protein-7 (IGFBP7) was a differentiated expressed protein which overexpress in colorectal cancer compared with matched normal mucosa by RT-PCR and immunohistochemistry.

Methods. This phenomena was obtained from colonic adenocarcinoma (CRC) and normal mucosa suppression subtraction hybridization (SSH) cDNA libraries. However, in vitro experiments performed in 10 CRC cell lines showed that IGFBP7 expressed only in SW480 and Caco2 cell lines, which implied an underlying reversible regulatory mechanism. Using methylation-specific PCR (MSP) and bisulfite sodium PCR (BSP), it was found that its expression was associated with DNA hypomethylation of exon1.

Results. This was further supported by the in vitro study which showed restored IGFBP7 expression after demethylation agent 5-aza-2'-deoxycytidine treatment. Investigation of the functional role of IGFBP7 through transfection studies showed that IGFBP7 protein could inhibit growth rate, decrease colony formation activity, induce apoptosis and senescence in colon cancer cells, suggesting it a potential tumor suppressor protein in colorectal carcinogenesis. In normal colon epithelium, IGFBP7 strongly expressed in the differentiated cells at the surface of the colon epithelium, while weakly expressed at the crypt base. IGFBP7 strongly expressed in low grade colorectal carcinoma and weakly expressed in high grade colorectal carcinoma. Expression of IGFBP7 in CRC tissue correlated with favourable survival.

Conclusions. The addition of IGFBP7 in colon cancer cells induced more pronounced anterior-posterior polarity morphology, accompanied by upregulation with alkaline phosphatase (AKP) activity, indicating IGFBP7 a potential molecule associated with colon cancer differentiation. Expression changes are associated with EMT of colorectal cancer cell lines. Through Affymetrix 133 plus 2.0 expression chip and two-dimensional gel electrophoresis (2-DE) analysis, it was found that downregulation of IRS1, SOX9 and HSP60 may the possible key downstream genes involved in the process. Interestingly, the expression of IGFBP7 in CRC tissue was found to be correlated with the fasting glucose level, suggesting the potential important role of IGFBP7 in diabetes mellitus. The further work is now going on.

SO-023

The role of Dicer in the colorectal carcinogenesis

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Aims. The incidence for colorectal cancer increases continuously and meanwhile colorectal cancer is the second most cause of cancer death in Europe. The endoribonuclease Dicer plays an important role in cellular processes like development, differentiation, proliferation or cell death via micro RNA (miRNA)- processing. miRNAs, processed by Dicer, regulate many oncogenes as well as tumor suppressor genes which are involved in the carcinogenesis of the colorectal carcinoma. Therefore, we asked ourselves which role Dicer has in the colorectal carcinogenesis.

Methods. 117 cases of colorectal cancer patients were stained for Dicer expression. These represented the following stages: adenoma with low or high grade dysplasia and adenocarcinoma UICC stage 1, stage 2, stage 3 and stage 4. Immunohistochemical staining was done with a specific anti-Dicer antibody. The intensity of specific Dicer staining was analysed and graded into no—0, weak—1, moderate—2 or strong cytoplasmic -3 staining. Additionally, a mouse model was generated. Therefore, C57BL/6-mice with the following alleles were crossed: Lgr5-EGFP-IRES-creERT2, Rosa26-lacZ, APCfl, Dicerfl.

Results. We showed that Dicer expression is reduced during the process of colorectal carcinogenesis. Adenomas displayed a significantly higher expression of the endoribonuclease Dicer than adenocarcinomas. A gradually reduction in the Dicer expression from adenomas with low grade dysplasia up to the adenocarcinomas UICC stage 4 was seen. Thus, Dicer seems to influence colorectal carcinogenesis. Therefore the role of Dicer in the pathogenesis of colorectal cancer should be analysed in a mouse model. Thus we bred mice with an inducible Cre-recombinase under the promoter of the stem cell marker Lgr5. To trace these recombined cells we crossed these mice with Rosa26-lacZ reporter mice. Finally these mice were crossed with floxed Dicer alleles and APC alleles, because APC plays a central role in colorectal cancer.

Conclusions. Due to the reduced Dicer expression during the colorectal carcinogenesis process, Dicer might play a role in colorectal cancer. Thus we will next investigate the influence of Dicer employing a mouse model. In this mouse model, the combined knockout of the tumor suppressor APC, which is a component of the Wnt pathway and frequently mutated in colorectal cancer, and Dicer should unravel the role of Dicer in the colorectal carcinogenesis.

SO-024

Expression and significance of leptin receptor and phosphorylation of signal transducer and activator of transcription 3 in diffuse large B-cell lymphoma

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Aims. To investigate the expression and clinical significance of leptin receptor (OBR) and phosphorylation of signal transducer and activator of transcription (p-STAT3) in patients with diffuse large B-cell lymphoma (DLBCL)

Methods. Immunohistochemical analysis was used to detect the expression of OBR and p-STAT3 in 80 patients with DLBCL and 10 patients with reactive lymphoid hyperplasia (RLH). Using a panel of immunohistochemical markers (CD10, bcl-6 and Mum-1), all cases of DLBCL were further divided into two groups, GCB (germinal center B-cell-like) or non-GCB.

Results. Immunohistochemistry revealed expression of OBR and p-STAT3 in 45.0% (36/80) and 28.8% (23/80) cases of DLBCL, respectively, and minimal straining in 100% (10/10) cases of RLH (p<0.05). Compared with GCB group (8.7%, 2/23), non-GCB group has higher p-STAT3 expression rate (36.8%, 21/57, p<0.05).There was no significant difference in the expression of OBR between these two groups. Compared with clinical stage I–II [46.2% (18/39) and 25.6% (10/39)], stage III–IV has higher OBR and p-STAT3 expression rate [61.9% (13/21) and 38.1% (8/21), p>0.05]. The expression of OBR and p-STAT3 were not correlated with age, gender, extranodal infiltrations, LDH level, B-symptoms and IPI (international prognostic index, p>0.05). The expression of OBR was positively related with that of p-STAT3 in DLBCL patients (r=0.232, p=0.039).

Conclusions. OBR stimulates the JAK/STAT signaling pathway and induces the phosphorylation of STAT3. This may be involved in carcinogenesis and prognosis of DLBCL.

SO-025
Complex phosphorylation dynamics control the composition of the Syk interactome in B cells

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Aims. Spleen tyrosine kinase Syk provides catalytic activity for a variety of ITAM-based immune cell receptors and is essential for the development and activation of B cells. Moreover, Syk has been identified as a tumor suppressor an as an oncogene in diverse tissues. Its catalytic activity and the ability to interact with other signaling elements depend critical on dynamic phosphorylation of Syk. Thus comprehensive understanding of the phosphorylation and the interaction of Syk with specific effector proteins is important and purpose of this study.

Methods. The analysis of Syk phosphorylation and its interactome was performed in a three-step experiment. Firstly we used stable isotope labeling with amino acids in cell culture (SILAC) to distinguish between specific Syk interaction proteins an unspecific background and furthermore to compare the phosphorylation state of Syk at different time points of B cell antigen receptor (BCR) stimulation. Secondly we analyzed purified Syk in a global and unbiased manner by high-resolution mass spectrometry. Thirdly we investigated the impact of selected phosphorylation sites and binding partners on Syk regulation by mutational analysis.

Results. We have identified the full spectrum of phosphoacceptor sites in human Syk and quantified its complex dynamics in response to BCR stimulation. While the majority of inducible phosphorylations occurred on tyrosine residues, one of the most frequently detected phosphosites encompassed serine 297 located within the linker insert distinguishing the long and short isoforms of Syk. Furthermore we could elucidate the Syk interactome in resting and activated B cells, consisting of more than 25 interacting proteins. One newly discovered group of interacting partners is the 14-3-3 family of adapter proteins, which bind directly to phosphoserine 297 after BCR stimulation. The latter complex attenuates inducible plasma membrane recruitment of Syk, thereby limiting antigen receptor-proximal signaling pathways. Part of this work was published as frontline article in the "European Journal of immunology".

Conclusions. Collectively, the established ligand library provides a basis to understand the complexity of the Syk signaling network. The described regulation of Syk by 14-3-3 represents the first reported serine dependent inhibition of Syk.

SO-026
EBV positive T-cell lymphoproliferative diseases of childhood and adolescents: a clinicopathological study of 40 Chinese patients

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Aims. To observe and investigate the clinical and pathological features of EBV+ T-cell lymphoproliferative diseases of childhood and adolescents in South-West China, with a review of literature

Methods. Related clinical and follow-up data were collected for all of the patients. Morphology review was down, and pathologic diagnosis was based on the WHO Classification for Tumors of Hematopoietic and Lymphoid Tissue (2008). Immunophenotype analysis, EBER1/2-ISH and antigen gene rearrangement analysis was performed. Overall sur-

vival (OS) was plotted using Kaplan-Meier method. Prognosis related agents of the tumor were estimated as well.

Results. The median age of patients was 23 years (range 1–44), and 19 patients were less than 18 years old. The male-to-female ratio was 3:1.6. The major clinical manifestations included fever, lymphadenopathy, splenomegaly or hepatosplenomegaly and pancytopenia. Biopsy material included lymph node (30 samples), skin (seven), spleen (3), liver (1), and soft tissue (1). Seven patients presented the skin lesions, hydroa vacciniforme-like lymphoma was diagnosed, and two of them also suffered from the systemic disease simultaneously. Thirty-five cases were systemic diseases. According to the proposed categorization of CAEBV from the CAEBV study group by K. Oshima, 21 (60%) cases fevered grade A1 (polymorphic and polyclonal) and the remaining 14 cases (40%) was included in A2 (polymorphic and generally monoclonal) or A3 (monomorphic and monoclonal). For the patients with systemic disease, the prognosis of both adult and children patients was poor. Whenever for the patients with skin lesion alone, the prognosis was better.

Conclusions. EBV positive T-cell lymphoproliferative diseases are relatively more common in China and it may involve both children and adults. There are some problems presented in diagnosis and sub-classification of the tumor.

SO-027
Prevalence of novel human polyomaviruses in the buffy coats of healthy blood donors

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Aims. Merkel cell polyomavirus (MCPyV) DNA sequences have recently been detected in the buffy coats of healthy blood donors, suggesting that MCPyV persists latently in blood cells in a fraction of healthy patients. In long term, viral persistence may allow MCPyV to generate mutants that can participate in the cell transformation process, as detected in CLL and MCC. Here we assessed the presence of some of the recently discovered human polyomaviruses, i.e. MCPyV, HPyV6, HPyV7 and HPyV9.

Methods. DNA quality was assessed by SCS ladder. PCR for MCPyV, HPyV6, HPyV7 and HPyV9 was performed on the DNA isolated from the buffy coats of 30 healthy blood donors. All PCR products were sequenced. In addition, HPyV6-FISH and HPyV7-FISH were performed.

Results. None of the buffy coats revealed detectable MCPyV DNA (0/30). In contrast, specific HPyV6 DNA was detected in 20% (6/30), specific HPyV7 DNA in 23% (7/30) and HPyV9 DNA in 6% (2/30) of the buffy coats as confirmed by sequence analysis. FISH confirmed the presence of HPyV6 and HPyV7.

Conclusions. Here we report the presence of novel polyomaviruses in the buffy coats of healthy blood donors. Especially the finding of HPyV9 which is closely related to the lymphotropic polyomavirus is of interest. In as much these polyomaviruses might contribute to the pathogenesis of transfusion associated diseases remains to be established.

SO-028

Expression of APOBEC3B and its interaction with hnRNP K in hepatocellular carcinoma

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Aims. The cytidine deaminase apolipoprotein B mRNA editing catalytic subunit-3 (APOBEC3) proteins have been identified as potential DNA mutators and potent inhibitors of diverse retroviruses, retrotransposons and hepatitis B virus. Heterogeneous nuclear ribonucleoprotein K (hnRNP K), a positive regulator of HBV expression, has been identified as a major interaction partner of a member of APOBEC3---APOBEC3B(A3B) protein. This study is to investigate the expression levels of A3B and hnRNP K in hepatocellular carcinoma (HCC) tumor tissues, and study if deaminase activity of A3B could affect its interaction with hnRNP K.

Methods. mRNA and protein expression analysis of A3B and hnRNP K were carried out by semi-quantitative RT-PCR, real-time PCR and Western Blotting. We construct HA-tagged A3B expression vector and its three mutants with loss of cytidine deaminase activity, myc-tagged hnRNP K expression vector. Cotransfection and co-immunoprecipitation was done to investigate the effect of deaminase activity of A3B on hnRNP K expression and its interaction with A3B.

Results. A3B was found widely up-regulated in HCC tumor tissues compared with their normal counterparts. Overexpression of A3B or its mutants won't change hnRNP K mRNA or protein expression. But cotransfection of A3B mutants A3B C100S and C100/289S with hnRNP K expression vector would decrease the interaction between these two proteins.

Conclusions. In this study, we showed that A3B mutant C100S could decrease the interaction of A3B with hnRNP K, whereas deaminase inactivation of A3B won't affect hnRNP K expression

SO-029

Characterization of long non-coding RNAs in hepatocellular carcinoma

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Aims. The vast majority of the human genome is represented by non-protein-coding RNAs (ncRNAs), which are ribonucleic acids of different lengths without an open reading frame. Many well known ncRNAs are involved in all stages of protein biosynthesis or in the regulation of gene expression. Recently, different functions have been attributed to the few well-characterized ncRNAs, e.g. in epigenetics and cancer. However, the function of most of the newly discovered long ncRNAs is still unknown and a detailed analysis is lacking. Since cancer research has focused on protein-coding genes for the last decades, the potential of involvement of ncRNAs in the pathogenesis and prognosis of hepatocellular carcinoma (HCC) is not known so far.

Methods. We screened for the expression of 17,000 ncRNAs in 32 cases of HCC and 7 control tissue samples. After identifying tumor-specific candidates, their expression was validated in HepG2 and Huh7 cells. Their impact on cell viability was uncovered after siRNA-mediated

ncRNA knockdown in liver cancer cell lines. By using RNA affinity purification (RNA-AP), protein interaction partners were identified.

Results. Statistical analysis unraveled 187 upregulated and 278 downregulated ncRNAs in HCC. One ncRNA, LOHC (Long non-coding RNA Overexpressed in Hepatocellular Carcinoma), was highly expressed in liver cancer compared to normal liver patient samples. Validation of differential expression by quantitative PCR in an independent cohort confirmed significantly higher expression in HCC versus normal liver. Knockdown of LOHC expression significantly reduced cell viability and influenced cell cycle progression of HepG2 and Huh7 cells. Using RNA-AP, IGF2 mRNA binding proteins (IMPs) were identified as LOHC interaction partners. Moreover, interaction of LOHC and IMPs was largely different in diverse stages of cell cycle, which additionally influenced LOHC expression and stability over time.

Conclusions. LOHC is an important ncRNA in HCC, which regulates cell viability and cell cycle. It was found to be an interaction partner of IMPs, which can regulate LOHC stability and have a major role in the pathogenesis of liver cancer. These data show that besides protein-coding genes, the expression of ncRNAs could be highly and specifically regulated in HCC, which will allow conclusions about the use of ncRNAs as potential diagnostic and prognostic markers. Most importantly, ncRNA expression profiling in cancer has identified functionally important players in liver tumorigenesis.

AG Orthopädische Pathologie

SO-030

Identification of molecular target structures in myxoid liposarcoma in order to establish new therapeutic strategies

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Aims. Liposarcomas belong to the most common soft tissue tumours (10–15%), but therapeutic options are limited and specific agents with defined molecular target structures are not available. As myxoid liposarcoma is caused by a gene translocation leading to the formation of an aberrant transcription factor, expression of many genes may be altered in these tumours. Therefore, we implemented gene and microRNA expression analyses in myxoid liposarcoma in order to reveal key molecules and signal transduction pathways involved in tumour formation and progression. We aimed at identifying candidate genes that may serve as diagnostic or prognostic biomarkers or even as therapeutic target structures.

Methods. Whole genome and microRNA microarray analyses were performed on a well characterised cohort of tumour samples and fat samples as control. The results could be verified by qPCR. Expression of candidate genes was analysed by qPCR and immunohistochemistry in primary tumour samples. Furthermore, inhibition experiments in the myxoid liposarcoma cell lines MLS402 and MLS1765 were performed in order to examine the functional relevance of selected candidates. In addition to their separate analysis, whole genome and microRNA microarrays were evaluated together using the bioinformatics package BIRTA (Bayesian Inference of Regulation of Transcriptional Activity). This analysis integrates mRNA and microRNA expression data to infer the activities of transcription factors and microRNAs.

Results. Among the significant differentially expressed genes and microRNAs we could identify interesting candidates with high clinical relevance such as the microRNAs miR-29 and miR-181 or the genes TOP2A and FGFR2. Together with other members of the FGF/FGFR

family FGFR2 was detected to be overexpressed in myxoid liposarcomas compared to fat tissue. Overexpression was confirmed by qPCR in the whole tumour cohort. FGFR2 expression in primary myxoid liposarcomas was also shown on protein level by immunohistochemical staining. By BIRTA analysis transcription factors and microRNAs with differential activity in myxoid liposarcoma samples compared to fat tissue could be identified.

Conclusions. Our study revealed several new candidate genes and microRNAs in myxoid liposarcoma which represent starting points for the establishment of specific therapeutic strategies. As one approach we could demonstrate a potential role of FGFR signalling in the pathogenesis of myxoid liposarcoma.

SO-031
MicroRNA profiling of primary high-grade soft tissue sarcomas

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Aims. Soft tissue sarcomas define a group of highly aggressive, histologically and genetically heterogeneous malignant tumors of mesenchymal origin that account for approximately 1% of all adult malignancies. More than 60 different sarcoma subtypes are recognized by pathological examination, frequently on the basis of minor differences but with significant clinical impact. MicroRNAs are considered as attractive candidates that may improve diagnostic, prognostic, and predictive characterization of this group of malignancies.

Methods. We performed a comprehensive miRNA expression analysis in a series of 76 untreated, primary high-grade soft tissue sarcomas representing eight subtypes, and in a panel of 15 representative sarcoma cell lines using microarray technology.

Results. This screening revealed unique miRNA expression patterns for synovial sarcomas (SS), myxoid liposarcomas (MLS), and leiomyosarcomas (LMS), and defined unique sets of miRNAs discriminating the different liposarcoma subtypes from non-neoplastic adipose tissue. The over-represented miRNAs included miR-133a, miR-133b, and miR-1 in LMS, members of the miR-200 family in synovial sarcomas, and the tumor-associated miR-9 and miR-9* in myxoid liposarcomas compared to adipose tissue. Moreover, we found co-expression of 63 miRNAs clustering in a genetically imprinted chromosomal region 14q32.2 separating primary sarcoma samples and sarcoma cell lines into two molecular subgroups. In conclusion, our profiling approach has identified sets of deregulated miRNAs in a unique series of eight histological subtypes of primary, untreated high-grade sarcomas. Furthermore, we identified sarcoma cell lines for in-depth evaluation of these miRNA candidates with regard to their molecular and biological function.

Conclusions. Taken together, our comprehensive miRNA profiling identified a novel set of miRNAs that might contribute to sarcomagenesis and provide a starting point for experimental modulation of relevant targets for new therapeutic strategies in high-grade sarcomas.

SO-032
Salinomycin enables pro-apoptotic NF-κB signalling in soft tissue sarcomas

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Aims. Chemotherapy for advanced soft tissue sarcomas remains unsatisfactory due to their low chemosensitivity, despite combination chemotherapeutics. Therefore, alternative therapeutic strategies are required. In this study, we investigated the effect of salinomycin, an appropriate agent to deplete cancer stem like cells (CSCs), in combination with doxorubicin on soft tissue sarcoma cell lines. The aim of this study was to elucidate if salinomycin enables a more effective strategy to deplete CSCs in soft tissue sarcoma cell lines.

Methods. Based on the three soft tissue sarcoma cell lines A204 (rhabdomyosarcoma), HT-1080 (fibrosarcoma), and SW872 (liposarcoma) we analyzed the effect of mono (doxorubicin; salinomycin alone) and combined treatment regimes. The level of the CSC maker CD133 was determined by flow cytometry. The treated cells were analyzed by cell viability and caspase (3/7; 9) activity assays. We investigated further, the impact of the different treatment regimes by reporter assays for NF-κB, qRT-PCR to determine the levels of TP53 and PUMA. In addition, p53 expression and the phosphorylation state of serine 15 were analyzed by Western blot.

Results. The sensitivity of soft tissue sarcoma cells on salinomycin correlated with the CD133 status of the corresponding cell lines. Furthermore, salinomycin mono-treatment was not sufficient to induce any of the analyzed parameters. In contrast, the combination with doxorubicin led to an overall significant increase in caspase and NF-κB activity, as well as in TP53, PUMA transcription, and an increased sub G1 fraction compared to doxorubicin alone.

Conclusions. This study demonstrates that the combination of a low salinomycin dose with doxorubicin is a more effective strategy for the treatment of soft tissue sarcoma cells than doxorubicin alone.

SO-033
Loss of P16(INK4a) indicates a senescence barrier in soft tissue and bone sarcomas and is associated with shorter patients survival

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Aims. P16(INK4a) is an important factor in carcinogenesis and its expression is linked to oncogene-induced senescence. Very recently it was shown that up and downregulation of p16 indicates a senescence barrier. However, in soft tissue and bone sarcomas this phenomenon has not been described so far. In this study we analysed a well characterized cohort of soft tissue and bone tumours for p16(INK4a) expression and correlate the results with clinicopathological parameters including survival.

Methods. 136 soft tissue tumours and bone tumours of the Ludwig-Maximilians-University (LMU) were revisited and categorized in the actual WHO classification system.

Results. P16(INK4a) upregulation was seen in low grade sarcomas (G1) and downregulation in high grade sarcomas (G2 and G3). Loss of p16 correlates with shorter patients survival in G2-tumors (p=0.02). The tumour collective comprises undifferentiated pleomorphic sarcomas (n=40), Leiomyosarcomas (n=29), synovial sarcomas (n=16), Liposar-

comas (n=20), angiosarcomas (n=2), malignant solitary fibrous tumors (n=2), chondrosarcomas (n=3) and other sarcomas (n=24).

Conclusions. Upregulation of p16 might be associated with the induction of senescence and indicates a senescence barrier. Downregulation of p16 is found in malignant progression and is correlated significantly with shorter patients' survival.

SO-034
A comprehensive study of 214 osteosarcomas of the jaws

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Aims. Osteosarcomas of the jaws account for approximately 5% of all osteosarcomas and seem to represent a biologically and clinically distinct subgroup. However, due to the rarity of the disease most of the studies described in the literature so far are of rather small size. We therefore studied a large series of gnathic osteosarcomas and correlated our findings with clinico-pathological parameters with special emphasis on factors affecting the prognosis of patients.
Methods. 214 gnathic osteosarcomas registered in the Bone Tumor Reference Center in the past 40 years were histologically re-evaluated and graded. Additionally, the corresponding clinical files were collected and analyzed for clinico-pathological parameters.
Results. Our series included 136 mandibular and 78 maxillary osteosarcomas with a median patient age of 39 years and an average follow-up of 59 months. The overall survival at 5 years was 66.8% and at 10 years 59.2%, respectively. The prognosis of patients differed significantly with regard to tumor grade (p=0.027), metastatic (p<0.0001) and recurrent (p<0.0001) disease as well as with the achievement of a complete resection (R0) at any time during the course of the disease (p<0.0001, 5-year survival 79.9% vs. 24.3%). Tumor size and site, however, did not proof to be of statistical significance. Interestingly, (neo)adjuvant therapy did not prolong survival.
Conclusions. Osteosarcomas of the jaws have distinct clinico-pathological properties compared with their counterparts in the peripheral skeleton. The mainstay of therapy seems to be the complete surgical resection of the tumor resulting in an excellent prognosis of patients.

SO-035
Establishing the foundation for targeted proteomics of protein networks in osteosarcoma

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Aims. In a time of feasible and cost effective whole genome sequencing, the hopes of understanding tumor biology are currently mainly focused on genomics. However, diagnostically and prognostically relevant biomarkers as well as therapeutic targets generally represent proteins. Therefore, protein signatures are urgently needed in the majority of tumors, including osteosarcomas. Our aim is to establish proteome extraction from calcified tissues to analyze the proteome of osteosarcomas and to potentially identify tumor specific and therapeutically relevant proteins or protein signatures.
Methods. As a model for proteome extraction from calcified tissues we used a barocycler on cancellous bone from the spine of autopsy cases. Here, we present an expedited workflow to extract and consistently monitor peptides, and therefore proteins, from cancellous bone using bottom up mass spectrometry and selection reaction monitoring mass spectrometry (SRM-MS).
Results. We optimized extraction procedures from the above mentioned tissue specimens using a barocycler. The extracted proteome was dige-

sted with sequence specific endopeptidases and peptides fractionated using isoelectric focusing and strong cation exchange chromatography. Individual fractions were analyzed using shotgun proteomics and a spectral library for SRM-MS for cancellous bone was established.
Conclusions. The presented methodology will lay the foundation for extending protein signature analyses in osteosarcoma. Perspectively, patient samples will be analyzed to potentially discover new biomarkers and therapeutic targets in this rare neoplastic disease.

SO-037
First histologic results of a scaffold-free strategy for cartilage regeneration in humans

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Aims. Traumatic articular cartilage lesions bear a significant risk to result in osteoarthritis and its regenerative capacity is highly limited. Although various tissue engineering strategies are available, none of the used procedures provides satisfying results. Autologous chondrocytic spheroids (Co.don, Teltow) represent an innovative strategy in the treatment of articular cartilage defects. We present the first study of histologically investigated second-look biopsies of this procedure.
Methods. Articular cartilage lesions of four patients were treated with autologous chondrocytic spheroids. In a second-look intervention specimens were taken between four and sixteen months after implantation. The indication for the second look arthroscopy was independent from the spheroid based autologous chondrocyte transplantation (ACT). Needle biopsies were taken using a 1.5 mm Yamshidi needle and fixed in buffered formalin. Following decalcification and paraffin embedding specimens were prepared for histological (HE, alcian-blue) and immunohistological (collagen II, collagen X and aggrecan) examination according to standardized methods.
Results. The specimens revealed typical hyaline cartilage characteristics showing numerous round shaped chondrocytes embedded in huge amounts of alcian-blue positive extracellular matrix components. No vascularization or fibrous tissue was detectable. The typical articular cartilage architecture consisting of flat cells in the superficial zone was seen. In the immunohistological staining great amounts of Collagen II and Aggrecan were visualized, whereas no collagen X could be detected. No significant differences were evident within the different specimens representing the different time-points after spheroid based ACT.
Conclusions. The present study represents the first histomorphological and immunohistochemical evaluation of scaffold-free spheroid-based ACT in humans. The results of this study demonstrates the high impact of the use of spheroids for the regeneration of hyaline cartilage and show that the implantation of these Scaffold-free autologous chondrocytic spheroids is a successful procedure, leading to the regeneration of hyaline cartilage.

SO-038
Differenzierung von Arthrose und rheumatoider Arthritis in Synovialmembranen mittels proteomischer Techniken

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Aims. Rheumatoid arthritis (RA) is a systemic, inflammatory, autoimmune disorder with progressive articular damage that may result in lifelong disability. Despite the existence of ACR criteria for the definition of the disease exact histological diagnosis and evaluation of disease

activity in tissues remains a challenge. The aim of this study is to search for proteins that can distinguish between synovial tissue from patients with osteoarthritis (OA) and RA by proteomic techniques.

Methods. Based on the synovitis score we have compared 3 groups of synovial tissues of patients with osteoarthrosis, low grade synovitis and high-grade synovitis in patients with rheumatoid arthritis. Synovial samples of each group were pooled and differential proteomic technology was applied for the quantitative and statistical analysis of protein biomarkers by dual radioisotope labelling, 2D gel electrophoresis, and subsequent analysis by MALDI-TOF mass spectrometry. Differential proteomics was performed in three paired groups- OA versus RA, low grade RA versus high grade RA and OA versus low grade RA.

Results. 619 different protein spots could be detected and 129 proteins were identified. 9 proteins discriminate OA from RA as well as OA from low grade RA of which 5 proteins were statistically highly significant (p<0.0001).

Conclusions. MALDI MS is able to discriminate OA and RA in synovial tissue regardless of low and high synovitis score. Despite the similar histology of OA and low grade RA, the diagnosis of both conditions can be established by identification of only 5 proteins.

AG Oralpathologie

SO-040
Comparison of HPV prevalences in HNSCC patients with regard to regional and socioeconomic factors

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Aims. A distinct subset of head and neck squamous cell carcinomas (HNSCCs) can be attributed to the oncogenic effects of persistent oral infection by human papilloma virus (HPV). This typically affects a certain population of younger patients independent of classic risk factors. Predilection site of HPV positive tumors are tonsillar squamous cell carcinomas with an HPV prevalence of up to 93%. Numerous studies have investigated HPV prevalences in HNSCCs with highly variable results. This study aims to elucidate the disparities of HPV prevalence by analyzing two different patient collectives.

Methods. 85 patients of an otolaryngology private practice in a rural area and 11 private health insured patients of the Department of Otolaryngology of the University Hospital Regensburg were screened for p16 overexpression and consecutively tested for HPV infection.

Results. The total HPV prevalence of private practice patients (PPP) was 7.06% (6 patients) with the highest infection rate in tonsillar carcinomas (33.33%) and a larger percentage of female patients in the HPV positive group than in the HPV negative group (p=0.037). Predilection site specific HPV prevalence was 16.27% in the PPP collective in contrast to 72.73% in the private health insured patients collective (PHIP). An analysis of Uvula resection specimen from healthy individuals did not yield any positive results. HPV positive patients of both collectives were, on average, younger (55 years) than HPV negative patients (60 years) and less likely to have a history of alcohol or tobacco abuse. Histologically, besides lower differentiation, HPV positive tumors were less often keratinizing and more frequently of basaloid morphology.

Conclusions. While patient and histological characteristics of HPV positive tumors are concordant with former studies, a huge variation in HPV prevalences was noted amongst the different patient collectives. Therefore highly variable HPV infection rates in HNSCCs can be attributed (besides selection of screening locations and detections methods) to the choice of particular patient collectives with a significantly lower incidence rate in rural areas and a higher rate in private health insured patients.

SO-041
The role of histone deacetylases in combination therapy of HPV-positive and negative HNSCC cell lines

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Aims. Head and neck squamous cell carcinoma (HNSCC) is the sixth most common malignancy worldwide and associated with a high mortality and morbidity. Over the recent years, it has become clear that HPV-positive/p53-negative tumors form a distinct disease entity that differs from HPV-negative/p53-mutated tumors in terms of biological behavior and clinical response. Treatment options are very limited and novel strategies that consider the distinct HNSCC subtypes are urgently needed to improve outcome. Histondeacetylases (HDAC) are enzymes which modify histone-proteins by removing the acetyl moieties from lysine residues. This ultimately leads to condensation of chromatin, preventing access of transcription factors, which in turn leads to transcriptional repression. HDAC over activity have been associated with tumorigenesis and consequently several inhibitors have been developed and are currently being tested as anticancer agents in a variety of solid and hematologic malignancies.

Methods. Two different HNCSS cell lines (FaDu: HPV-positive/p53-wt and UD-scc2: HPV-negative/p53-mut) were treated with the HDACi suberoylanilide hydroxamic acid (SAHA) alone and in combination with established chemotherapeutics and targeted drugs as well as with conventional radiation therapy. Therapy efficacy and corresponding molecular alterations were detected by clonogenic assays, kinetic cell response profiling and immunoblotting, respectively.

Results. We could show that a single therapy with SAHA alone is not very effective in HPV-positive as well as HPV-negative cell lines. However, combination therapy with conventional chemotherapeutics such as cisplatin or mitomycin as well as combined HDAC/EGFR-inhibition or HDACi/radiation result in strong additive effects on cell viability in comparison to single chemotherapy, targeted therapy or radiotherapy alone. Very interestingly, combined SAHA/cisplatin treatment was much more effective in HPV-positive cells than in HPV-negative cells.

Conclusions. The histone deacetylase inhibitor SAHA enhances very effectively conventional cisplatin-based chemotherapy as well as radiotherapy and EGFR-targeting approaches in HNSCC cell lines. Moreover, our data may help to establish a therapy which balances unwarranted side effects and therapeutic benefit better than current treatment approaches. Next, we plan to transfer our findings into the in vivo situation to prove the clinical applicability of our findings.

SO-042
Distinct inflammatory patterns in HNSCC patients predict treatment specific outcome

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Aims. Patterns of inflammation are well known to be associated with the clinical outcome in unselected cohorts of various cancers including head and neck squamous cell carcinoma (HNSCC). However, data on the association of distinct inflammatory cells in HNSCC with outcome and response in specific therapeutic settings is sparse.

Methods. We investigated a small but well characterized cohort of 72 HNSCC treated either by definite radiochemotherapy or radioimmunotherapy. Patterns of overall and intratumoral total T-cell (CD3), T-helper cell (CD4), cytotxic T-cell (CD8), regulatory T-cells (Treg; Foxp3), B-cell (CD20), mast cells (CD117) and macrophages (CD68) were investigated with immunohistochemistry and correlated to tumor type, response and patient outcome.

Results. The density of inflammatory infiltrate was generally higher in p16-positive HPV associated tumors than in their p16-negative counterparts. High overall and intratumoral macrophage density was associated with non-response to radiotherapy, this was not seen for any other inflammatory parameter. High intratumoral T-helper and Treg cell numbers were associated with decreased overall survival. High overall T-cell and B-cell infiltrate were associated with slightly better overall progression-free survival which was mainly due to a significant impact of both parameters on distant progression-free survival.

Conclusions. The predictive value of inflammatory cells in patients treated by definite radiotherapy differs widely from results reported from unselected patient cohorts, with a clear loss of the predictive value for cytotoxic T-cells. Patterns of inflammation in these therapeutic settings influenced the risk of distant but not local disease recurrences.

SO-043

The secretome of activated fibroblasts plays a critical role in phenotype transition and EGFR signalling in OSCC cells

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Aims. The reciprocal interaction of stromal fibroblasts with oral squamous carcinoma cells (OSCC) is suggested to mediate epithelial to mesenchymal transition (EMT) of cancer cells as a prerequisite for invasion and metastasis as well as effectiveness of EGFR tyrosine kinase inhibitors. Therefore, we investigated if and how activated fibroblast mediated OSCC phenotype transition is accompanied by changes in EGFR signalling in OSCC cells in vitro.

Methods. As a model for activated stromal fibroblasts, hTERT-BJ1 fibroblasts were stimulated with TGF-B1 and PDGF-AB to generate a myofibroblast or a proliferative phenotype, respectively. Conditioned medium (FKMunstim, FKMTGF, FKMPDGF) was used to stimulate OSCC cells in vitro up to 4 days. Phenotype modulation was assessed by mRNA analysis, immunohistochemistry, and Western blotting for E-cadherin, N-cadherin, Vimentin, and Cytokeratin. Changes in cell cycle as well as in expression and phosphorylation of EGFR signalling proteins were analysed by flow cytometry and Western blotting.

Results. The OSCC cells used for stimulation experiments were characterized by an abundant E-cadherin and Cytokeratin expression, whereas N-cadherin or Vimentin expression was virtually absent. 4 day stimulation with the different FKM lead to an up-regulation of Vimentin and a loss of Cytokeratin in the OSCC cells with the most pronounced effect in case of applying FKMTGF. In contrast, E-cadherin expression seemed to be unaltered. With respect to EGFR signalling, both ERK and PI3K/AKT were strongly activated by FKMTGF > FKMPDGF. These effects were well correlated with a significant increase in the S phase as revealed by cell cycle analysis. In addition, we found a decrease in the expression of the proapoptotic protein BIM which represent a downstream mediator of ERK as well as AKT.

Conclusions. In summary, soluble factors particularly of activated myofibroblasts are capable to induce EMT like phenomena in OSCC cells. These phenotype changes are associated with an activation of EGFR signalling and enhanced cell proliferation and provide preliminary evidence for increased cell survival. Results speak well for a possible influence of activated fibroblasts also on EGFR-inhibitor therapy. Therefore,

carcinoma associated fibroblasts may be promising novel targets for combined therapy strategies.

SO-044

Value of pathohistological parameters for the individual response prediction to induction docetaxel, cisplatin, and 5-fluorouracil (TPF) chemotherapy in advanced oral cancer

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Aims. Although TPF therapy improves tumour response and survival in advanced HNSCC, there is an individual variability with respect to therapy sensitivity. Therefore the study was aimed to investigate if pathohistological parameters with focus on stroma induction can contribute to therapy response prediction in TPF treatment.

Methods. Biopsies taken prior to induction chemotherapy and postsurgical specimens of 21 HNSCC patients receiving TPF chemotherapy followed by curative surgery were available. In the pre-treatment samples, tumour localization, grading, extent of stroma formation, invasive front structure and the extent of inflammatory reaction was analysed. Moreover, the expression of E-cadherin, N-Cadherin and αSMA was immunohistochemically assessed. Data were correlated to chemotherapy response determined by clinical examination and CT imaging. Additionally, αSMA expression was exploited in postsurgical specimens.

Results. The study group included carcinomas of the tonsils (8), the flour of the mouth (3), the tongue (7), and the oropharynx (3); all of staging group IVa; clinical responders: 14/21. All tonsil carcinomas could be allocated to the responder group. Analysis of the pre-treatment biopsies revealed no clear difference between responders and non-responders with respect to stroma formation, inflammation, structure of the invasive front and the expression of αSMA, E-cadherin and N-cadherin. In tendency, responders seemed to have a higher malignancy grade associated with disseminated tumour growth. Investigating tonsillar carcinomas and oral cavity carcinomas separately, there was a trend in terms of increased αSMA and reduced membranous E-cadherin in tonsillar carcinomas. In contrast, in oral cavity tumours, non-responders exhibited a lower extent of membranous E-cadherin and an increased αSMA positivity in the post-therapeutic specimens.

Conclusions. 1) tonsillar carcinomas show a better TPF therapy response compared to oral cavity carcinomas, 2) markers reflecting therapy response are required to be separately evaluated in tonsillar and nontonsillar carcinomas, and 3) in non-tonsillar carcinomas chemotherapy resistance seems to be associated with stroma activation and phenotype transition of cancer cells which may serve as a predictive marker. However, when interpreting the findings of the study, the small number of patients, use of biopsy material or the preselection of advanced stage patients must be critically considered.

SOX2 as a potential therapeutic target in squamous cell carcinoma of the head and neck

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Aims. Recently, we identified SOX2 as lineage specific oncogene in squamous cell carcinomas of the lung (LSCC). Since LSCC are morphologically and clinically related to head and neck squamous cell carcinomas (HNSCC), our aim was to assess whether SOX2 amplification/over expression occurs in HNSCCs and if SOX2 could serve as a potential target for HNSCCs.

Methods. We assembled a cohort of 503 patients with HNSCCs, including 260 metastases and 141 recurrences. All samples were assessed for SOX2 amplification by FISH and SOX2 expression by IHC. HPV status was detected by PCR-ELISA. Molecular parameters were correlated with each other and clinico-pathological data. Furthermore, SOX2 expression was modulated in the SCC25 HNSCC cell line via lentiviral vectors carrying shSOX2 and SOX2 over expression constructs to investigate the role of SOX2 in apoptosis resistance.

Results. 20% of primary HNSCCs displayed a SOX2 amplification and results in SOX2 over expression. In almost all cases, metastatic and recurrent tumor samples shared the same SOX2 amplification status as the corresponding primary tumor. SOX2 amplification/over expression was mutually exclusive with HPV infection. Amplification/over expression of SOX2 was significantly associated with pathological parameters of poor outcome. Efficient modulation of SOX2 gene and protein expression was obtained in the SSC25 cell line following treatment with lentiviral vectors. Inhibition of SOX2 expression enhanced apoptosis sensitivity of SSC25 cells to apoptosis-inducing agents while SOX2 over expression had the converse effect, inducing therapy resistance.

Conclusions. SOX2 amplification frequently occurs in primary HNSCC and is a clonal event in metastatic disease. Furthermore, SOX2 amplification/over expression is associated with worse prognosis, possibly related to enhanced therapy resistance of SOX2 expressing cells. Targeting SOX2 and related molecular pathways may enhance therapy efficacy in HNSCC. However, SOX2-amplified tumors could potentially comprise a subset of HNSCC against which may hold therapeutic efficacy.

AG Herz-, Gefäß-, Nieren- und Transplantationspathologie

Prognostic value of matrix metalloproteinase-9, tissue inhibitor of metalloproteinase-1, B+ Tenascin-C and ED-A+ Fibronectin in dilated cardiomyopathy with left ventricular dysfunction

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Aims. Patients suffering from dilated cardiomyopathy (DCM) often present with severe heart failure symptoms and show increased mortality rates. Hitherto there is no reliable biomarker to estimate patients'

prognosis at the time point of first clinical presentation. Cardiac tissue remodeling in DCM is accompanied by a structural and functional reorganization of the extracellular matrix (ECM). Our current study was aimed to investigate matrix metalloproteinase 9 (MMP-9), tissue inhibitor of metalloproteinase 1 (TIMP-1) as well as fetal Tenascin-C (B+ Tn-C) and Fibronectin (ED-A+ Fn) variants known to be crucially involved in that process.

Methods. 187 patients with DCM, chronic myocarditis or inflammatory cardiomyopathy were included in the study at the time point of endomyocardial biopsy (EMB) and prospectively followed at 3- to 6-months intervals between 1999 and 2007. Serum levels of MMP-9, TIMP-1 and B+ Tn-C were assessed using ELISA. Tissue deposition of B+ Tn-C and ED-A+ Fn was quantified using confocal laser scanning microscopy.

Results. For all three serum markers, concentrations above a calculated threshold value (60 ng/ml for MMP-9, 168 ng/ml for TIMP1 and 1200 ng/ml for B+ Tn-C) were associated with significantly decreased survival rates (MMP-9: p=0.008, TIMP-1: p=0.001, B+ Tn-C: p<0.001) and a higher risk to die or undergo heart transplantation (MMP-9: p=0.006, TIMP-1: p<0.0001, B+ Tn-C: p=0.002). In cardiac tissue, a reexpression of B+ Tn-C and ED-A+ Fn not occurring in healthy tissue could be shown. Patients with protein deposition levels of ≥4.5% for B+ Tn-C and ≥2.1% for ED-A+ Fn had a significantly decreased survival (p=0.001 for B+ Tn-C, p=0.031 for ED-A+ Fn) and the risk to die or undergo heart transplantation was significantly increased (p=0.003 for B+ Tn-C, p=0.015 for ED-A+ Fn).

Conclusions. Elevated serum concentrations of MMP-9, TIMP-1 and B+ Tn-C and increased levels of B+ Tn-C and ED-A+ Fn protein deposition in cardiac tissue obtained by EMB are new promising markers for risk stratification in DCM patients. Additionally, against the background of the reexpression of ED-A+ Fn and the availability of a human recombinant antibody usable as a vehicle for targeted drug delivery, novel innovative therapeutic strategies can be developed for DCM.

The impact of ventricular assist device on acute cellular rejection and antibody-mediated rejection in cardiac allografts—a prospective study

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Aims. This study evaluated the impact of bridge-to-transplant ventricular assist device support on development of acute cellular (ACR) and antibody-mediated rejection (AMR) in cardiac allografts.

Methods. We studied 358 consecutive right ventricular endomyocardial biopsies (EMB) between 01/2011 and 07/2012 prospectively. Paraffin-embedded sections were evaluated for acute cellular rejection, endothelial cell swelling and capillary deposition of C4d, C3d. The effects of VAD (n=138) on ACR and AMR, classified according to the ISHLT, were studied and compared to results of EMB harvested from patients without VAD support (n=220).

Results. A positive correlation was found for endothelial cell swelling and capillary C4d deposition; the data failed to show a correlation between C4d and C3d deposition or C3d deposition and endothelial swelling. Our results did not demonstrate significant differences between the two groups in any given parameter.

Conclusions. The use of VAD did not predict development of AMR or ACR. The C3d staining does not add to the pathological diagnosis of AMR.

SO-050

Clinical grade next generation sequencing and SNP analysis for detection of pathogenic mechanism in primary cardiac angiosarcomas

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Aims. Primary cardiac angiosarcomas are very rare and constitute the largest group of malignant cardiac tumors. The prognosis is extremely adverse due to rapid growth, inoperable localization as well as the lack of sensitivity for chemotherapeutics and radiotherapy. To gain insight into the pathogenesis, 9 of these rare tumors were systematically investigated for genomic aberrations.

Methods. To detect larger genomic aberrations SNP-arrays (Infinium HD Bead Chip Technology of Illumina) were used and for detection of mutations in 409 frequently mutated oncogenes and tumor suppressor genes multiplex PCR and next generation sequencing (Comprehensive Cancer Panel of Ion Torrent) was applied.

Results. The SNP-array analysis revealed a complex karyotype of cardiac angiosarcomas and a recurrent small gain encompassing 3 receptor tyrosine kinase genes. The sequence analysis was performed with 6 matched samples of tumor and reference tissue. All detected mutations were verified with sanger sequencing. Two groups of genes with recurrent mutations were identified: genes contributing to receptor tyrosine kinase signaling and chromatin modifiers.

Conclusions. This study demonstrates the potential of clinical grade next generation sequencing combined with SNP analysis for detection of pathogenic mechanism in malignancy.

SO-051

Glomerular microRNA expression profiles in transplant-associated thrombotic microangiopathy

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Aims. Thrombotic microangiopathy is a serious complication of renal transplantation. Besides recurrence of the primary disease (RecTMA) renal transplant-associated thrombotic microangiopathy (rTx-TMA) can emerge de novo by humoral rejection (HR-TMA) or calcineurin inhibitor toxicity (CNI-TMA). These different forms of rTx-TMA require different therapy. Prior analysis showed differential expression of pro- and antithrombotic mRNA transcripts in rTMA. We hypothesized that glomeruli with rTx-TMA might have different glomerular capillary microRNA profiles that might explain the shift in mRNA transcripts contributing to microthrombosis and could also allow etiological differentiation.

Methods. Renal transplant biopsies from 19 patients with TMA (RecTMA, n=6; CNI-TMA, n=7; HR-TMA, n=6) were compared to 8 renal transplant control biopsies without any histological signs of TMA or humoral rejection. RNA was isolated from 50 laser microdissected glomeruli of paraffin-embedded biopsies. After cDNA preamplification, the expression of selected pro- and antithrombotic mRNA transcripts and microRNA profiles were examined using TaqMan real time PCR. Putative target genes were analyzed in silico. Differentially expressed RNAs were identified with Tukey tests. Correlations between miRNAs and mRNAs were determined with Spearman's test.

Results. Out of 675 examined microRNA four microRNA were differentially expressed: Glomerular miR-150 was overexpressed in both RecTMA and CNI-TMA compared to controls. Glomerular miR-222-3p and miR-223 were overexpressed in all subgroups compared to controls. Glomerular miR-513-3p was downregulated in RecTMA compared to CNI-TMA. miR-150, miR-222-3p and miR-223 correlated inversely with KLF2 and KLF4. miR-513-3p correlated inversely with ADAMTS13.

Conclusions. microRNA could cause alterations in the microvascular expression levels of pro- and antithrombotic mRNA transcripts and may contribute to microthrombosis in rTx-TMA. Particularly miR-223 may affect fibrinolysis through induction of PAI-1 and suppression of tPA via suppression of endothelial protective KLF2. microRNA transcripts identified as possibly relevant for the differential diagnosis and for the pathogenesis of rTx-TMA will be verified in a second, independent cohort and in functional studies.

SO-053

Molecular profiling in human pulmonary allografts

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Aims. Obliterative remodelling of the small airways (bronchiolitis obliterans, BO) in human pulmonary grafts is one of the main reasons for chronic allograft dysfunction and represents a largely unsolved challenge in pulmonary medicine. Transbronchial biopsies do not reproducibly detect BO because of its discontinuous and patchy nature. Up to now, no tissue based predictive markers for the onset of obliterative remodelling are available in routine clinical practice.

Methods. We analyzed the mRNA-expression of 45 tissue remodeling-associated genes using target-gene specific preamplification and TaqMan-based low-density/high throughput arrays (LDA) in transbronchial lung biopsies from lung-transplanted (LTx) patients. 186 transbronchial biopsies from patient groups with rapid or late onset of bronchiolitis obliterans syndrome (BOS) after LTx respectively were analyzed. Results were correlated with previous molecular findings from compartment-specific analyses of explanted lung allografts by laser-assisted microdissection.

Results. Low-density/high throughput (RT-PCR) array-based analyses of transbronchial biopsies yield reproducible and reliable results. We could identify characteristic expression patterns not only in remodelled airways, but also in biopsies of patients suffering from BOS that showed no tangible morphological changes. Upregulation of genes like MMP9, RANTES (CCL5), TIMP1, EDN1, BMPR2 und IL-6 correlated with the clinical onset of BOS often preceding deterioration of clinical function tests.

Conclusions. We established a distinct mRNA signature diagnosing/predicting airway remodeling in lung-transplanted patients even in morphological inconspicuous biopsies. Further prospective longitudinal molecular profiling in surveillance programs is warranted to elucidate the clinical benefits and potential use for predicting the response to therapy in lung transplant patients.

SO-054

Molecular characterization of bronchiolitis obliterans after stem cell transplantation as a manifestation of GvHD

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Aims. Obliterative remodeling of the pulmonary small airways (Bronchiolitis obliterans, BO) presumably starts as an inflammatory process initiated by epithelial damage. Consecutively myofibroblasts are activa-

ted producing a surplus of extracellular matrix and profibrotic, inflammatory cytokines. BO can present as a harmless epiphenomenon or a life-limiting disease by severely limiting lung function. This dramatic course has been best described as chronic allograft dysfunction in lung transplanted patients. A very similar clinical course and morphology can be seen as a manifestation of graft-versus-host disease (GvHD) following hematopoietic stem cell transplantation (HSCT). Adequate animal models for the elective study of BO, predictive markers for the onset of BO and a causal therapy are not established yet. The objective of the present study was to elucidate the morphological and molecular changes in BO lesions occurring after HSCT in comparison to "conventional" BO in lung allografts.

Methods. Isolation of BO lesions from formalin-fixed and paraffin-embedded (FFPE) lungs was performed by laser-assisted microdissection. mRNA expression of 45 fibrosis-associated genes was measured using cDNA preamplification and TaqMan-based low-density/high-throughput arrays. The gene expression of 14 lung explants following HSCT, 10 explanted lung allografts and 4 lung explants following radiation/chemotherapy were compared with each other. Furthermore, bronchioli from 16 downsized donor lungs were used as references.

Results. We found significant up-regulation of BMP-4, RANTES, TIMP-1, THBS1, MMP-9 and -14, BMPR2, LOX and PLAU in GvHD lungs. No significant differences regarding expression signatures, cellularity and inflammation were delimitable between transplanted and non-transplanted lungs.

Conclusions. BO in lung transplants and lung parenchyma from HSCT patients show similar morphological and molecular changes. These distinct expression patterns warrant further investigation as potential predictive markers for BO in hematologic patients following HSCT.

SO-055

Glomerular endothelial microRNA expression profiles in an in-vitro model of acute humoral rejection

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Aims. Acute humoral rejection (AHR) is mediated by alloantibodies against transplant endothelial cells with and without complement activation. This results in functional changes of endothelial cells subsumed under the ambiguous term "activation". Little is known about AHR pathophysiology and the histological diagnosis remains challenging. Therefore, identification of a diagnostically useful miRNA signature for complement-mediated and complement-independent humoral rejection is most desirable. The aim of this study was to identify diagnostic microRNA signatures of different forms of AHR that could also elucidate the "activation" of glomerular endothelial cells.

Methods. Human renal glomerular endothelial cells (HRGEC) were HLA-typed and treated with binding (HLA-A1 Ab) or non-binding (HLA-A2 Ab) antibody with and without addition of complement. Using different in vitro settings, we analysed expression of 384 microRNAs by Taqman Low densitiy arrays in a model for 1. complement-mediated AHR, 2. complement-independent AHR and 3. the additional effects of complement in AHR.

Results. Several microRNAs were differentially expressed in our in vitro model of AHR. 1) Two thirds of miRNAs in complement-mediated AHR were down-regulated. microRNAs of the miR200-cluster (miR-200a, miR-200b and miR-429) were down-regulated most strongly. Interestingly, only seven microRNAs were upregulated, five of these microRNAs were located on chromosome 19 (miR-517a, miR-517c, miR-519a, miR-525-3p and miR-125-3p). In addition, miR-124a was slightly up-regulated. 2) complement-independent AHR showed a signature of upregulated let-7a, miR-505 and miR-182 and downregulated miR-124a, miR-502 and miR-597. 3) additional effects of complement in AHR show

a signature of upregulated miR-124a and downregulated miR-200b, miR-139-5p and miR-342-3p.

Conclusions. With microRNA profiling in an in vitro model we identified a glomerular endothelial-specific microRNA signature of complement-mediated and independent forms of AHR. These miRNA signatures will be validated for the tissue-based diagnosis of complement-mediated and independent forms of AHR. Furthermore, up-regulation of microRNAs located on chromosome 19 appears remarkable and should stimulate further research.

SO-056

MicroRNA expression profile in Epstein-Barr virus-associated post-transplanted smooth muscle tumours

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Aims. Epstein-Barr virus(EBV)-associated post-transplant smooth muscle tumours (PTSMT) are rare complications after solid organ or stem cell transplantation. Aberrant microRNA expression patterns have been identified in several neoplasms and are known to contribute to the deregulated cell homeostasis in tumour cells. In this current analysis we wanted to characterize the expression profiles of several hundred microRNA in PTSMT.

Methods. Analysis of five PTSMT samples from four patients and seven EBV- benign uterine leiomyomas. A set of 365 mature microRNA and corresponding endogenous controls were analyzed by quantitative real-time PCR.

Results. Cluster analysis of the expression profile revealed that PTSMT and leiomyomas share a highly similar microRNA profile. EBV-associated microRNA (miR-10a, miR-21, miR-29b, miR-34a, miR-146a, miR-155, miR-127, miR-200b, miR-203 and miR-429) are not deregulated in EBV-infected PTSMT.

Conclusions. Expression pattern of microRNA in PTSMT is not associated with EBV infection but reflects the leiomyomatous differentiation of the tumour cells.

SO-057

Typing of renal amyloidosis in formalin-fixed paraffin-embedded (FFPE) biopsies specimens by MALDI imaging mass spectrometry

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Aims. Amyloidosis is a heterogeneous group of metabolic diseases characterized by deposition of amyloids in various tissues. The most common forms of amyloidosis are AL- and AA-amyloidosis. Current diagnosis of amyloidosis is made by histopathologic examination and Congo red-histochemistry. Further typing requires immunohistochemistry. While specificity and sensitivity of commercially available antibodies against Amyloid AA is high, antibodies against Amyloid AL show nonspecific reactivity because of conformational changes of light chain antigens in AL amyloidosis. Precise identification of the amyloid subtype is mandatory to ensure diagnosis of underlying disease and has therapeutic impact. The aim of the present study is precise diagnosis of AL-amyloidosis by matrix-assisted laser desorption/ionization (MALDI) imaging mass spectrometry (IMS).

Methods. Renal FFPE biopsy specimens with AL-amyloidosis (n=12) and AA-amyloidosis (n=10) were subjected to trypsin and matrix deposition using the ImagePrep device (Bruker, Bremen), and were subsequently analyzed by MALDI MS in imaging and profiling mode using a

Bruker Autoflex Speed. Statistical analysis was done with ClinProTools 3.0. Each analysis was performed as an independent experiment to ensure statistical independence.

Results. Eleven predictive peptide signatures for AA and 10 for AL type amyloidosis were detected. MS images were compared with optical images of the Congo-red stained serial tissue sections. Both AL and AA-type amyloidosis MS images were in good concordance with the histochemistry images demonstrating regions with amyloid deposition.

Conclusions. IMS is able to discern AL amyloidosis from AA-amyloidosis. Identification of various peaks is required to provide a reliable diagnostic test which can be applied in histopathological routine. MALDI IMS represents a rapid and sensitive application for the accurate characterization and typing of renal amyloidosis, and it is of diagnostic utility in addition to standard histochemical techniques.

SO-058

Macrophage Migration Inhibitory Factor (MIF) deficiency attenuates renal damage in experimental glomerulonephritis

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Aims. Macrophage migration inhibitory factor (MIF) is a pleiotropic, pro-inflammatory cytokine playing pathogenetic roles in sepsis, atherosclerosis and glomerulonephritis. Upon kidney injury, MIF expression is induced in renal and infiltrating cells. Antibodies directed against MIF reduced glomerulonephritis in rats, but genetic and murine studies are missing. Here we studied the effects of genetic deletion of the MIF gene (Mif-/-) in mice with nephrotoxic nephritis (NTN).

Methods. NTN was induced by a combined i.p. injection of sheep anti-mouse glomeruli serum and mouse TLR9 ligand CpG ODN1668 in Mif-/- mice and wild type littermates (WT). All animals were sacrificed on day 14 and renal function and pathology were examined. Regulation of MIF secretion was examined in glomerular cells in vitro.

Results. On day 14, compared to WT, Mif-/- mice had significantly reduced proteinuria, serum creatinine and blood urea nitrogen. Histology revealed marked reduction in crescents, reduced glomerular fibrinogen deposition and a reduced activation of mesangial cells in Mif-/- mice. Inflammatory cells mainly infiltrated the tubulointerstitial space or periglomerular areas. This was significantly reduced in Mif-/- mice compared to wildtype littermates. Correspondingly, tubulointerstitial injury was significantly decreased in the Mif-/- mice. Binding of sheep IgG and mouse IgG was mostly restricted to the glomerular basement membrane and was not different between both groups, suggesting that MIF deficiency had no effect on disease induction. In vitro incubation of glomerular endothelial cells, mesangial cells and podocytes and to lesser extent also parietal epithelial cells with NTN serum induced MIF secretion.

Conclusions. In conclusion, MIF deficiency ameliorated the development of glomerulonephritis in mice. These data suggest MIF as a therapeutic target in inflammatory renal disease.

SO-059

Lipid droplet accumulation is associated with an increase in hyperglycemia induced renal damage. Prevention by LXR

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Aims. Abnormal lipid metabolism and renal accumulation of lipids play a role in the pathogenesis of diabetic nephropathy. Liver X receptors (LXR), members of the nuclear receptor superfamily, regulate gene expression linked to lipid and carbohydrate homeostasis, inhibit inflammatory gene expression in macrophages and attenuate the development of atherosclerosis in animal models; this ability of LXRs makes them potential targets for intervention in diabetic nephropathy. In the present study we investigated the effects of LXR activation on the hyperglycemia and hyperlipidemia induced renal disease.

Methods. Kidneys from WT, LDLR-deficient and mice with macrophage overexpression of LXR α with and without LDLR-deficient background were examined after 20 weeks of experiments. Diabetes was induced by administration of streptozotozin and mice were randomly assigned to either standard chow or Western diet. In addition the effects of LXR activation by the synthetic agonist GW3965 (GW; 20 mg/kg/day) on the development of renal changes were evaluated by histological, immunhistological and functional analyses.

Results. In LDLR-deficient hyperlipidemic and streptozotozin-induced diabetic mice hyperglycemia and hyperlipidemia reciprocally accentuated renal injury and altering renal function. In hyperglycemic-hyperlipidemic kidneys the accumulation of Tip47-positive lipid droplets in glomeruli, tubular epithelia and macrophages was accompanied by the concomitant presence of the oxidative stress markers xanthine oxidoreductase (XOR) and nitrotyrosine, findings which could be evidenced also in renal biopsies of diabetic patients. LXR stimulation by GW3965 upregulated genes involved in cholesterol efflux, downregulated proinflammatory/profibrotic cytokines (e.g.TNF-α, TGF-β) inhibiting the pathomorphology of diabetic nephropathy, renal lipid accumulation and improving renal function. XOR and nitrotyrosine were reduced. In macrophages GW3965 or LXR α-overexpression significantly suppressed glycated or acetylated-LDL induced cytokines and reactive oxygen species. Specifically, in mice transgenic expression of LXR α in macrophages was able to significantly ameliorate hyperlipidemic-hyperglycemic nephropathy.

Conclusions. The results demonstrate the presence of lipid droplet induced oxidative mechanisms and the pathophysiologic role of macrophages in diabetic kidneys and indicate the potent regulatory role of LXRs in preventing renal damage in diabetes.

SO-060

A new therapeutic approach to Fabry disease: substrate reduction therapy through Gb3-depletion

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Aims. Fabry disease is an inherited lysosomal storage disease characterized by deficiency in α-galactosidase A (αGalA), which is responsible for degradation of glycosphingolipids and in particular of globotrihexosylceramide (Gb3). As a consequence of the storage, lysosomal dysfunction develops in different organs. Until today, the only available therapy is a life-long administration of the recombinant αGalA which is connected to considerable costs and loses its efficacy due to production of neutralizing antibodies in part of the patients. The aim of this study was to explore an alternative therapeutic approach for Fabry patients by reducing the amount of the stored glycosphingolipids by substrate reduction.

Methods. For the study a murine model of Fabry disease (αGalA-/-) was used. Reduction of the stored Gb3 was achieved by crossing these mice with mice deficient for Gb3-synthase (Gb3S-/-) generated by our group. The organs of αGalA-/-, Gb3S-/- and of αGalA-/-/Gb3S-/- double-knockout mice were analyzed by thin layer chromatography, immune overlay technique and conventional as well as electron microscopy.

Results. In Gb3S-/- mice, thin layer chromatography of kidney extracts, which are rich in globosides in WT mice, showed absence of Gb3 thereby demonstrating the successful targeted inactivation of the Gb3S gene. Gb3-deficient mice exhibited no overt developmental or behavioral defects; their body and organ weights were normal. Histological examination of bone marrow, thymus, lymph nodes, spleen, brain, eye, heart,

lung, intestines, liver, pancreas, and kidney did not reveal differences between Gb3S-/- and WT mice. Thin layer chromatography of organs of αGalA-/- mice showed accumulation of glycosphingolipids and Gb3 was identified as the major accumulating glycosphingolipid. Electron microscopy demonstrated an abnormal lysosomal morphology in the αGalA-/- mice. In the αGalA-/-/Gb3S-/- double-knockout mice, the storage was abolished. Moreover in these mice, the lysosomal morphology returned to normal in the absence of Gb3-synthesis.

Conclusions. In Fabry mice, Gb3 is the major accumulating glycosphingolipid and the storage phenotype of these mice can be fully reverted by interference with Gb3-synthesis. Therefore a pharmacological inhibition of the Gb3-synthase represents a novel therapeutic option for the treatment of Fabry disease.

SO-061
Glomerular mRNA expression of PLA2R in primary and secondary membranous glomerulonephritis

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Aims. Recently, the main target antigen in primary membranous glomerulonephritis (MGN) was identified as the M-type phospholipase A2 receptor (PLA2R). The subepithelial immune deposits in primary membranous glomerulonephritis have been reported to be PLA2R-positive on immunohistochemical evaluation. Comparison of glomerular PLA2R mRNA expression levels between primary and secondary membranous glomerulonephritis could be diagnostically useful to distinguish between primary and secondary MGN.

Methods. Glomeruli from three cases each of primary and secondary MGN were isolated from paraffin embedded tissue and subjected to mRNA quantification with RT PCR after preamplification.

Results. Relative glomerular PLA2R mRNA expression was higher in secondary (208±307) than in primary (15±4) MGN.

Conclusions. The decreased glomerular mRNA expression of PLA2R in primary membranous GN could represent protective downregulation, limiting immune complex formation with PLA2R antibodies and could be used to distinguish between primary and secondary MGN. These mRNA expression levels will be compared to normal glomeruli and validated in a larger cohort.

AG Dermatopathologie

SO-062
Reliability of pyrosequencing for detection of rare BRAF mutations in melanoma patients

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Aims. The detection of V600E BRAF mutations has fundamental clinical consequences since the treatment option with BRAF inhibitors such as vemurafenib or dabrafenib yields response rates of approximately 50%. Since clinical responses have been seen also in patients with non-V600E BRAF mutations, it is essential to acquire knowledge about these rare mutations and to define reliable methods to detect them. We aimed to validate the pyrosequencing method in comparison to other screening techniques regarding their reliability to detect rare non-V600E mutations.

Methods. DNA was prepared from formalin-fixed paraffin embedded sections of melanoma tissues. A new BRAF assay was designed to determine BRAF mutations by pyrosequencing. Rare mutations were confirmed by capillary sequencing and compared to findings by COBAS test and immunohistochemistry using a novel BRAF antibody. Clinical data with respect to melanoma type, tumor site, and survival were summarized for patients with rare mutations.

Results. Among our study population, a total of 14 patients exhibited rare BRAF mutations. The V600EK601del and V600DK601del mutations (1 case each) have not been described before. Furthermore, V600K (6 cases), V600E2 (GAA; 2 cases), and V600D, V600G, V600R, and L597S mutations (one case each) were detected. Mutations were not found by COBAS test in 7 out of 11 patients. Data were correlated with immunohistochemical findings.

Conclusions. Accurate diagnosis of rare mutations is crucial since vemurafenib is approved for treatment of melanoma with V600 mutant BRAF and not limited to V600E mutations. Patients with rare BRAF V600 mutations could respond to treatment with BRAF inhibitors. We show that pyrosequencing is a reliable and time-saving alternative to detect rare BRAF mutations.

SO-063
Application of pyrosequencing for mutation detection of GNAQ and GNA11 mutations in uveal melanoma

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Aims. Uveal melanoma is the most common and most aggressive intraocular tumor in adults. Recently activating mutations in GNA11 and GNAQ genes have been identified in the majority of this malignancy. The heterogeneity pattern of such mutations in primary and metastatic tumors as well as tumor-derived cell lines is not well investigated. Our study aimed to examine the GNAQ/GNA11 and BRAF mutation status in 18 samples of 15 patients with uveal melanomas using the pyrosequencing methodology.

Methods. DNA was prepared from formalin-fixed in paraffin embedded tissues of two primary tumors and 16 metastases along with two established uveal melanoma cell lines using the NucleoSpin®Tissue kit (Macherey-Nagel, Düren). PCR primers and sequencing primers for the two hot spots in GNAQ and GNA11 (codon 209 and 183) and for the V600E BRAF mutation were designed to be used on the pyrosequencing system PyroMark Q24 (Qiagen, Hilden). Pyrograms were analyzed with the PyroMark Q24 (Qiagen) software. All sequences were confirmed by conventional Sanger sequencing.

Results. We identified 13 mutations in 18 samples (72.2%). In GNAQ we discovered two mutations (R183Q, 11.1%) and in GNA11 we detected 11 mutations (Q209L, 61.1%). In total, 10 of 15 patients carried one of the analyzed mutations. We found the same GNA11 (Q209L) mutation in the two primary tumors, their corresponding metastases, and the tumor-derived cell lines. For the first time we identified one GNA11 Q209L as a substitution of CAG by CTC. BRAF mutations were not detectable. All results could be verified by Sanger sequencing.

Conclusions. Our data support the theory of a mutually exclusive nature of GNAQ and GNA11 mutations and show a consistency of the mutation status between primary and metastatic tumors. Pyrosequencing is a reliable and time-saving method to detect GNA mutations in uveal melanomas.

SO-064
Recurrent BRAF V600K mutations in spindle cell melanoma

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Aims. Spindle cell (SCM) and desmoplastic melanoma (DM) are clinically, histologically and prognostically distinct melanoma subsets. Several studies included individual cases and some series report the presence and frequencies of BRAF mutations in these rare subsets of melanoma. However, BRAF mutation frequencies in SCM and DM have not been compared. Here, we assessed the BRAF mutation frequencies in two independent cohorts of SCM and DM.
Methods. Computer-based archival searches for SCM and DM were performed at both institutions from Ulm and Columbia University. Cases were included when formalin-fixed paraffin-embedded tumor tissue was available for microdissection/DNA-extraction of tumor regions and subsequent PCR/pyrosequencing of the BRAF V600 codon. For validation, we applied Sanger sequencing. Besides comparison of BRAF mutation frequencies between histotypes and cohorts, we compared to reported frequencies derived from COSMIC- and medline queries.
Results. The Ulm cohort showed a total of three V600K mutations out of 18 samples (2/9 SCM, 1/9 DM) whereas the NYC cohort demonstrated only one V600E mutation (1/7 SCM, 0/11 DM, 0/6 SCM/DM). All detected BRAF mutations were heterozygous by pyro- as well as Sanger sequencing. Although mutation frequencies by histological subtype differed between the cohorts, we interpreted this discrepancy as a side effect of small case numbers resulting from highly resolved histological sub-stratifications. Nonetheless, the high frequency of V600K in our series (16.7% of Ulm or 7.1% of all tested cases) was not anticipated and significantly higher when compared to all annotated V600K mutations in the COSMIC database (status Oct 2012; n=118 V600K-mutated melanoma of all 9871 melanoma entries ~1.2%; p=0.016, Ulm and p=0.014 Ulm+NYC; both p values from Fisher's exact test). These findings suggest that in contrast to all melanomas, BRAF V600K mutations are more frequent in SCM/DM.
Conclusions. To our knowledge, this is the first report describing recurrent BRAF V600K mutations in SCM. In conventional melanoma, BRAF V600K mutations are associated with a more aggressive clinical course, shorter overall survival and decreased efficacy of BRAF inhibitors (e.g. vemurafenib). Thus, recurrent BRAF V600K mutations in SCM are of significant clinical and potentially therapeutic relevance.

SO-065
Comprehensive biomarker assay in spindle cell- and desmoplastic melanoma reveals a diagnostic signature and potentially targetable subsets

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Aims. Spindle cell melanoma (SCM) and desmoplastic melanoma (DM) differ clinically in terms of prognostic and therapeutic implications; however, due to partially overlapping histopathological features, diagnostic distinction is not always straightforward. Although numerous studies have assessed individual markers in each subtype, a direct comprehensive characterization of diagnostically and therapeutically relevant biomarkers has not been performed. Here we present an array-based analysis of 44 biomarkers in two independent case-cohorts.
Methods. The archives of both institutions were searched for SCM and DM. Tissue-microarrays were generated and examined using a literature-based set of 44 commonly available biomarkers. These biomarkers included at least 16 potentially drugable targets and were assessed by either fluorescence in situ hybridization, immunohistochemistry or special stains.
Results. A total of 42 cases were assessed. These were composed of 16 SCM, 20 DM and 6 SCM/DM in two subsets: Ulm (9DM, 9SM) and NYC (7DM, 11SM, 6SCM/DM) were assessed. Intraclass correlation derived a 12 component biomarker signature (named CBS: 7 significant, 5 distinct). Five markers were positive in DM (COL-IV; CD68; p75; Trichrome; CNL2p) and 7 biomarkers were present in SM (LAM; MelanA/MART1; HMB45; KIT; MDM2, p53; CNG8q24). Cross-validation experiments (Ulm/NYC) the most reliable markers were Melan-A/MART-1 and HMB45 (DM–; SM+). Marker analysis of the 16 potentially targetable molecular aberrations revealed (with one exception, a MET+KIT-positive) mutually exclusive labeling for CKIT, HER2, EGFR, MET, and ALK in an aggregate of 16 of 42 cases (38%).
Conclusions. To our knowledge, this is the first comprehensive screening study that compares SCM vs. DM based on the expression profile and gene/CEP-status of common tissue-based diagnostic biomarkers. When histopathological distinction is not definitive, the presented staining algorithm may be helpful for diagnostic classification as well as identification of drugable targets in these rare subsets of melanoma.

SO-068
Proximal-type epithelioid sarcoma: a study of two cases

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Aims. Proximal-type epithelioid sarcoma (PES) is a rare aggressive neoplasm that features undifferentiated epithelioid cells with rhabdoid features and strong immunoreactivity for pankeratin and vimentin. PES differs from classical epithelioid sarcoma by its morphology and more proximal localization but it shares with classical epithelioid sarcomas the immunophenotype including loss of nuclear INI-1 expression.
Methods. The clinicopathological and immunohistochemical features of two cases of PES including extended follow-up are presented.
Results. The tumors originated in the mons pubis and the trunk near the iliac spine in two young women (21 yrs and 35 yrs). The pubic tumor recurred twice locally at 6 and 9 months post-operatively and was treated by radical re-excisions and radiotherapy. She is currently disease-free (40 months after last recurrence). The second case experienced rapid wide-spread metastasis and died of progressive disease 2 yrs later. Histologically, both tumors displayed rhabdoid morphology with frequent bi/multinucleation. One tumor showed alveolar growth pattern and contained numerous osteoclast-like giant cells, thus mimicking clear cell sarcoma. Immunohistochemistry revealed strong paranuclear expression of pankeratin and vimentin and complete loss of nuclear INI-1. Electron microscopy in one case displayed complex paranuclear lamellar inclusions of intermediate filaments.
Conclusions. Awareness of this rare presentation of PES is mandatory to avoid misinterpretation as metastatic carcinoma of unknown primary (CUP) or (in case of alveolar pattern) as clear cell sarcoma.

SO-069

FHL2 plays an important role in migratory capacity and keratinisation of outer root sheath cells in hair

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Aims. TGF-β1 stimulation may translocate Four-and-a-half LIM domain protein 2 (FHL2), a component of the focal adhesion structures, to the nucleus, where it serves as transcriptional cofactor. The present study analysed the role of FHL2 in hair sheath.

Methods. Paraffin-embedded human skin and dorsal skin of FHL2-deficient mice were analyzed for expression patterns of TGF-β1, FHL2 and estrogen receptor alpha. To analyse the functional role of FHL2 expression in ORS cells in vitro studies were performed using human keratinocytes (HaCaT), which show large similarity with human ORS cells.

Results. In human anagen hair, nuclear FHL2 was co-expressed with TGF-β1 in distal outer root sheath (ORS) keratinocytes, which showed dissociated cell arrangement and low proliferation. TGF-β1 treatment of cultured keratinocytes increased FHL2 expression in focal adhesions and in the nucleus, increased migratory capacity, enhanced "basal" (cytokeratin 14+) keratinisation, induced dissociated cell layering and suppressed proliferation. FHL2 knockdown of TGF-β1-stimulated keratinocytes reduced migratory capacity and keratinisation. In mouse model, female FHL2 KO mice retained telogen hair phase until postnatal day 49. In contrast, male FHL2 KO mice showed regular telogen-anagen transition on postnatal day 22.

Conclusions. In conclusion, TGF-β1 induces nuclear translocation of FHL2 in distal ORS keratinocytes of human anagen hair. In vitro data of the present study indicate that FHL2 enhances migratory capacity and stabilises keratinisation of these bulge progeny. Female FHL2 knock out mice do not initialise anagen until postnatal day 49, probably due to deficient migratory capacity and keratinisation of cells forming the secondary hair germ, which shows nuclear FHL2 expression in WT mice. The telogen arrest in female mice might be explained on the basis of an interaction of nuclear cofactor FHL2 and estrogen receptor alpha, which was co-expressed with FHL2 and TGF-β1 in ORS keratinocytes.

SO-070

Does P16 immunostaining correlate with the presence of Merkel cell polyomavirus in Merkel cell carcinomas?

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Aims. P16 expression is a well known surrogate marker for high risk human papillomaviruses (hrHPV) in anogenital and oropharyngeal carcinomas. The Merkel cell polyoma virus (MCV) associated with ca. 80% of Merkel cell carcinomas (MCC) shares many similarities with hrHPV. The DNA of both virus families is composed of double stranded circular DNA, their genome size is small with ca. 5600 bp (MCV) and ca. 8000 bp (hrHPV). Importantly, there exists also the homological LxCxE motive in the encoded Rb binding site. In hrHPV infection the Rb protein is blocked which results in cell cycle progression. As a consequence, due to this oncogenic stress, p16 expression is upregulated. Here we investigated if p16 overexpression correlated with the presence of MCV in MCC tissues.

Methods. We performed immunohistochemical p16 staining on formalin-fixed, paraffin- embedded (FFPE) MCC cases (n=65) on both tissue microarrays (TMA) and whole tissue sections. The immunohistochemical stainings were evaluated in intensity and percentage of stained cells by three investigators (cut off for positivity: 2+/3+ and ca. 60% cells stained). The p16 expression in the MCC cases on the TMAs (n=45) were correlated with available MCV FISH and PCR data. The p16 expression in the MCC cases on whole tissue sections (n=20) were correlated to immunohistochemical staining of the MCV large T Antigen (LTag) indicating virus presence and the MCC marker cytokeratin 20 (CK20).

Results. Immunohistochemistry on TMAs revealed 89% (40/45) positive p16 cases. P16 positivity was not correlated with MCV PCR- (71%; n=32/45) or FISH-positivity (84%; n=38/45). Next, we investigated MCC whole tissue sections (n=20) by immunohistochemistry staining of p16, MCV LTag and CK20. P16 positivity was found in 95% (19/20) of MCC, whereas 85% (17/20) of the cases were positive for MCV presence by LTag expression and 95% (19/20) for CK20. No significant correlation was found for MCV presence and p16 expression. Overall, p16 was detected in 89% (58/65) of the MCC cases.

Conclusions. In conclusion, our data show that 89% of MCC are p16-positive, and that most (45) MCV-positive tumors are also p16-positive, in line with the situation found in anogenital and oropharyngeal cancers for hrHPV and p16. However, no significant correlation between MCV presence and p16 positivity was found, due to additional p16-positive MCC with no MCV present. P16 expression thus rather may serve as an additional tool in MCC diagnostic, or may point to other viruses yet to be identified.

Hungarian-German cooperation meeting

SO-071

Expression of tight junction proteins in human tumors

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Aims. Epithelial cells are attached to each other by intercellular junctional complexes, as tight junctions (TJs) composed of transmembrane (claudins, occludin, tricellulin, etc.) and cytoplasmic proteins. Altered expression of TJ proteins is detected in many diseases and the changes are cell type and tissue specific. During tumor development/progression, altered expression of TJ proteins is associated with grade, stage, type of tumors. In many tumors however expression of certain claudins increases. Due to the high specificity of the changes the possible use of claudins as molecular markers in cancer diagnostics was raised.

Methods. Formalin fixed paraffin embedded and frozen material from human tumors were studied by immunohistochemistry/immunofluorescence. Primary antibodies were used for detection of TJ protein (claudins, occludin, tricellulin). Results were evaluated semiquantitatively or by digital slide scanners and analysed statistically. mRNA was detected by RT-PCR reaction.

Results. Claudins, tricellulin, occludin were visualized as membrane-bound linear reactions or dots by immunohistochemistry and morphometry results were parallel with mRNA expression in most cases. Generally TJ protein expressions decreased in tumor cells in correlation with progression of the tumor. There were however exceptions, with detection of increased expression of certain TJ proteins. Claudin1 was increased in cervical/esophageal squamous carcinoma, seropapillary endometrial cancer, colorectal/pancreatic adenocarcinomas, etc. Claudin4 was increased in cholangiocarcinoma, ovarian, pancreatic/prostate carcinoma, etc. Overall survival was associated with claudin4 expression in prostate cancer and tricellulin expression in pancreatic ductal carcinoma. Distinct types of tumors of certain organs could be differentiated based on claudin pattern as pancreatic endocrine/exocri-

ne tumors, fetal/embryonal components of hepatoblastomas, histologic subtypes of lung cancer, metastatic liver cancer. Claudins seem to differentiate low- and high grade transitional cell carcinomas of urinary bladder.

Conclusions. TJ proteins are expressed in cell- and tissue-type specific way in tumors following the expression pattern of tissue and cell type of origin. Generally expression of TJ proteins decreases during carcinogenesis, there are exceptions however, since certain members of the claudin family increase even in early stages of carcinogenesis, giving potential for their use as biomarkers or target molecules for therapy of tumors.

SO-072

Adipophilin immunostain visualizes lipid droplet-accumulation following hypoxia and may therefore help in the diagnosis of organ infarcts

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Aims. Lipid droplets (LDs) are dynamic storage compartments for energy-rich fats nearly ubiquitously present in eukaryotic cells. LDs exert tissue-specific functions in metabolically active cell types and are increased in conditions following lipid overload or cellular damage as e.g. in hypoxia. The LD/cytoplasm interface is stabilized and decisively regulated by amphiphilic proteins of the PAT/perilipin-family with its main constituents perilipin (perilipin-1), adipophilin (perilipin-2), TIP47 (perilipin-3) and MLDP (perilipin-5).

Methods. We evaluated LD-associated proteins in cell culture systems of experimental hypoxia as well as the value of adipophilin immunohistochemistry for the diagnosis of organ infarcts.

Results. In experimental hypoxia, induced by dimethyloxaloylglycine (DMOG) in cultured cells of the line HuH7 or directly by incubation in a hypoxic chamber, hypoxia-inducible factor 1 alpha (HIF-1 alpha) was already detectable after a few hours, and small LDs coated by adipophilin started to increase after 1 to 2 days as measured by immunofluorescence microscopy and immunoblot. Perilipin was not significantly detected. In formalin-fixed, paraffin-embedded human tissues, adipophilin immunostain marked microvesicular LDs as e.g. in hepatocytes of liver specimens with ischemia-reperfusion injury following liver transplantation not detectable by routine H&E light microscopy. Concerning organ infarcts, adipophilin marked increased amounts of larger LDs in the vital border zone of subacute myocardial infarctions (n=14), kidney (n=11) as well as liver infarcts (n=8) as compared with respective normal cell types. Areas of subacute infarcts were generally better visible with adipophilin immunostain when compared to routine H&E. In colon ischemia (n=11), adipophilin-positive LDs accumulated at the basal side of colon epithelia, whereas normal epithelia were not stained or showed only very few dot-like LDs. In brain (n=6), adipophilin stained especially macrophages and microglia in grade II cerebral infarcts, whereas grade I infarcts as well as normal brain were virtually not stained.

Conclusions. Adipophilin visualizes small LDs in experimental hypoxia in cell culture as well as in the vital border zone of subacute organ infarcts. Immunohistology for adipophilin may thereby facilitate the histomorphologic diagnosis of subacute organ infarcts.

SO-073

Proteoglycans in the development and progression of liver cancer

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Aims. Our studies on the glycosaminoglycan pattern of hepatocellular carcinomas revealed profound alterations of these components in liver carcinomas. The aims of our ongoing investigations are to uncover the consequences related to the altered glycosaminoglycan and proteoglycan expression.

Methods. Human hepatocellular carcinoma specimens were studied to assess the alteration in glycosaminoglycan composition. Subsequently, sulfation of heparan sulfate (HS), changes in the expression of HS synthetic enzymes, syndecan-1, decorin and agrin content and distribution of tumorous livers were determined. The effect of heparan sulfate on cell nuclear function was studied by gel shift and reporter gene assays. Stable syndecan-1 transfectant hepatoma cells were used to study the action of syndecan-1. Thioaetamide-induced hepatocarcinogenesis models were applied on decorin-/- mice.

Results. The major glycosaminoglycan of the normal liver is heparan sulfate, which increases about ten-fold, whereas chondroitin sulfate (CS) increases around a hundred-fold in liver cancers. Heparan sulfate chains originated from cancer are longer in size and they are moderately undersulfated. As they can enter the cell nucleus, they interfere with the regulation of transcription by inhibiting topoisomerase I and II, as well as binding transcription factors to DNA. Not only HS chains, but HS proteoglycans can be detected in the nucleus. Transfection experiments with full-length and truncated syndecan-1 lacking the extracellular domain resulted in differentiation of HepG2 and Hep3B cell lines by downregulating the Ets-1 transcription factor. Decorin, a CS/DS proteoglycan protects against hepatocarcinogenesis by interacting with PDGF and inhibiting downstream signaling of its receptor.

Conclusions. Normal composition and structure of liver proteoglycans is indispensable for the homeostasis of liver function. Their sugar chains and also their protein core participate in signal transduction and regulation of transcription. Therefore, their structural alterations facilitate the development of regulatory disorders characteristic for cancer.

SO-074

MicroRNA expression profile in surgically resected or Sorafenib treated HCCs

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Aims. MicroRNAs (miR) are negative post-transcriptional regulators of gene expression. Malignancies bear with characteristically altered miR profile. In vitro data suggest that miR-122 expression of HCC cells sensitizes them to Sorafenib treatment. We aimed to explore unique pretreatment miR expression pattern of HCCs showing slow and fast progression under Sorafenib treatment and of HCCs which underwent surgical resection and were not treated with Sorafenib. We correlated the obtained results with survival.

Methods. 15 unresectable HCC patients were diagnosed by fine needle aspiration biopsy (FNAB) and were consequently treated with Sorafenib until progression. 20 HCC patients were treated with surgical resection (SR) only. Sorafenib treated patients were divided into slow (treated more than 9 months) and fast (treated less than 9 months) progression subgroups. Expressions of miR-17-5p, miR-18a, miR-21, miR-34a, miR-122, miR-195, miR-210, miR-214, miR-221, miR-222, miR-223, miR-224, miR-140, miR-328 and U6 were analyzed (ABI Taqman MicroRNA re-

verse transcription kit) following isolation of total RNA (RNeasy FFPE kit, Qiagen). NormFinder software was used to select proper reference miR (mir-195).

Results. The range of expression of investigated miRs was similar in FNABs and SRs. FNAB samples revealed significantly higher expression of miR-18a, while lower expressions of miR-34a, miR-122, miR-214, miR-222 were found in comparison to SR. Interestingly, Sorafenib treated patients with low miR-223 expression showed slow progression (p=0.013) and higher overall survival (p=0.051). Survival of surgically resected HCCs, however, did not reveal association with miR-223 expression.

Conclusions. Our results suggest that pretreatment mir-122 expression does not predict sensitivity to Sorafenib treatment, while low miR-223 expression of preoperative FNAB samples was associated with slow progression. Overall survival of Sorafenib treated patients with low pretreatment miR-223 expression is also significantly longer in comparison to those with high pretreatment miR-223 expression, therefore, low level of pretreatment miR-223 expression might predict the success of Sorafenib treatment.

SO-075
Loss of chromosome 18 in neuroendocrine tumors of the midgut

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Aims. The genetic alterations in neuroendocrine tumors (NET) of the midgut, in particular of the appendix, are poorly characterized. The most frequent chromosomal aberration in ileal NET is the loss of one chromosome 18. The relevance of this alteration is unclear. In this study we investigated the status of chromosome 18 and the expression of chromosome 18 related tumor suppressor proteins in appendical NET in comparison to ileal NET, in order to identify factors that could explain the different clinical behavior of appendical versus ileal NET.

Methods. The loss of chromosome 18 was examined using fluorescence in situ hybridization in 34 primary appendical and 77 primary ileal NETs. The expression of tumor suppressor proteins Smad2, Smad4, Maspin and DCC was assessed by immunohistochemistry. Tumor suppressors were also stained in normal endocrine cells by immunofluorescence double labelling.

Results. Chromosome 18 was lost in 1 of 34 appendical NET (3%) in contrast to 43 of 68 ileal NET (63%). 3 cases of appendical and 5 cases of ileal NET showed mosaicism for chromosome 18. Smad2 and DCC were expressed in all ileal and appendical NET, while Smad4 protein was absent in two cases of appendical and ileal NET. Maspin was not expressed.

Conclusions. Loss of chromosome 18 in ileal NET is probably related to tumor initiation and to the more aggressive behavior in comparison to appendical NET. The loss of chromosome 18 seems to play no role in tumor progression. Smad2, Smad4, DCC and Maspin seem to play no significant role in midgut NET tumorigenesis. Thus additional chromosome 18 associated tumor-related factors have to be explored.

SO-076
Comparative expression analysis of chromosome 18 related miR-NAs in ileal NETs with and without Chr18 loss

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Aims. MicroRNAs (miRs) play important roles in many kinds of biological processes, including acting as oncogenes and/or tumor suppressors. Because the (partial) loss of one chromosome 18 (Chr18) is a frequent event in ileal NETs (iNET) we assessed the potential deregulation of miR-expression in these tumors. We investigated the expression of 15 miRs localized on Chr18, in iNETs with and w/o Chr18 loss. For a robust quantification of miRs in NETs, we wanted to identify the best endogenous control for expression studies in iNETs and pancreatic NETs (pNET).

Methods. MiR expression in FFPE iNETs (10 with, 10 w/o Chr18 loss) was analyzed by real-time PCR. To identify the best normalizer, 9 small RNAs were tested in 10 iNETs. The stability of expression of the best three RNAs was further confirmed in 10 pNETs. Data analysis was obtained using the Δ Cp comparative Cp method, significance values were determined by Mann-Whitney test.

Results. No significant difference of expression between the cohorts concerning the 15 investigated miRs was observed. Regarding the potential control RNAs, SN61 and SN96a (fold changes of 0.41 and 0.05, respectively) showed the most stable expression throughout the i/pNET samples.

Conclusions. The role of Chr18 losses in iNETs is not yet understood. Our previous studies showed no loss of Chr18 related tumor suppressors. We extended this observation to Chr18 related miRs, but the significance of Chr18 loss in iNET remains enigmatic. The investigation of endogenous control RNAs points out the importance of the proper tissue specific normalization in miR-expression studies.

Postersession Gastroenteropathologie (oberer GI-Trakt)

FR-052
Expression and clinical significance of Notch 1 and Notch 2 in human adenocarcinomas of the oesophagus

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Aims. The identification of novel prognostic indicators is of greatest interest for the management of esophageal adenocarcinomas. The aim of our study was to investigate the prognostic relevance of Notch 1 and Notch 2 expression in these tumors.

Methods. 100 primarily resected tumors were analysed. Cytoplasmic and nuclear expression of Notch 1 and Notch 2 were determined by immunohistochemistry using tissue microarrays. Expression patterns were correlated with pathological features (pT, pN, G) and overall survival (OS) of the patients.

Results. Moderate/strong cytoplasmic Notch 1 and Notch 2 expression was detected in about half of the cases (48.9% and 50%, respectively). Negative/low Notch 1 expression was associated with lower tumor differentiation grade (p=0.039), but no association with OS of the patients was found. Regarding Notch 2, negative/low cytoplasmatic expression

was associated with lymphatic tumor spread (p=0.004), lower tumor differentiation grade (p=0.002) and worse OS of the patients in univariate (p<0.001) and multivariate analysis (p=0.052). In addition, a high prognostic relevance was found for cytoplasmic Notch 2 expression in the group of completely resected patients (n=83) in both, univariate (p=0.002) and multivariate analysis (p=0.014). Nuclear expression was only observed for Notch 2 in 17% of the tumors and was associated with lower tumor stage (p=0.001).

Conclusions. Notch 2 expression is an independent prognostic factor in adenocarcinoma of the esophagus. Our results further suggest an important pathophysiological role of Notch signalling for this tumor type and they point to a prominent role of the Notch 2 receptor in the pathway.

FR-053
The impact of tumor budding in oesophageal adenocarcinomas

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Aims. Tumor budding has prognostic significance in many cancers and is defined as the presence of detached isolated single cells or small cell clusters (up to 5 isolated cells) scattered in the stroma. Tumor budding can be observed at the peripheral invasion front (peritumoral budding, PTB) and/or within the tumor (intratumoral budding, ITB). For esophageal adenocarcinomas there are currently only scarce data about the impact of this findings, which indicate an adverse prognostic impact of high degree of tumor budding. Moreover, there is no standardized approach for the assessment of tumor budding in general. In the present study we investigated PTB and ITB in a well characterized collective of primary resected esophageal adenocarcinomas, using a scoring method which recently was successfully applied on colorectal carcinoma.

Methods. A retrospective study was carried out whole tissue sections of 86 resection specimens. Tumor buds were highlighted by pancytokeratin staining. PTB and ITB were scored across 10 high-power fields (HPF) by two independent observers. Results were correlated with clinicopathological and follow-up data.

Results. Interobserver agreement was excellent (p<0.001, intraclass correlation coefficient=0.8 for PTB and 0.78 for ITB). The median count of tumor buds HPF was 126/10 HPF for PTB (range 7–593) and 78/10 HPF for ITB (range 2–656). PTB and ITB correlated significantly with each other (r=0.9; p<0.001). High PTB/ITB was associated with advanced tumor stages (p=0.04/0.005), higher rate of R1 resection (0.003/0.01), lower tumor differentiation (grading; p=0.014/0.007) and non-intestinal/diffuse tumor type according to Lauren (p>0.001/0.005). Survival analysis showed only a trend to worse survival for high grade budding (both PTB and ITB; p=0.15/0.13).

Conclusions. Peripheral tumor budding and intratumoral budding can be observed in esophageal adenocarcinomas in various degrees. Our results indicate an association with aggressive tumor behavior. However, the prognostic role of tumor budding in esophageal adenocarcinoma remains unclear and warrants further investigation.

FR-054
Predictive potential of miR-192 in the therapy response of advanced esophageal cancer

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Aims. Multimodality therapy options have been widely promoted in the treatment of locally advanced esophageal cancer. Mostly neoadjuvant chemotherapy and combined radiochemotherapy are frequently used in this therapeutic setting. In order to identify possible predictive markers for the therapeutic outcome, this study aimed to characterize miRNA profiles in pre- and post-therapeutic biopsies of responsive and non-responsive patients.

Methods. Experimental design: Initially a microarray-based approach was performed including eight patients with esophageal cancer. Patients received neoadjuvant chemoradiation followed by surgical resection. Major histopathological response was defined if resected specimens contained less than 10% vital tumor cells (major/minor response: 4/4 patients). Intratumoral RNA was isolated from both, pre-therapeutic tissue biopsies in addition to corresponding surgical specimens. The profile of 768 miRNAs was analyzed in 16 specimens (pre- and post-neoadjuvant therapy). Selected miRNAs were then on pre- and post-therapeutic biopsies of 80 patients with esophageal cancer, who have undergone multimodality therapy (major/minor response: 30/50 patients).

Results. Comprehensive miRNA profiling identified miRNAs in pre-therapeutic biopsies that were significantly different between major/minor responders. Based on the microarray results, miR-192, miR-194, and miR-622 were selected and the dysregulated miRNAs were studied on an extended series of esophageal cancer patients. The expression of miR-192, miR-194, and miR-622 was significantly reduced after neoadjuvant therapy confirming the array profiling data. Importantly, the pre-therapeutic intratumoral expression of miR-192 and miR-194 was significantly associated with the histopathologic response of esophageal squamous cell carcinoma to multimodal therapeutic treatments.

Conclusions. In patients with locally advanced squamous cell carcinoma of the esophagus undergoing neoadjuvant chemoradiation and esophagectomy, miR-192 and miR-194 in pre-therapeutic biopsies are considered as indicators of major histopathologic regression.

FR-055
Epigenetic therapy of esophageal cancer cells by HDAC inhibitors and azacytidine

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Aims. Next to genetic alterations, also epigenetic alterations influence carcinogenesis and progression of esophageal squamous-cell carcinomas (ESCC) and Barrett's adenocarcinomas (BAC), such as promoter hyper-methylation of tumor suppressor genes and/or hypo-acetylation of histones. Here, we analyzed the efficacy of targeting of epigenetic modifiers in esophageal cancer cells and whether this differs for ESCCs and BACs.

Methods. ESCC (OE21), BAC (OE33) and normal esophageal epithelial (Het-1A) cell lines were examined for HDACs (-1, -2, -3) and DNMTs (-1, -3A, -3B) protein expression (western blot, immunofluorescence). Three different HDAC inhibitors (HDACi; Vorinostat, Entinostat, Romidepsin) and Azacytidine (AZA) were tested in dose-response curves alone and in combination by measuring cell viability (MTS-assay) and apoptosis (flow cytometry). Moreover, selected FFPE tissue specimens of ESCCs and BACs were examined by immunohistochemistry for HDACs and DNMTs.

Results. Both OE21 and OE33 cell lines showed a decreased expression of HDAC1 and HDAC2, compared to the normal epithelial cell line Het-1A. For HDAC3 no differences were observed. Treatment with Entinostat reduced cell viability (to about 20%) of both OE21 and OE33, but not Het-1A. Vorinostat and Romidepsin showed no selective tumor growth inhibition. AZA alone was only effective in OE21 cells. In contrast, combination of AZA with either Entinostat, Vorinostat or Romidepsin resulted in synergistic growth inhibition predominantly in OE21 cells (all to about 70%). No synergistic effects were observed with respect to apoptosis, except for the combination of Entinostat and AZA in OE33 cells. In tissue specimens, HDAC1 and HDAC2 were highly expressed in both ESCCs and BACs, but also in normal esophageal epithelium. In ESCCs, marked down-regulation of DNMT1 was observed in the invasive tumor cells as compared to normal esophageal squamous epithelium. In contrast, in BACs there was a prominent tumor cell specific up-regulation of DNMT3a.

Conclusions. This study determined of expression of epigenetic modifiers in both ESCC and BAC cell lines and tissue specimens. Accordingly, treatment with different HDACi and AZA combinations revealed that both ESCC and BAC cell lines responded to epigenetic therapy, but normal epithelial cells were not affected. Thus, targeting of epigenetic alterations in esophageal cancers by HDAC inhibitors and AZA may be a novel therapeutic approach.

FR-056
Interaction of cathepsin X with RPLP0 in gastric carcinogenesis

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Aims. Our previous studies have shown an association between Helicobacter pylori infection, a strong up-regulation of cathepsin X (CTSX), and the development of gastric cancer. The physiological function of CTSX still needs to be clarified. We identify RPLP0 as a novel interaction partner of CTSX and presume that CTSX and RPLP0 play an interactive role in modulation of the immune system in response to H. pylori.

Methods. Screening of a yeast two-hybrid system resulted in identification of RPLP0 as one potential interaction partner of CTSX. Western blots and immunohistochemistry revealed elevated RPLP0 levels and predominant cytoplasmic location in gastric cancer samples known to express high CTSX levels. Immunohistochemistry on cancer cell samples indicated a shuttling between cytoplasm and nucleus of CTSX and RPLP0. Direct interaction of CTSX and RPLP0 was clearly characterized by immunoprecipitation and double immunofluorescence.

Results. Functional consequences of the interaction of CTSX and RPLP0 were tested on siRNA-treated human gastric carcinoma cells followed by cell cycle analysis. RPLP0 knockdown revealed an G1/S arrest, whereas knockdown of CTSX reflects no noteworthy effects, Apoptosis increased minimally. Surprisingly knockdown of both proteins resulted in increased apoptosis. In addition, we checked cell cycle proteins like p21, cylins and CDK's using western blot. Expression of p 21 increased whereas CDK2 expression in siRNA treated human gastric carcinoma cells decreased.

Conclusions. Here RPLP0 together with CTSX appear to play a role in cell cycle and tumor progression. The interaction of CTSX and RPLP0 seems to be a major regulatory element disregulating cell cycle processes indicated by G1 Arrest, senescence or apoptosis. Further experiments will focus on possible effects of these cell cycle changes on the CTSX-dependent activation of the immune system against H. pylori colonization and cancer development.

FR-057
Expression of Notch-signaling genes in chemo-resistant gastric cancer cells

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Aims. We recently analyzed the differential expression of cancer stem cell related genes in residual gastric tumour cells after platinum based neoadjuvant chemotherapy. NOTCH2-expression was significantly increased after chemotherapy, while NOTCH1-expression was decreased. The aim of this study was to further assess the differential expression of Notch-signaling genes between corresponding pre- and post-therapeutic tumour specimens and in vitro between cisplatin resistant cells and their isogenic parental gastric cancer cell lines.

Methods. Expression of NOTCH1, NOTCH2, JAG1 and HES1 was compared by real time PCR (qRT-PCR) using TaqMan low density arrays between corresponding pre- and post-therapeutic tumour specimens from patients treated with neoadjuvant chemotherapy demonstrating partial (TRG2, n=22) or minimal/no tumour regression (TRG3, n=22). Cisplatin resistant gastric cancer cell lines (AGSC2, MKN28C2) were derived from the cell lines AGS and MKN28 by continuous treatment with 2 μm cisplatin. XTT-tests, colony formation assays and cell-cycle analyses were used to compare the resistant with their parental cell lines. Gene expression of Notch-signaling genes was measured by qRT-PCR.

Results. Differential expression analysis had revealed an increase of NOTCH2 from pre- to post-therapeutic specimens in tumours with TRG2 (p=0.002) and TRG3 (p=0.062). In contrast a decrease in expression was observed in both tumour groups for NOTCH1 (p=0.072 and 0.001), JAG1 (p=0.067 and 0.026) and HES1 (p=0.050 and 0.007). The resistant cell lines AGSC2 and MKN28C2 showed increased IC50-values for cisplatin, enhanced colony formation capacity under cisplatin-treatment and a markedly reduced cisplatin induced G2/M-arrest in comparison with their parental cell lines. The expression of NOTCH2 was significantly increased in AGSC2 and MKN28C2 (p=0.005 and 0.032). NOTCH1 expression was reduced in MKN28C2 (p=0.006). The expression of the target genes HES1 and HEY1 was reduced in both resistant cell lines (AGSC2: p=0.049 and 0.061, MKN28C2: p=0.002 and 0.044).

Conclusions. The comparison of mRNA expression between corresponding pre- and post-therapeutic tumour specimens revealed differential expression of Notch-signaling genes. Selection of cisplatin resistant cell lines led to similar alterations in vitro. This suggests that alterations of Notch-signaling in tumours might be related to chemoresistance in neoadjuvant treated gastric cancer. In addition, our results point towards divergent functions of Notch1 and 2 in this context.

FR-058
Lymph node recovery after gastrectomy for gastric adenocarcinoma is influenced by clinical and pathological factors

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Aims. As the prognosis of epithelial malignancies of the stomach is classically staged by the TNM system, one important predictor of survival for patients with resectable adenocarcinoma of the stomach seems to be the regional lymph node status, and their adequate assessment for

staging. Up to now clinical and pathological factors influencing lymph node recovery are poorly understood.

Methods. We reviewed pathology reports of patients who underwent partial or total gastrectomy for gastric adenocarcinoma between 1997 and 2012 to evaluate possible impacts on lymph node recovery.

Results. Until now, 108 patients underwent gastrectomy for gastric adenocarcinoma at different level hospitals. Overall, 29/27 lymph nodes per gastrectomy were detected in mean/median with a standard deviation of 15 lymph nodes and a range of a minimum 2 to a maximum of 86 lymph nodes. Correlation analysis revealed a significant association between number of tissue samples and assessed lymph nodes. Univariate analysis displayed a coherence in/between the number of lymph nodes recovered and the extension of gastrectomy extent, levels of medical care, pathologists, and pathology technicians ($p < 0.01$). Multivariate analysis identified the number of embedded tissue by pathologists as well as the experience/education of the pathology technicians as the most important healthcare-related variables contributing to the variation of lymph node recovery ($p < 0.05$).

Conclusions. Present study demonstrates that depending on external (surgeons) and internal (pathologists) factors the number of lymph node recovered varied extremely. Therefore, stepwise refinement of lymph node recovery due to standardized surgery techniques for surgeons and appropriate macroscopically training programs for pathologists may increase the maximal number of lymph nodes assessed and improve accurate staging, respectively, and thus patients' prognosis.

FR-059
Primary Merkel cell carcinoma of the stomach

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Aims. Extracutaneous manifestation of Merkel cell carcinoma is extremely rare; location in salivary glands, nasal cavity, lip, lymph nodes, vulva, vagina and esophagus has been described. Location in stomach hasn't been found yet. Diagnosis of Merkel Cell Carcinoma of extracutaneous sites requires special immunohistochemical and molecular analysis.

Methods. Tumor tissues of sigma, inguinal metastasis, biopsy specimen of stomach and the surgical specimen of the resected stomach were examined histologically and by immunohistochemistry. Resected gastric tumor tissue was examined by FISH and PCR (Polyoma virus).

Results. In our case of an 83 year old patient an adenocarcinoma of the sigma, in which the immunohistochemical reaction was typical of colorectal carcinoma with positivity for CDX 2 and CK 20, was resected. Neuroendocrine markers failed to be positive. 18 regional lymph nodes of sigma were free of metastasis (pT3, pN0, R0, G2). Synchronously an enlarged inguinal lymph node of the right side with infiltration by an unusual differentiated neuroendocrine carcinoma was removed. Immunohistochemical reaction was typical of Merkel cell carcinoma; however no skin tumor could be detected by extensive clinical and radiological examination. In ultrasound examination and in CT thickened wall of pyloric region was found. Gastroscopic biopsy was taken. Immunohistochemical examination of biopsy specimen was typical of Merkel cell carcinoma again. Stomach resection succeeded. Resected distal stomach with 19 regional lymph nodes without metastasis showed infiltration from mucosa to subserosa and fat-tissue, corresponding to pT3, pN0, pM1 (LYM), R0, G3. We obtained typical immunohistochemical pattern of Merkel cell carcinoma in inguinal lymph-node metastasis, biopsy specimen of stomach and in surgical specimen of the resected distal stomach with positivity for CK 18, CK 20, Synaptophysin, Chromogranin and NSE. No translocation with chromosome 16 or chromosome 22q12 as partner of translocation in FISH. In PCR

(MCPyV DNA-detection clinical factors associated with merkel cell polyomavirus infection in Merkel cell carcinoma; Harri Sihto et al; J Natl Cancer Inst 2009;101: 938–945) polyomavirus-DNA could be positively identified in resection specimen of the stomach.

Conclusions. To our knowledge of literature, this is the first published case of Merkel cell carcinoma of the stomach. Our case reflects the plasticity of genetic program in tumor cells independent of normal organ specifity.

FR-060
Detection of novel miRNA/mRNA interactions in gastrointestinal stromal tumors (GISTs)

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Aims. Gastrointestinal stromal tumors (GISTs) harbour gain-of-function mutations in the receptor tyrosine kinases KIT and PDGFRA, with constitutive activation of downstream intracellular signalling cascades PI3K/AKT1 and RAS/RAF/ERK pathways. Notably, GISTs from different anatomical localisations (stomach, small intestine, rectum) display characteristic histologic growth patterns, and there are also distinct phenotypic features associated with KIT and PDGFRA mutations, respectively. Finally, stimulation of cell proliferation and eventually evasion of apoptosis are central elements in GIST tumor progression. Previously, we reported on different miRNA expression and also on different mRNA expression patterns in GISTs according to anatomical localisation and genotype. The aim of the current study was to decipher novel miRNA/mRNA interactions in GISTs using data sets from identical tumor samples, and to compare these with clinico-pathological parameters.

Methods. Genome-wide mRNA expression analyses were performed using Sentrix HumanWG-6 arrays (Illumina, San Diego, CA) in a series of 12 GISTs (4 with PDGFRA mutation from the stomach, 4 with KIT mutation from the stomach, and 4 with KIT mutation from the intestinum. For determination of miRNA expression, miRBase set v10.1 (miRXplore Microarray, Miltenyi Biotec GmbH, Bergisch Gladbach) comprising a total of 734 human miRNAs was employed.

Results. Both data sets were compared and searched for significant inverse correlations between miRNAs and mRNAs over all 12 samples, as well as for the three subgroups independently. Different tools for the prediction of miRNA/mRNA interactions were employed to predict possible miRNA targets, and this was compared to the data sets. Pairs of inversely correlated miRNAs/mRNAs were mapped to known pathways of relevance in GISTs, namely PI3K/AKT and RAS/RAF/ERK pathways, as well as to regulatory hubs of cell proliferation/apoptosis.

Conclusions. Using a combined approach of a two-dimensional data set and in-silico analysis, we were able to identify novel interactions between miRNAs and mRNAs in GISTs, with potential regulatory effects on major signalling pathways and cell cycle control hubs.

FR-061

High expression of the long intervening non-coding RNA HOTAIR predicts disease free survival in gastrointestinal stromal tumours (GIST)

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Aims. Long intervening non-coding RNAs (lincRNAs) comprise a recently discovered class of functionally and structurally heterogeneous transcripts that are increasingly recognised as key regulators of diverse cellular processes. Few characterised lincRNA species are involved in maintaining pluripotency, embryonic development, orchestration of epigenetic programmes, nuclear organisation and other core processes. While clear indications exist for the role of certain lincRNA species in carcinogenesis, this field remains largely unexplored to date. Only recently, upregulated expression of the HOTAIR has been reported to correlate with shorter disease-free survival in GISTs.

Methods. To investigate tumour specific transcriptional deregulation of lincRNAs in GIST, we performed array-based profiling of over 11,000 lncRNAs in 40 GIST samples.

Results. This is the first study addressing aberrations of lincRNA expression in GIST on a genome-wide scale. We included clinically characterised specimens of different risks of malignant behaviour and anatomic locations (stomach, small intestine and rectum). Specific lincRNA and mRNA expression signatures were revealed based on disease risk and location. Earlier reported epigenetic-related lincRNA HOTAIR, which is capable of epigenetic reprogramming of multiple cancer related genomic sites, was among most prominent candidates, and its high expression significantly correlated to risk. To further investigate prognostic potential of the transcript, expression analysis of HOTAIR was performed by qRT-PCR in a validation set of GIST specimens. HOTAIR abundance was significantly associated with disease free survival and can be used as independent prognostic factor of the disease.

Conclusions. Finding of high prognostic relevance of the epigenetic-related lincRNA HOTAIR suggests its functional contribution to pathogenesis presumably by epigenetic mechanism. Mechanistic details as well as functional characterisation of other prominent lincRNA candidates are currently under investigation.

FR-062

Robust linear regression model of Ki-67 for determination of the mitotic rate in gastrointestinal stromal tumors

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Aims. Risk stratification of gastrointestinal stromal tumors (GIST) by tumor size, lymph node and metastasis status is crucially influenced by the mitotic activity. Currently, there are no quantitative studies comparing mitotic activity on hematoxylin-eosine (H&E)-stained tissue sections with immunohistochemical markers such as phosphohistone H3 (PHH3) and Ki-67.

Methods. According to the TNM guidelines, the mitotic count on H&E sections and immunhistochemical PHH3-stained slides was assessed

per 50 high power fields (HPF) for 154 GIST samples. The Ki-67-associated proliferation rate was evaluated on three digitalized hot spot areas using the commercially available image analysis software ImageAccess®.

Results. The H&E-based mitotic rate correlated significantly better with Ki-67-assessed than with PHH3 assessed proliferation activity (r=0.780, p<0.01). A linear regression model (ANOVA, p<0.001) allowed reliable prediction of the H&E-associated mitoses based on the Ki-67 expression only.

Conclusions. Our data indicate that the mitotic rate could be reliably and time-efficiently estimated by immunhistochemistry of Ki-67 using only three hot spots.

FR-063

High expression of p16 is correlated with shorter patients' survival in gastroenteropancreatic neuroendocrine tumors (GEPNETs)

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Aims. We examined in gastroenteropancreatic neuroendocrine tumors (GEPNETs) whether the expression of p16 correlates with clinicopathological parameters including WHO-grading, Ki67 expression and overall long term survival of patients.

Methods. GEPNETs from 98 patients were analyzed immunohistochemically for protein expression of p16 and Ki67. Primary tumors were gastric (n=5), small bowel (n=27), colorectal (n=17), gallbladder (n=3) and unknown origin (n=6). Follow up data for all patients were available (median follow-up time: 44.5 months).

Results. High expression of p16 was observed in 19% (n=19) of the tumors (score 2/3 vs. 0/1) and correlated with WHO-Grading. It occurred in 15% and 36% of well differentiated and poorly differentiated specimens respectively (p=0.032, G1 and G2 versus G3). High expression of p16 was statistically associated with a shorter long term survival (p<0.001). Also in the subgroups of WHO-grading, low grade (NET, G1/G2) and high grade (G3) shorter patients' survival was associated with high p16 status (p=0.004 and p=0.091, respectively). Ki67<10% (low) and Ki67≥10% (high) was observed in 75 and 23 specimens (19%) respectively. In 19 cases with high expression of p16 survival depended on Ki67 expression. All patients with Ki67≥10% died within 14 months (median survival 4 months), whereas patients with Ki67 <10% had a 55% ten year survival rate (p<0.001).

Conclusions. High expression of p16 is correlated with shorter patients' survival in GEPNETs and might be useful as a prognostic marker.

FR-064

T-cell spectrum analysis in celiac disease patients: a deep sequencing approach

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Aims. Coeliac disease (CD) is a gluten-sensitive enteropathy and causes chronic inflammation of the small intestine. Affecting between 0.5% to 1% of the general population in Europe and the USA, CD has emerged as a major health-care problem. Published complications of CD include refractory CD (RCD) which does not improve by a gluten free diet and the development of an enteropathy associated T-cell lymphoma (EATL). The common standard in the diagnostic of CD, RCD and EATL is histopathological analysis of small intestinal biopsy samples which de-

monstrate characteristic small intestinal lesions: villous atrophy, crypt hyperplasia, and an increased number of intraepithelial lymphocytes (IEL). However, the specificity of histological lesions is variable and a precise diagnosis is not possible in a substantial number of cases.

Methods. Considering that the pathogenesis of CD, RCD and EATL involves a T-cell mediated immune response, our study focused on determining the entire T-cell repertoire in affected tissue samples. Therefore, we utilized a multiplex T-cell receptor beta (TCRβ) PCR-assay followed by next generation sequencing (NGS, Illumina HiSeq 2000) of the generated amplicons. To this end, we analyzed biopsies of CD, RCD and EATL cases, some of which were derived from the same patients at different time points.

Results. After elaborated bioinformatics analysis of our TCRβ NGS data we gained approximately one million sequence reads per sample. PCR reads were aligned to the germline Vβ- and Jβ-segments (IMGT) and identical rearrangements were clustered in order to define their percentage composition. All samples included dominant T-cell clones comprising 3% to 75% of all T-cells with an average percentage of 22%. Interestingly, most RCD cases displayed very extensive clonal T-cell populations. Most strikingly, the analysis of samples from the same patients at different time points revealed a preserved consistency of the most dominant T-cell clones over time.

Conclusions. Our TCRβ NGS approach is an innovative tool to provide an extremely deep insight into the mechanism of T-cell response in patients with CD, RCD and EATL, and will help to identify gluten-specific T-cell clonotypes. We are convinced that based on our very detailed sequence information, we will be able to identify T-cell repertoires with a risk of developing RCD or EATCL at a very early stage in addition to the dominant and consistent T-cell populations displayed in this work.

FR-065
Non-apoptotic caspase-8 dependent mechanisms in the intestinal mucosa

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Aims. Caspase-8 is a cysteine protease that plays a critical role in regulating cellular apoptosis of intestinal epithelial cells and thus the disturbance of caspase-8 expression or function may contribute to development of several intestinal diseases. The pathogenesis of inflammatory bowel diseases is related to important molecular pathways such as Wnt, Notch, Hippo, BMP or Hedgehog in controlling cell fate determination. Therefore, this study focuses on a putative interaction between caspase-8 function and pathways in the establishment of the intestinal mucosal barrier and the pathogenesis of inflammatory bowel diseases.

Methods. Two types of caspase-8 knockout mice and special cell culture systems were used to examine effects of intestinal caspase-8 depletion on molecular pathways using a spectrum of molecular techniques and functional approaches.

Results. Intestinal depletion of caspase-8 was associated with development of an inflammatory phenotype, impaired molecular signalling and intestinal barrier leakage. Disturbances of intestinal cell-cell contacts were characterised.

Conclusions. These data identify a critical function of caspase-8 in established signalling cascades regulating intestinal homeostasis. In addition, a putative role of caspase-8 in the pathogenesis of inflammation-related intestinal disorders is suggested.

FR-066
Association of ACSL5-derived Wnt2B palmitoylation and differentiation of intestinal adenocarcinomas

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Aims. The acyl-CoA synthetase 5 (ACSL5) functions as a bidirectional modifier referring to apoptosis and intestinal carcinogenesis and is a possible interface between the cross-linked intestinal signalling pathways. Because of its function in fatty acid metabolism and its mitochondrial localisation is there a possible influence of the enzyme activity of ACSL5 on Wnt2B, which was also found mitochondrial localised. The interaction results in a modified translocation of Wnt2B between mitochondria and cytoplasm and an inhibition of the activation of Wnt signalling and is effected by palmitoylation. The results we have found in cell culture models and murine tissue should now be translated into more cell lines and human carcinomas of different malignancy status (G1–G3).

Methods. To verify the results from HEK293 (intact Wnt signalling) and CaCo2 (colon carcinoma) cell lines, we have added HT29, SW480 and HCT116 cell lines to our study. The ACSL5-derived modification of the Wnt signalling was analysed by realtime PCR, immunohistochemistry, luciferase assay and an assay to detect palmitoylation. The observations from the cell culture were proved in the APCmin/+ mouse model and human tissue of intraepithelial neoplasia and adenocarcinomas of the intestinal mucosa.

Results. In vitro, ACSL5-derived palmitoylation of Wnt2B decreased with the status of malignancy and Wnt activity. The findings are partially reflected by mice experiments. In human adenocarcinomas of different malignancy Wnt2B palmitoylation is found, but strong baseline Wnt activity is given. Human adenomas (IEN) show a more heterogeneous pattern. An increased activation of Wnt signalling (beta-catenin translocation) and the proliferation rate was shown in spite of simultaneous decrease of ACSL5 and Wnt2B. These results indicate in a synopsis of the in vitro findings a distinctive feature of ACSL5 dependent Wnt regulation in vivo.

Conclusions. A so far unknown molecular signalling pathway was identified, showing the antiproliferative modifying function of ACSL5 in intestinal mucosa. This pathway seems to be of central relevance for the intestinal homeostasis. In carcinogenesis other Wnt-driving mechanisms are overcome activities of the Wnt2B modifier ACSL5.

FR-067
Liquid or solid—the role of the consistency of peritoneal adhesion barriers for the biocompatibility

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Aims. Peritoneal adhesions remain a serious clinical problem after surgery. The only promising prevention is based on various barrier materials consisting of different liquid and solid materials. However, the optimal device has not yet been found. Also, the influence of the materials' consistency in adhesion prevention is unknown. Therefore, it is necessary to investigate the processes of adhesion formation and the influence of implanted materials on a cellular level (2). In the present study, the biocompatibility of three liquid and two solid barrier materials as well as one untreated control group were compared.

Methods. Wistar rats remained untreated (n=14) or were treated with liquid (n=55; Adept®, Intercoat®, Spraygel®) or solid (n=43; Seprafilm®, SupraSeal®) barriers after standardized peritoneal damage. After 14 days the animals were sacrificed and the treated areas were explanted and processed according to standardized methods for histological examination. Inflammation was evaluated by the presence of granulocytes (ASD stain) and lymphocytes (HE stain), the foreign body reaction by the amount of foreign body giant cells (HE). Also, the extent of fibrosis was assessed (EvG stain). The histological evaluation occurred according to the ISO score for local effects after implantation.

Results. Regarding biocompatibility in accordance to the ISO score no differences were found between the groups. The inflammatory response was minimal in both treatment groups as well as in the control. No foreign body reaction was detected in all of the groups. Minimal fibrosis was seen in the control as well as the treatment groups.

Conclusions. These are interesting and important findings concerning the application of material-based devices to prevent adhesion formation. Since there are different advantages and disadvantages within both liquid and solid approaches, it is important to know that the consistence seems not to influence biocompatibility. However, this is the first investigation of the biocompatibility of various materials with different consistence. In further studies degradation, easy application during surgery and the tissue reaction on a cellular and molecular level should analysed more in detail.

FR-068

Extracellular ATP stimulates EMT and acts as a pro-invasive factor in tumor microenvironment

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Aims. ATP, the key intracellular energy currency, accumulates within the tumor microenvironment and is closely involved in cancer cell metabolism and in antitumor immunity. The established role of ATP as a growth modulator and a proinflammatory mediator endue ATP and other purines with potential players in host-tumor interaction. Our previous study demonstrated that extracellular ATP stimulated human cancer invasion via P2Y receptors. The aim of this study is to elucidate the mechanism of ATP's pro-invasive effect, and characterize the function of ATP receptor subtypes.

Methods. A number of different types of human carcinoma cell lines were used. cDNA microarray screening, RT-PCR, Western blotting, and RNAi technology were used.

Results. Increased migration and invasive ability across Matrigel was observed in some human carcinoma cell lines, including prostate, breast, colon, melanoma and lung, when stimulated with ATP or its analogues. Given that tumor microenvironment is rich in ATP and other purines, we have hypothesized that ATP might be a potential invasion stimulator in tumor microenvironment. We screened the ATP-stimulated candidate genes by cDNA microarray and found that expression of a panel of invasion/metastasis-related genes was significantly changed. Validation was done both at mRNA and protein levels. Expression of IL-8, MMP-3 and HB-EGF was up-regulated after ATP treatment. ATP also induced EMT by modulating the expression of snail, E-cadherin and claudin-1. Multiple P2Y receptors subtypes were expressed on tumor cells, but P2Y2 receptor was found to be mainly responsible for the pro-invasive effect of ATP. Further we found that P2Y2 receptor transactivated with EGFR and co-activated ERK1/2 signaling pathway, which was involved in regulating expression of EMT and other related genes.

Conclusions. In summary, our results, for the first time, revealed that ATP is a potential pro-invasive factor in tumor microenvironment. P2Y2 receptor, together with EGFR, acted as a mediator in the regulation of ATP-induced EMT and invasion of cancer cells.

FR-069

Rhabdoid carcinomas of the gastrointestinal tract: three cases of a highly aggressive neoplasm with emphasis on INI-1-status

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Aims. Malignant rhabdoid tumors are rare and highly lethal neoplasms that mainly arise in the kidney and less frequently at extra-renal sites. They resemble the atypical rhabdoid/teratoid tumors of the central nervous system in childhood.

Methods. We describe the clinicopathological and immunohistochemical features of three cases of carcinomas with rhabdoid features originating in the pancreas, stomach and colon (one each).

Results. Patient's age was 66, 68 and 80 yrs. All presented with advanced disease and they died of progressive disease no later than 10 months after initial diagnosis. Histological examination showed highly anaplastic dyscohesive cells with excentric vesicular nuclei, prominent nucleoli, rhabdoid paranuclear inclusions and frequent bi-/multinucleation. Immunohistochemistry revealed strong coexpression of pankeratin and vimentin but no staining with other lineage-specific markers. Two cases showed loss of nuclear INI-1 expression. Electron microscopy of one case revealed paranuclear whorls of intermediate filaments that corresponded to the immunostaining pattern for keratins and vimentin.

Conclusions. Rhabdoid phenotype in carcinomas of the GI tract is associated with a highly aggressive clinical course irrespective of INI-1 status. This strictly uncommon variant of gastrointestinal carcinoma might represent a distinct pathway of dedifferentiation of epithelial neoplasms. Exploration of the molecular mechanisms is necessary for potential targeting these highly aggressive neoplasms.

Postersession Molekulare Pathologie

FR-070

Distinguishing pancreatic cancer from cholangiocarcinoma using MALDI imaging mass spectrometry

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Aims. Pancreatic adenocarcinoma (PDAC) and cholangiocarcinoma (CC) are both cancers with dismal prognosis originating in close proximity to each other. Distinguishing CC from PDAC by means of histomorphology or immunohistochemistry is very difficult. Thus, elucidating differences at the proteome level between them would provide valuable information for the diagnostic and therapeutic decision-making process. Imaging mass spectrometry (IMS) applied to CC and PDAC tissue samples enables the visualization of the spatial distribution of cancer specific protein/peptide expression profiles in correlation with histological features.

Methods. In this study, twenty-five PDAC and fifteen extrahepatic CC samples from a tissue microarray (TMA) constructed using formalin-fixed, paraffin-embedded (FFPE) tissues were analyzed by MALDI imaging mass spectrometry (IMS). Briefly, sections were mounted onto conductive glass slides and underwent paraffin removal as well as antigen retrieval. On-tissue digestion was achieved by spotting trypsin and matrix onto the tissue in an array pattern. Samples were analyzed utilizing an Ultraflextreme MALDI-TOF/TOF mass spectrometer. Additionally, MS/MS measurements of selected peptides were acquired. Data analysis was performed by using the ClinProTools 2.2 and FlexImaging 3.0 software.

Results. Distinctive differences in peak patterns could be identified in CC and PDAC. By combining five peaks in a genetic algorithm based model, 88.9% of all CC samples and 92.9% of all PDAC samples could be diagnosed correctly. Further identification of tryptic peptides revealed significantly higher expression of a peptide from keratin type II in PDAC samples and significantly higher expression of a peptide from collagen alpha-2 chain in CC samples. Proteins of keratin type II, namely cytokeratin 1, 4, 7 and 8 were chosen for further validation by immunohistochemistry.

Conclusions. MALDI imaging mass spectrometry using FFPE tissues enables the identification of entity-specific protein profiles that upon validation might be useful for the differential diagnosis between morphological similar tumors.

FR-071

Identification and validation of novel protein biomarkers for the development of a chip-based early breast cancer detection system

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Aims. Currently, mammography is the gold standard in breast cancer screening. However, this method may lead to late tumor detection in some instances, e.g. in women with dense breast tissue. Novel techniques that could improve early breast cancer detection are thus highly desirable. The EU-funded joint research project "MicroBioMed" (Microtechnologies for biomedicine applications) aims at developing a cost-effective, specific and sensitive biochip-based detection system for early breast cancer detection. As a non-invasive method, blood serum will be used as the analytical medium. Our aim and contribution to the project is the identification and validation of novel protein serum biomarkers with sufficient breast cancer specificity.

Methods. By means of DNA array expression profiling and 2D-DIGE protein analysis, a set of potential protein serum biomarkers was identified and subsequently validated by three different steps: First, mRNA expression was determined in different benign and malignant breast cancer cell lines. Next, expression of selected candidate genes was measured on mRNA as well as on protein level using a collection of fresh frozen breast cancer specimens and healthy breast tissues. For candidates confirmed as upregulated in breast cancer tissue, Western Blot analysis and ELISA assays are being performed using human serum samples. The performance of a 3-marker-panel will be optimised using receiver operator characteristics (ROC) curve analysis.

Results. Performing mRNA and protein expression analysis on breast cancer cell lines and breast cancer tissues, we identified five novel candidate molecules exhibiting abundant expression solely present in malignant tumor cells. Furthermore these proteins have also been successfully detected in human serum samples. By using quantitative ELISA assays for selected candidates we are now testing their specificity and sensitivity in a cohort of serum samples from breast cancer patients compared with healthy donors.

Conclusions. So far, we have identified five novel biomarker candidates potentially useful for early breast cancer detection. The best biomarkers will be combined in a multi-marker panel and integrated into the biochip system, developed by the engineer partners of the MicroBioMed network. Assays like this may be used in near future to complement mammography in breast cancer screening.

FR-072

Prevalence of the Washington University polyomavirus in human cancers

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Aims. The Washington University polyomavirus (WUV) was discovered in nasopharyngeal aspirates of children in 2007. An association of this virus with human cancers and its tissue tropism has not been described yet. In this study we aimed to identify the presence of WUV in human cancers such as lung cancers, lymphomas, renal cancers, thymomas and in non neoplastic tissues like tonsils, lymph nodes and renal tissue by DNA PCR.

Methods. DNA from formalin fixed and paraffin embedded (FFPE) tissue samples was isolated and checked for the size of amplifiable DNA fragments by the housekeeping genes [Human AF4 exon 3 (600bp), exon 11 (400bp), PLZF exon1 (300bp), RAG 1 exon 2 (200bp) and TBXAS exon 9 (100bp)] PCR. An oligonucleotide primer set which amplifies a 244 bp fragment of large T-Ag region of the WUV was used to screen the DNA samples for the presence of WUV by qualitative PCR.

Results. DNA PCR on 471 DNA samples which included 371 neoplastic (74 lung cancers, 81 lymphomas, 43 thymomas, 173 renal cancers) and 100 non neoplastic (40 reactive lympnodes, 30 tonsils and 30 renal tissue) patient specimens revealed that WU polyomavirus is not associated with any of the tested human cancers and non tumor tissue samples.

Conclusions. WU polyomavirus DNA was not detected in human cancers suggesting that it does not play a role in human tumorigenesis. Absence of WUV DNA in human non neoplastic tissue samples suggests that tissue tropism of WUV does not involve reactive lymphnodes, tonsils and renal tissue.

FR-073

MicroRNA expression in parathyroid tumours

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Aims. Differential diagnosis between atypical adenomas of the parathyroid (AA) and parathyroid carcinomas (PCA) may be challenging using conventional histologic criteria except for the classical features of malignancy. MicroRNAs (miRNAs) are endogenous, non-coding, small RNAs that regulate gene expression posttranscriptionally. Recent studies in other malignant tumors (e.g. breast carcinoma) reveal characteristic miRNA profiles along the cancer progression path. The aim of the study was to determine the miRNA profile in benign [normal parathyroid tissue (NP), parathyroid adenoma (PA)] and malignant (PCA) as well as in AA.

Methods. Three samples each of NP, PA, AA and PCA were analyzed using 384 well TaqMan low-density array real-time qPCR. Total RNA isolation was done with the miRNeasy FFPE' kit from Qiagen. For the low-density array, preamplification products were pressed through microchannels into wells fixed in the card and preloaded with immobilized, deshydrated target-specific primers-probe pairs. PCR analysis was run on the ABI PRISM 7900 System. Analysis was performed with TaqMan Array Human MicroRNA Card A and TaqMan Array Human MicroRNA Card B v3.0 (both Applied Biosystems), respectively. For PCR-conditions, the ready-to-use TaqMan Universal PCR Master Mix (Applied Biosystems) was applied. qPCR analysis was performed in concordance to the MIQE-guidelines.

Results. miRNA expression analysis shows a characteristic profile along the progression path from NP, PA, AA to PCA in 58 out of 765 miRNAs.

Among them, miRNAs 25, 29a and 151-5p are significantly downregulated in PCA vs. NP whereas no significant upregulation could be demonstrated. Expression of 13 miRNAs (186, 200c, 551a, 586, 293, 599, 581, 29b-2, 10a, 214, 181c, 92b, 765) out of those 58 miRNAs reveal a similar profile between AA and PCA, whereas 5 (miRNAs 25, 200b, 429, 30a-5p, 944) show a similarity between AA and PA.

Conclusions. The miRNA expression profile confirms the histologically "intermediate" position of atypical adenomas of the parathyroid between parathyroid adenomas and carcinomas. Further analyses with more samples are needed to determine a subgroup of miRNAs that is required to favour a benign or rather malignant behaviour of atypical adenomas of the parathyroid.

FR-074
Differential diagnostic value of miRNAs in thyroid nodules

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Aims. MicroRNA (miR) expressions play important roles in multiple biological processes and are consistently found to be altered in thyroid tumors. For pathologists it is often a challenge to differentiate between overlapping subtypes, especially between follicular variants of papillary and follicular carcinoma of the thyroid. The current study explores whether miRNA expressions can be routinely used as an additional differential diagnostic tool in thyroid pathology.

Methods. Based on literature, three different TaqMan microRNA assays (miR-146b, miR-221 and miR-222) were used to determine the miRNA expression level by means of quantitative real-time PCR (Q-PCR). The miRNA strand was first reverse transcribed into its single stranded cDNA (complementary DNA) using the TaqMan MicroRNA Reverse Transcription Kit (ABI). miR-16 and U47 were as well as an Universal miRNA Reference used as controls. The miRNA expression analysis was expanded to a series of 10 subtypes of benign and neoplastic thyroid lesions. The ΔCT value was calculated for each specimen.

Results. MiR-146b (cut-off ≤ -0.285) shows highly increased expression levels in papillary thyroid variants and is predictable. miR-221 and miR-221 were not differently expressed in normal tissue versus adenomas, papillary and follicular carcinomas.

Conclusions. As compared to the literature miR-146b as well as miR-221 and miR-222 seem to play important roles in tumorgenesis of thyroid carcinomas. A close association is implicated between the elevated miR-146b in papillary thyroid carcinomas which could potentially be exploited and utilized as practical additional routine biomarker for differential thyroid cancer diagnosis, especially for follicular variants of papillary carcinoma.

FR-075
A new isoform of human plakophilin 3 gene is differentially expressed and regulated by its own promoter

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Aims. Plakophilins (PKP1-3) are essential parts of desmosomal adhesion, with differentiation-dependent expression patterns. Moreover, PKP3 seems to be involved in malignant transformation, with possible oncogenic or tumor-suppressive functions. Here we present evidence for the existence, differential expression and individual regulation of a new variant of the PKP3 gene, PKP3b.

Methods. Using purified polyclonal antibodies specific for PKP3b or the known PKP3a, we analyzed the expression patterns in normal and tumorous tissues or cell lines by immunofluorecence microscopy. Promoter analyses were performed by transfection of reporter gene constructs into human cultured cells bearing genomic sequences in front of the PKP3b-specific exon 1b. Finally, employing electromobility shift assay (EMSA) we wanted to identify potential binding sites for transcription factors.

Results. PKP3b differs from PKP3a only at the very N-terminus due to splicing in of the exon 1b. Immunofluorescence analysis of normal skin revealed that both variants localize at the desmosomes throughout all epidermal cell layers. Small intestine exhibited a desmosomal staining of all epithelial cells for PKP3a. In contrast, staining of PKP3b was focally and restricted to apical desmosomes. Similar results were obtained when analyzing tumors. Squamous cell carcinomas revealed extended desmosomal staining for both variants. Colorectal adenocarcinomas exhibited a desmosomal staining of all tumor cells for PKP3a, while PKP3b showed a focal pattern with apical restriction. Similarly, in cancer cell lines PKP3b was abundant in stratified epithelial cells, but heterogeneous or absent in simple-epithelial cells. For the identification of regulating elements we used a 3kb sequence in front of exon 1b for reporter gene analysis in different cell lines and obtained evidence for the existence of an alternative promoter for PKP3b. Finally, we identified possible binding sites by EMSA for transcription factors (C/EBP or HSF2, AP1) that may be responsible for differential expression of PKP3b.

Conclusions. With PKP3b, we identified for the first time desmosomal protein variants regulated by independent promoters. The new variant PKP3b is expressed differentiation dependent, mainly in stratified epithelia and corresponding carcinomas but more restricted and heterogeneously in simple epithelia and adenocarcinomas. Potentially, the new variant may serve as a marker protein for analysis of differentiation and malignant transformation.

FR-076
Allelic variants of p53, MDM2 and PPP2R2B affect longevity in a gender specific manner

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Aims. Aging is thought to occur through the accumulation of molecular and cellular damages. A key regulator of the cell's stress response is p53. We were interested whether SNPs in members of the p53-pathway are associated with longevity in humans.

Methods. We genotyped the following SNPs: p53—Arg72Pro (rs1042522), MDM2—SNP309 (rs2279744), MDM4—SNP34091 (rs4245739), SNP31826 (rs1563828), PPP2R2B (rs319217) in 155 long-lived individuals (LLIs) who died at the age of 91 and over and in 171 ethnically-matched control subjects.

Results. In female LLIs, the Pro-allele of the p53 Arg72Pro SNP and the G-allele of SNP309 were significantly associated with an increased survival time (p=0.026, p<0.001, respectively, log-rank test). In contrast, there was no difference regarding the survival time in male LLIs (Arg72Pro: p=0.58, SNP309: p=0.503, log-rank test). There was no impact on longevity for the genotypes of the respective SNPs in the MDM4 gene.

Conclusions. Here we show for the first time that the Pro-allele at the Arg72Pro-SNP and the G-allele of SNP309 are correlated with increased lifespan in a gender-specific manner. Our data support the hypothesis that genetic variants that are associated with lower activity of p53—and therefore increased tumor risk—are correlated with prolonged lifespan.

FR-077
GHSR DNA methylation pattern in different cancers

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Aims. DNA methylation patterns have been recognised as cancer-specific markers that have high potential for clinical applications. Aberrant variations of methylation occur on early stages of carcinogenesis and, therefore, hold promise for early diagnostics. We have earlier reported on the high sensitivity and specificity of the growth hormone secretagogue receptor (GHSR) hypermethylation for detection of breast cancer. Given the fact that aberrant loss of GHSR expression was functionally involved in cancer cell invasion, we asked if this epigenetic aberration may also occur in other common cancers and, thus, have broader biomarker potential.

Methods. A total of 163 samples were included in this study. These were carcinomas of lung, colorectum, prostate, stomach, breast and pancreas, chronic lymphocytic leukaemia, glioblastoma as well as respective normal tissues. To characterise the GHSR DNA methylation pattern, we investigated the methylation status of 27 CpG sites in the promoter region and first exon of GSHR quantitatively by bisulphite pyrosequencing. To ascertain the impact of hypermethylation on regulation of expression, mRNA levels were analysed by qRT-PCR in patient samples of lung and pancreas cancer as well as in respective cell lines after treatment with DNA hypomethylating agent 5-aza-2'deoxycytidine.

Results. A high degree of GHSR methylation was recorded in all types of interrogated malignancies. The overall methylation load was significantly higher in neoplastic compared to respective normal specimens. Aberrant hypermethylation exhibited no difference with regard to tumour staging, and could be detected in early stage tumours. Cancer samples could be reliably discriminated from normal samples by using the methylation signature with area under the ROC curve values of 1.00 (pancreas, breast, CLL), 0.97 (lung), 0.89 (prostate), 0.83 (colorectum) and 0.81 (stomach). A negative correlation of GHSR mRNA levels and methylation degrees was shown for specimens of lung (correlation coefficient −0.71) and pancreas (−0.66), where RNA samples were available. The gene was in part demethylated in cell lines upon treatment with 1 μm 5-aza-2'deoxycytidine with concomitant re-activation of expression.

Conclusions. In conclusion, the results show that epigenetic deregulation of GHSR is a common feature of different cancers. Quantitation of GHSR methylation may be valuable as a diagnostic marker in different types of cancer.

FR-078
MLH1, MSH2, MSH6 and ERCC1: DNA-mismatch repair proteins associated with response to platin-based chemotherapy in MPM?

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Aims. Platin-based chemotherapy is, besides the 'gold-standard' antifolate therapy with pemetrexed, the today most common chemotherapy for MPM. Some published data suggests that the efficacy of this therapeutic option depends on the individual power of DNA-repair mechanisms and it seems likely that enzymes involved in these pathways could be important markers in the context of 'tailored' anti-cancer therapy. A study was designed to clarify the impact of DNA-repair enzyme expression and activity, potentially resulting in a marker panel predicting patients response to a platin-based chemotherapy, resulting in a preselection of patients.

Methods. 146 patients were tested immunohistochemically for protein expression levels of MLH1, MSH2, MSH6, TUBB3 and ERCC1. In addition, these patients were sequenced by pyrosequencing for ERCC1 codon118 and C8092A SNPs. For correlation analysis between numeric variables, Spearman's rank correlation test was used; for association between protein expression and dichotomous variables the exact Wilcoxon Mann-Witney Rank Sum test was used. Survival analysis was done by Cox-regression (COXPH model). Statistical significance was set at $p=0.05$.

Results. 783 TMA scores were evaluated by an experienced pathologist, 36 lead to no result. All enzymes show a strong staining intensity. 204 ERCC1 sequence analysis were accomplished. For codon 118, 13 (12.75%) C/C, 39 (38.24%) C/T and 50 (49.02%) T/T genotypes were affirmed; for C8092A SNP 11 (10.78%) A/A, 58 (56.86%) A/C and 33 (32.35%) C/C genotypes could be approved. For OS, the most predictive marker in our collective was gender, but also ERCC1 expression would be a good predictor for differences in OS. Gender is also the best parameter for prognosis of PFS. Furthermore, ERCC1 C8092A A/A genotype is clearly associated with poor patients' outcome, codon118 C/C genotype also has a tendency for forecast of a platin-Cx response as well as TUBB5 protein staining intensity. In combination, TUBB5 expression level and C8092A A/A genotype can sustainable predict patients PFS.

Conclusions. Our results show that DNA-repair mechanisms are diagnostically relevant targets predicting patients' response to platin-based chemotherapy. Especially ERCC1 expression and mutational status are strong indicators when combined with patients' gender. In future, ERCC1 has to be established in the pathologically routine diagnostics.

FR-079
Mutation status of β-catenin is correlated to its nuclear accumulation in deep (aggressive) fibromatosis/desmoid tumors

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Aims. Deep (aggressive) fibromatosis/desmoid tumor is a relatively common mesenchymal neoplasm characterized by spindle cell proliferations with locally aggressive behavior, but no tendency for metastasis. It can arise either in the abdominal wall, in the mesentery or in extra-abdominal localisations. Its etiology is heterogenous and probably multifactorial, including endocrine (estrogen receptor positivity) and physical factors (trauma, irradiation), as well as a genetic basis with mutations affecting the APC/β-catenin pathway. The aim of the current study was to evaluate the frequency of β-catenin mutations in a monocentric cohort of desmoid tumors, and to correlate the genotype with different clinico-pathologic parameters.

Methods. For the current study, we analysed a cohort of 56 deep (aggressive) fibromatoses for mutations in β-catenin, using both Sanger sequencing and a novel pyrosequencing assay. The mutation status of β-catenin was compared to clinico-pathological parameters and immunohistochemical staining for β-catenin, estrogen receptor and the β-catenin target gene cyclin D1 on tissue microarrays.

Results. Thirty-three of the patients were female, and 23 were male. Thirty-nine of the cases were located extra-abdominal, 9 in the abdominal wall, 5 cases in the mesentery, and 3 in the breast. Thirty-three (59%) of the cases harbored mutations in β-catenin, including 17 cases with p.T41A mutation, 11 cases with p.S45F mutation, 3 cases with p.S45P and 1 case with p.S45N mutation. One case displayed two mutations (p.T41A and p.S45F), 18 cases were of wild-type status for β-catenin (including

2 cases in the clinical setting of FAP), and in 5 cases the DNA was too degraded for mutation analysis. There was a significant association of gender and localization (p=0.006), in that all cases from the abdominal wall or breast occurred in females, while the mesenterial and extra-abdominal localization was relatively more frequent in males. There was a significantly higher nuclear β-catenin expression in the cases with β-catenin mutation (p=0.04).

Conclusions. Mutations in β-catenin are correlated to a higher nuclear accumulation of the protein, probably according to a lower degradation rate of the mutated protein. Pyrosequencing has a higher sensitivity in the detection of β-catenin mutations in deep fibromatosis compared to Sanger sequencing, which most likely reflects the low tumor cell concentration in some of the cases.

FR-080
FFPE-tissue is the issue as feasible source for gene expression analysis – a comparison of multiple reference genes

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Aims. Formalin-fixation, paraffin-embedding is the standard processing technique for tumor tissue in modern pathology. New techniques like cryo-conservation allow fast fixation and long-time storage but result in increased costs and enlarged storage complexity. However formalin-fixed, paraffin-embedded (FFPE) tissue is available in a large quantity making it the ideal material for retrospective studies. The following study was designed to investigate the influence of formalin-fixation on the quality of mRNA and applicability for gene expression analysis. Multiple potential reference genes for pulmonary tumors with neuroendocrine differentiation were included and tested for their robust expression.

Methods. Eighty specimens from 2005 to 2012 from the Department for Pathology and Neuropathology at the University Hospital Essen were analyzed for their gene expression by using TaqMan® gene expression assays on demand (AoD). Three distinct reference genes (ACTB, GAPDH, HPRT1) were evaluated for their expression and one prominent tumor marker (PSMA1, unpublished data, Mairinger, Walter et al.) was included in the analysis and functioned as internal technical control.

Results. For GAPDH and ACTB a phenomenal accordance was found making them prominent reference genes for further research. Additionally the feasibility for a FFPE tissue-based gene expression analysis was verified revealing that the mRNA quality is sufficient.

Conclusions. If FFPE preparation was performed carefully under standardized conditions isolated mRNA can be used for reliable and successful gene expression analysis allowing for large, retrospective studies that connect patient material with full follow-up data.

FR-081
A breast cancer specific panel of mutation hotspots for focused next generation sequencing

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Aims. Focused next generation sequencing (NGS) platforms covering some hundreds to some thousand of amplicons are offered by several manufacturers. Together with the machines, cancer panels covering selected exons in oncogenes and tumor suppressor genes are commercially available. However, these panels turn out cover only a minority of the genes that have been recently found to be mutated in breast cancer. Therefore, we set out to develop a more specific gene panel based on recently generated NGS data.

Methods. A large data set of sequence variants in 778 breast cancers was downloaded from The Cancer Genome Atlas (TCGA) project. The data were filtered for somatic mutations and mutations that are non-silent when located in the coding region. After filtering, we were left with a total number of 38,692 non-overlapping mutated genomic loci.

Results. 56 loci were mutated in at least 1%, 437 loci were mutated in at least 0.5% of the 778 tumors. The most frequently mutated loci were found in 24.0% (PIK3CA, exon 20), 10.2% (PIK3CA, exon 9), 6.0% (PIK3CA, exon 9), 3.9% (GATA3), 3.3% (AKT1, exon 11), 3.2% (TP53, exon 7), 2.9% (GATA3, exon 5), 2.8% (PIK3CA, exon 4) and 2.6% (AOAH, exon 0) of the tumors. We selected 112 loci that were (i) mutated in at least 1% of the 778 tumors or (ii) mutated in at least 0.5% of the 778 tumors and were located in 57 genes that were found frequently mutated in breast cancer or molecular subtypes of breast cancer before.

Conclusions. The mutations found in cancer cells are extremely diverse among different tumor entities. Using commercially available gene panels for focused NGS, the coverage of a specific cancer entity can be poor. On the other hand, the great majority of covered loci are usually not relevant in the tumor entity under consideration. To improve sensitivity and specificity of a NGS assay, we analyzed the mutation landscape of breast cancer and isolated a panel of 112 frequently mutated loci.

FR-082
HPV detection in cytological specimens: about what the method lets us know

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Aims. The screening for human papillomavirus (HPV) in order to detect patients at risk for cervical cancer/precursor lesions is widely used in developed countries. The test is usually performed with a well-established and commercially available method provided by different enterprises. Our aim was to compare 4 different methods in order to identify advantages and weaknesses of each method by using the same extracted DNA.

Methods. One hundred eighty one consecutive specimens (SurePath and ThinPrep) showing HPV-related cytological alterations were preliminary analysed using the HPV Sign® PQ (Diatech) and the Linear Array® (Roche) method. The discrepant results were further studied with the Real time® High Risk HPV (Abbott®) method and the INNO-LiPA (Innogenetics®) method.

Results. Of 181 cytological specimens studied 61 (34%) were discrepant. Not analysable samples were due to lack of DNA detection or problems in interpreting the result. The number of non-detected high risk HPV was 11 (HPV Sign PQ®), 11 (Linear Array®), 13 (Real Time® High Risk HPV) and 3 (INNO-LiPA), respectively. Not analysable samples due to lack of DNA detection or problems in interpreting the result was 9 (HPV Sign PQ®), 8 (Linear Array®), 2 (Real Time® High Risk HPV) and 2 (INNO-LiPA) respectively. Interestingly, there were also samples showing different HPV types when analysed with different methods.

Conclusions. This study shows that the choice of detection method has direct influence on the sensitivity and specificity for detecting HPV subtypes. Each method can have its advantages and limitations concerning handling and data interpretation, however most sensitive results seem to be achieved with the INNO-LiPA method.

FR-083

Determination of MDM2 amplification status using chromogenic in-situ hybridization (CISH) is superior to MDM2/CDK4 immunohistochemistry in differential diagnosis of liposarcomas

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Aims. Liposarcomas are classified into four subtypes, comprising well differentiated liposarcoma/atypical lipomatous neoplasm (WDL/ALN), dedifferentiated liposarcoma (DL), myxoid/round cell liposarcoma (MRCL) and pleomorphic liposarcoma (PL). Both the WDL/ALN and the DL share a similar genetic background of giant marker chromosomes and ring chromosomes with amplification of a chromosomal region including the genes encoding for the cell cycle regulators CDK4 and MDM2. This specific genetic background has been suggested as a diagnostic marker for WDL/ALN in comparison to true lipomas, as well as for DL in comparison to other mesenchymal spindle cell neoplasms. The aim of the current study was to compare two different methods of MDM2/CDK4 analysis in different groups of liposarcomas.
Methods. Using tissue microarrays, a cohort of 77 liposarcomas was retrospectively analysed for MDM2 amplifications using a novel chromogenic in-situ hybridization probe (CISH), and this was compared to immunohistochemical stainings against MDM2 and CDK4.
Results. The cohort consisted of 32 WDL/ALN, 11 DL, 25 MRCL and 9 PL cases. 24 (75%) of the WDL/ALN were positive for MDM2 CISH, compared to 11 (100%) of the DL, 1 (4%) and 6 (67%) of the PL cases. Using immunohistochemistry, 18 (56%), 11 (100%), 3 (12%) and 6 (67%) of the cases were positive for MDM2, compared to 15 (47%), 10 (90%), 0 (0%) and 6 (67%) cases that were positive for CDK4.
Conclusions. MDM2 CISH has a higher sensitivity and specificity than MDM2/CDK4 immunohistochemistry in the comparison of different groups of liposarcomas, especially in the group of WDL/ALN. Accordingly, further studies will be conducted comparing MDM2 CISH and MDM2/CDK4 immunohistochemistry in the differential diagnosis of WDL/ALN vs. true lipoma, and of DL vs. other spindle cell neoplasms.

FR-084

BRAFV600E is associated with overexpression of CK 19, HBME-1, and Cyclin D1 in papillary thyroid carcinoma

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Aims. In thyroid neoplasms BRAF V600E mutation is restricted mainly to papillary thyroid carcinoma (PTC) and a few undifferentiated carcinomas. In PTC the mutation is suggested to be responsible for a more aggressive biology with poorer outcome. Up to date no association with other immunohistochemical markers has been reported.
Methods. Among 197 consecutive PTC patients 143 were selected with documented age at diagnosis, gender, TNM stage (according to UICC 2009), extra thyroidal extension, nodal metastases, and recurrence or death from disease. Tissue micro arrays were constructed from formalin-fixed, paraffin-embedded samples. Immunostaining was carried out for clone VE1 (valid to detect BRAF V600E), CK 19, HBME-1 and Cyclin D1 in order to compare PTC with and without the mutation. Pyro sequencing validated BRAFV600E status in a representative subgroup (n=27).
Results. BRAF V600E positivity (VE) was found in 74/143 (52%), BRAF wild type (WT) in 69/143 (48%). VE PTC had increased CK 19 (66/68 VE vs. 27/64 WT), HBME-1 (58/68 VE vs. 22/64 WT) and expression of nuclear cyclin D1 (17% VE vs. 10% WT). Extra thyroid extension was more obvious in them (23/51 VE vs. 8/60 WT). Therefore, in pT3-level the mean size of a mutated PTC was smaller (2.14 cm VE vs. 3.58 cm WT). Lymph node metastases tend to occur twice as often in VE PTC (15/47 VE vs. 9/52 WT). A positive correlation to classical structured PTC was seen (49/62 VE vs. 13/62 WT; p<0.01), but a negative to follicular variant PTC (16/62 VE vs. 38/62 WT; p<0.01). No difference in age (mean age at diagnosis 52 a), gender (f:m=4.2:1), and follow up events was found.
Conclusions. This study demonstrates the correlation of the BRAF V600E mutational state with expression of CK 19, HBME-1 and Cyclin D1 in PTC, particular in the classical variant. Although an earlier extra thyroid extension and a trend for nodal metastases can be seen, there is no evidence of poorer overall or disease free survival.

FR-085

BRAF V600E-specific immunohistochemistry is complementary to BRAF sequencing—a validation study comparing manual and automated staining

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Aims. Detection of BRAF V600E has diagnostic (e.g. hairy cell leukemia) and therapeutic (e.g. melanoma) relevance. Recently, a BRAF V600E mutation-specific antibody has been developed and may represent a feasible alternative to DNA-analysis. The plethora of immunohistochemical (IHC) protocols makes manual implementation tedious and currently validation studies are lacking. Here, we tested a set of manual IHC-protocols and compared the test performance with sequencing results.
Methods. For all assays we employed formalin-fixed—in part decalcified—and paraffin-embedded tissue samples. Using the commercially available VE1 antibody, IHC-protocols included empiric testing with 9 variables in 17 protocols; pyrosequencing and automated staining served as independent test methods. Results and routine test performance measures were compared without considering one method as a standard.
Results. Staining patterns in 17 protocols indicated 2 correctly classifying procedures. Performance assessment employed thirty-three tissue samples composed of 27 leukemias (8 wild-type; 18 mutated; 1 non-informative) and 6 melanomas (V600E; V600K; wild-type, 2 each) that were genotyped by pyrosequencing. V600E staining was positive in 20 cases (19 of 20 V600E-containing samples + 1 non-informative) whereas all wild-type and V600K-cases were immunonegative. Thus, an equal number of V600E-mutated cases would have been missed by either technique. The correlation coefficient for both methods was 0.75–0.87 and the Youden index was 0.95.
Conclusions. Detection of V600E-mutated BRAF at the protein level using a manual IHC-protocol on routine and decalcified tissue samples is possible. The presented protocols should expedite the implementation process in routine diagnostic practice. In summary, our results indicate that both molecular techniques should be considered complementary rather than mutually exclusive.

FR-086

Comparison of three different PCR-based methods for B-RAF mutation testing

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Aims. Approximately 50% of malignant melanomas harbor activating mutations at codon 600 in the protein kinase BRAF. The new BRAF inhibitor Vemurafenib enables the treatment of patients with these tumors. Over 90% of the observed mutations are a single nucleotide mutation resulting in p.V600E followed by p.V600K, p.V600R, p.V600D and p.V600E2. Recent data have shown that these individual mutations result in different binding kinetics of the BRAF inhibitor. In addition already today, according to the European Summary of Product characteristics of Vemurafenib "no data are available in patients with melanoma harboring BRAF V600 mutations others than V600E and V600K". It remains to be seen, whether novel BRAF inhibitors currently in clinical trials might be approved with different dosages for different mutations. The aim of this study is to evaluate sensitivity and usability of 3 commercial tests to detect mutations in the BRAF gene for IVD.

Methods. The study comprised 90 FFPE tissues from patients with metastatic melanoma. Tumor material was manually microdissected. DNA was extracted using the cobas®DNA sample preparation kit (Roche). BRAF analysis was done by three different real-time PCR-based The cobas®V600 test (Roche), the AmoyDx BRAF V600E Mutation Detection Kit (Amoy Diagnostics) and the therascreen® BRAF RGQ PCR kit (QIAGEN). Non concordant results were additionally analyzed by bidirectional Sanger sequencing.

Results. Of the 90 melanoma tested 35 harbored a V600 mutation (39%): 33 p.V600E, and 2 p.V600K. AmoyDx BRAF V600E kit and the cobas®V600 test identified samples with p.V600E and p.V600K as p.V600 mutation positive. One sample reported by the cobas® test as negative was identified as p.V600E positive by the two other systems. Another sample showed a invalid result with the cobas® test but valid negative results with the other tests. Specific mutations were distinguished by the therascreen® BRAF RGQ kit only and were confirmed by Sanger sequencing.

Conclusions. Real-time PCR is a reliable routine tool for detection of the p.V600 mutations with a faster laboratory turnaround time than Sanger sequencing. The three systems tested showed no significant difference in sensitivity for the V600E mutation. Although the AmoyDx and the cobas®kits are primarily designed to detect the p.V600E mutation also p.V600K was detected through cross reactivity. The therascreen® BRAF RGQ PCR kit was the only system in the study capable of distinguishing between the individual p.V600 mutations.

FR-087

A novel microfluidic-based screening approach for KRAS mutation analysis in colorectal carcinomas

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Aims. KRAS mutation analysis has become a frequent task in molecular pathology laboratories. Here, we validated a novel microfluidic-based screening approach for testing KRAS mutations in microdissected tumor cells of patients with colorectal cancer.

Methods. Colorectal cancer cell lines (n=6) and formalin-fixed and paraffin-embedded (FFPE) tissue resection specimens (n=52) were analysed by dideoxy-sequencing as well as standard qPCR-based (qPCR) and microfluidic-based (mf-qPCR) real-time PCR for KRAS Exon 2 (codon 12, 13) mutations. For FFPE tissue specimens, microdissection of tumor cells prior to DNA extraction was performed.

Results. All 6 cell line and all 52 FFPE -samples resulted in acceptable ddSeq, qPCR (duplicate) and mf-qPCR (duplicate to quadruplet) runs and data. The three methods repeatedly provided the same, known KRAS mutation status in cell lines. In FFPE-samples, the frequency of KRAS mutations detected by ddSeq were KRAS wildtype (53.8%) and KRAS mutations of p.G12D (15.4%), p.G12V (9.6%), p.G13D (9.6%), p.G12C (3.8%), p.G12A (3.8%), p.G12S (1.9%) and p.G12R (1.9%). All three methods showed identical results in FFPE-samples in 38/52 (73.1%) cases. 1/52 (1.9%) cases had the rare KRAS p.G13C mutation, not represented in the qPCR and mf-qPCR design. Of the remaining 11 disputable cases, concordance was seen in 6/11 (54.5%) cases for ddSeq with qPCR and in 0/11 cases for ddSeq with mf-qPCR. In 5/11 (45.5%) cases results agreed for qPCR with mf-qPCR, but disagreed with ddSeq. This was related to the setting of cut-off's for qPCR and mf-qPCR detection

Conclusions. This study explored the value of a novel microfluidic-based approach of KRAS mutation screening in microdissected tumor cells of colorectal cancers. The mf-qPCR had a similar performance as the qPCR assay, both being potential "pre-screening" tools with fast throughput and little "hands-on". However, qPCR and mf-qPCR were limited in specificity due to the uncertainty of previously described and here newly set cut-off's of delta-CT values for bona-fine KRAS mutation detection by real time PCR.

FR-088

Detection of genetic aberrations involving the 3'-region of ALK in NSCLC samples: comparison of FISH, immunohistochemistry, qRT-PCR and a novel RT-PCR based 454 next-generation sequencing approach

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Aims. Non-small cell lung cancer (NSCLC) is the most common cause of cancer-related deaths in western countries. Recently, genetic aberrations involving the 3'region of the gene encoding for the anaplastic lymphoma receptor tyrosine kinase (ALK) located at chromosomal region 2p23 have been identified in 3 to 7% of adenocarcinomas. Patients whose tumors harbor this aberration will benefit from a targeted therapy with the tyrosine kinase inhibitor crizotinib. Genetic aberrations involving ALK are heterogenous, including several different variants of inversions with the echinoderm microtubule associated protein like 4 (EML4) gene located at 2p21, as well as rare translocations with other partner genes, e.g. kinesin family member 5B (KIF5B) located at 10p11 and TRK-fused gene (TFG) located at 3q12. The aim of this study was to generate a novel 454 next-generation sequencing approach for the detection of genetic aberrations of ALK, and to compare it with currently used and well-established methods (FISH, immunohistochemistry).

Methods. NSCLC lung cancer cell line NCI-H2228 with a known EML4-ALK inversion (variants 3a and 3b) was used as a positive control. 100 paraffin-embedded NSCLC samples were used as a test cohort. RNA was isolated from cells as well as from paraffin-embedded tumor tissue, and reverse transcribed using a mixture of random hexamer and oligo-dT primers. PCR amplification was performed from the cDNA, using a multiplex approach with MID-tagged primers in the 5'- and the 3'-region of ALK for 454 next-generation sequencing. qRT-PCR was done from the same cDNA, with differently labeled probes for 5'- and 3'-region of ALK. FISH was performed using the TriCheck probe (Zytovision), and immunohistochemistry was done using clone D5F3.

Results. NSCLC cell line H2228 as well as tumor samples with EML4-ALK inversion showed a higher expression of 3'-region of ALK com-

pared to 5'-region by qPCR, and this was also reflected by higher read counts using 454 next-generation sequencing. All positive samples were directly sequenced by Sanger sequencing to confirm the break point on cDNA level. There was a good correlation between the different methods, with FISH being the most sensitive method.

Conclusions. We developed a novel 454 next-generation sequencing approach for detection of genetic alterations involving the 3'region of ALK. This assay has a good sensitivity comparable to immunohistochemistry, and can be used as part of a multigene panel test for NSCLC using 454 next-generation sequencing.

FR-089
Detection of ALK positive non-small cell lung cancers on cytology specimens: high accuracy of immunocytochemistry with the 5A4 clone

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Aims. Lung cancer is often diagnosed by cytology making it necessary to perform predictive molecular marker analyses on cytological specimens. The gold standard for detection of predictive ALK rearrangements is fluorescence in situ hybridization (FISH), but FISH is both expensive and complex in its interpretation. The aim of our study was to investigate the accuracy of ALK immunocytochemistry (ICC) on cytological specimens of non-small cell lung cancers (NSCLC).
Methods. Forty-one conventional cytological specimens with available ALK FISH results were retrospectively analysed with the monoclonal 5A4 antibody (NovocastraTM, Leica Biosystems Newcastle Ltd., United Kingdom) on a fully automated slide stainer (BondMax, Leica). The specimens were enriched for ALK-FISH positive NSCLC (14/41, 34.1%). Evaluation of the ICC-staining was performed blinded to the FISH results. The staining intensity and the percentage of stained cancer cells were recorded. Any ICC-staining was regarded as a positive result. The ALK-ICC results were compared to the FISH results. In case of a discrepancy the ICC-stained slide and the FISH signals were reviewed.
Results. ICC was evaluable on 40/41 specimens. Fifteen out of 40 NSCLC (37.5%) were ALK-ICC positive with staining of the majority of cancer cells (median 100%, mean 82.3%). Twelve of the ALK-ICC-positive NSCLC (80.0%) showed an intense staining (3+). Compared to the ALK-FISH results only one NSCLC was false negative and one false positive by ICC, respectively. The sensitivity, specificity and positive and negative predictive values for ALK-ICC compared to ALK-FISH were 93.3%, 96.0%, 93.3% and 96%, respectively.
Conclusions. ALK-ICC is highly accurate for detecting ALK-rearranged NSCLC.

FR-090
Prognostic and predictive relevance of p.L576P KIT exon 11 mutations in gastrointestinal stromal tumors

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Aims. Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors of the gastrointestinal tract. Most tumors are driven by oncogenetic KIT and PDGFRA mutations. Mutational analysis has great impact on the clinical management of GIST patients being at high or intermediate risk of progressive disease. For example, the most common PDGFRA mutation in exon 18, i.e. p.D842V, is associated with primary resistance to imatinib which highlights the predictive impact of the mutational status of GISTs. Recently, an in vitro and in silico study reported KIT exon 11 point mutation p.L576P to be less sensitive to imatinib treatment compared to other KIT exon 11 mutations. We were therefore encouraged to investigate whether this specific mutational subtype might be of prognostic and/or predictive relevance.
Methods. We screened our GIST and Sarcoma Registry Cologne/Bonn with more than 3500 GIST tumor samples for p.L576P mutated cases. Additional cases were contributed by the different institutions. Clinicopathological characteristics and follow up data were collected.
Results. Altogether, 60 cases with KIT exon 11 point mutation p.L576P were identified. 48 cases presented as primary tumors. Among them, 23 cases were found in the stomach, 18 in the small bowel and five in the colon. Two cases were classified as extra-gastrointestinal GISTs. The further cases presented as metastases (e.g. in the liver or peritoneum). There was a slight male predominance (29 vs. 27). Tumor size was 4.7 cm (range: 0.7–17.0 cm; median: 4.0 cm). Mitotic count ranged from 0–42 (median: 1, mean: 5.5). Among 39 analysable cases, 7 cases were classified as being at intermediate and 9 cases at high risk of progressive disease. The rest belonged to the low/very low/no risk categories.
Conclusions. We report on a large and unique cohort of patients with gastrointestinal stromal tumors harbouring a KIT exon 11 mutation (p.L576P). Concerning clinicopathological standard parameters (e.g. age, size, gender distribution, mitotic count, localisation and risk classification) these tumors do not display a different phenotype compared to KIT exon 11 mutated GISTs in general. The analysis of follow up data is ongoing.

FR-091
Physical background of "magnification rule" for standardized Her2 and EGFR-IHC-scoring

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Aims. The detection of a HER2 overexpression and/or -amplification is a prerequisite for the targeted therapy of breast and gastric cancer with HerceptinTM. A major challenge is the interlaboratory variation of 12–26% (Piccart St Gallen 2009). In the context of the ToGA-study we developed the so-called "magnification rule" for standardization of HER2-IHC: according to this rule the magnification needed for detection of HER2 membrane staining and IHC intensity score are closely related. In a round robin test (RRT) the application of the magnification rule markedly improved the inter-observer concordance of HER2 IHC scoring (from κ<0.5 to κ>0.8; Rüschoff et al. Virch Arch 2010). The

current study analyzes the fundamentals of the magnification rule including optical-physical aspects.

Methods. In an international RRT (ESMO 2011, Abstr 9002) NSCLC samples were stained in 7 different institutes using the Dako EGFR pharmDxTM-Kit or the Ventana CONFIRM anti-EGFR (3C6) primary antibody. Using non-confocal brightfield microscopy and the software ImageJ, width and color intensity of intercellular DAB chromogen precipitates were measured semi-automatically at 40× magnification in 40 different TMA spots (20–40 tumor cells per spot, 4 measurements per cell). The data were compared with staining intensity scores (0–3+) determined by 10 international pathologists.

Results. The semi-quantitative staining intensity score is closely related to color intensity measured by image analysis and width of DAB precipitates. The mean width ±SD of precipitates was 0.78±0.21 µm for score 1+, 1.27±0.31 µm for 2+ and 2.60±0.85 µm for 3+. The width of precipitates from the intensity scores closely matched the optical resolution of the corresponding microscope objective lenses: Approximately 0.5 µm (40×), 1.0 µm (10×) and 2.0 µm (5×). This correlation was found independent of selected antibody and institute where the IHC stain was performed.

Conclusions. The perceived intensity, the width of the DAB chromogen precipitate, and the objective intensity determined by image analysis are closely related. These interrelations underlie the "magnification rule". This rule can be used to replace subjective estimation of staining intensity for scoring of membrane bound biomarkers by a quasi morphometric measurement of chromogen precipitate width using microscope objectives.

FR-092
Enzymatic assay for quantitative analysis of (D)-2-hydroxyglutarate

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Aims. Levels of (D)-2-hydroxyglutarate (D2HG, (R)-2-hydroxyglutarate) are increased in some metabolic diseases and in neoplasms (glioma, AML, chondrosarcoma and cholangiocarcinoma) with mutations in the isocitrate dehydrogenase 1 (IDH1) and isocitrate dehydrogenase 2 (IDH2) genes. Determination of D2HG is of relevance to diagnosis and monitoring of disease. Standard detection methods of D2HG levels are liquid-chromatography-mass spectrometry (LC/MS) or gas-chromatography-mass spectrometry (GC/MS). Here we present a rapid inexpensive and sensitive enzymatic assay for the detection of D2HG levels in tumor tissue (FFPE and fresh frozen tissue) and blood serum.

Methods. The assay is based on the conversion of D2HG to alpha-ketoglutarate (alpha-KG) in the presence of the enzyme (D)-2 hydroxyglutarate dehydrogenase (HGDH) from the anaerobic bacterium Acidaminococcus fermentans and nicotinamide adenine dinucleotide (NAD+). The determination of D2HG concentration is based on the detection of stoichiometrically generated NADH via diaphorase and resazurin which enables a fluorescent read-out on a spectrophotometer.

Results. The quantification limit of the enzymatic assay for D2HG enables detection of low basal D2HG levels in human tumor tissues and serum without IDH1 or IDH2 mutations. We estimated quantification limits of 0.44 µm in tumor tissue and 2.77 µm in serum. In contrast to the low basal levels concentrations of D2HG in tumor tissues containing IDH mutations or in serum from acute myeloid leukemia (AML)

patients with IDH mutations are significantly higher and can therefore be easily distinguished.

Conclusions. In summary we developed a sensitive enzymatic assay which allows a cost effective screening of human tissues and serum for D2HG. This will help to identify all kind of IDH1 and IDH2 mutations producing D2HG.

Postersession Pneumopathologie

FR-093
Experimental models of pulmonary arterial hypertension in humans

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Aims. Pulmonary arterial hypertension (PAH) is a potentially fatal disease which can occur independently, in the context of heart malformations or other diseases such as pulmonary embolism or COPD. Clinically, PAH is characterized by a discrepancy between dramatic clinical presentation and only minor histological findings. Characteristic histopathological changes in severe forms of PAH are plexiform lesions (PL), complex vascular neoformations bulging from the pulmonary arteries, as well as concentric lesions (CL), small arteries with dominant fibrotic obliteration. Animal experiments are widely used to gain further insights into the mechanisms of PAH. Particularly, rats which are treated with vascular endothelial growth factor (VEGF) receptor inhibitors represent a widely used model. This leads to VEGF overexpression and consecutively to PAH and vascular remodeling. Furthermore, different neoplastic models have been proposed such as glomeruloid-like lesions (GLL), circumscribed peritumoral vascular formations, in glioblastomas. This study investigates whether these models adequately reflect the changes seen in human PAH.

Methods. We performed laser-assisted microdissection of formalin-fixed, paraffin-embedded (FFPE) lung explants of patients suffering from PAH (n=13). Furthermore, we analyzed lungs of 20 rats, which had been exposed to hypoxia after receiving varying doses of a VEGF receptor blockers (SU5416) und subsequently developed PAH. From these specimens we isolated three different compartments: CL, PL and arteries next to PL. As a reference, we examined GLL from 5 patients and pulmonary arteries form downsized donor lungs (n=16). Microdissected samples were analyzed by quantitative real-time PCR based on TaqMan low-density arrays. We analyzed 45 angiogenesis-associated genes in each compartment using target gene-specific preamplification.

Results. Angiogenesis-associated genes, such as VEGF-alpha, THBS1, NOTCH4 and HIF-1a were up-regulated in human PAH lungs. Histologically, the remodeled rat lungs showed pathological changes comparable to those seen in human PAH lungs. The molecular microenvironment as well as the cellular composition of GLL differed significantly from plexiform vasculopathy.

Conclusions. Histological and molecular aspects of plexiform vasculopathy in humans and rats, particularly differences in expression of angiogenesis- and tissue remodeling-associated genes, are discussed. Neoplastic models of angiogenesis do not seem to be adequate settings for the study of PAH.

Abstracts

FR-094

Pulmonary-selective non-viral somatic gene transfer of COX2 and PGI2-synthase attenuates pulmonary arterial hypertension in a hypoxia induced mouse model

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Aims. Pulmonary arterial hypertension (PAH) is a life threatening disorder that has a multifactorial pathogenesis. Vasoconstriction and pulmonary vascular remodelling progresses to right heart failure. Several pharmacological concepts were introduced meanwhile. To increase the therapeutic half-life we applied a non-viral somatic gene transfer. Nano particles of DNA encoding prostacyclin(PGI2)-synthase and/or cyclooxygenase 2 (COX2) were complexed with polycationic polyethylenimine (PEI). Orotracheal aspiration allowed a pulmonary selective application. Our aim was to reduce pulmonary arterial pressure and right heart burden.

Methods. Four groups of 10 C57Bl/6N mice each were kept for 28 days in normobaric hypoxia (FiO2=0.1) while control group was kept in normoxia. Application of PEI-DNA particles was carried out at day 1, 7, 14, 21. The groups encompass single COX2 or PGI2-synthase encoding vector, combination of both and empty vector. After 28 days echocardiography and hemodynamic measurements were performed, heart weight was determined and lungs were isolated for vascular morphometry analysis.

Results. Application of PGI2-synthase or COX2 encoding vector each resulted in increased cardiac output and heart index while right ventricular systolic pressure, right ventricle enddiastolic diameter, myocardial performance index and weight of right ventricle decreased significantly. Co-application of both vectors led to a synergistic effect with additional increase of the cardiac output. Vascular morphometric analysis revealed reduction of completely muscularized resistance arterioles in all three groups (COX2, PGI2-synthase, and COX2+PGI2-synthase) indicating reduced vascular remodelling.

Conclusions. Somatic non-viral gene transfer of PEI-DNA nano-particles was successfully applied to a mouse model of hypoxia induced pulmonary hypertension. COX2 or/and PGI2-synthase encoding vectors could attenuate pulmonary vascular pressure as well as remodelling of pulmonary arterioles and right ventricle myocardium. In consequence, this pulmonary selective gene transfer was proven to be a suitable approach for PAH treatment.

FR-095

The contribution of p120-catenin modulated NF-κB activation in airway epithelial cells of acute lung injury induced by LPS

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Aims. The purpose of this study is to investigate the role of p120-catenin (p120) modulated nuclear factor kappa B (NF-κB) activation in airway epithelial cells of acute lung injury induced by LPS, and further to explore the molecular mechanisms.

Methods. In this study, the mouse model of acute lung injury (ALI) was established by one intra-tracheal injection of LPS. The lung tissue was collected at 1, 4, 7 and 14 days after stimulation. The morphological changes of tracheal epithelium were studied. SP immunohistochemistry was used to detect the p120ctn expression of in airway epithelial cells. Western blot was used to detect the expression of p120ctn, IκB and phosphoric IκB (pIκB) in lung tissues. ELSIA was used to detect NF-κB downstream inflammatory cytokines IL-8 and IL-1beta proteins levels in lung tissue lysates.

Results. In the present study, the experimental group of murine airway epithelial cells showed morphological changes of injury and repair after LPS injection. SP immunohistochemistry stain demonstrated that p120ctn expression on the lateral cell membrane was decreased at first, then cytoplasmic expression was present, finally, the linear membranous expression of p120 was restored. Western blot results were basically consistent with the morphologic changes. P120ctn expression in the lung tissue was firstly reduced and then recovered. The reverse change was found in NF-κB. IκB phosphorylation was present upon LPS stimulation; pIκB was increased and then decreased. ELSIA results showed that IL-8 and IL-1beta concentration was firstly increased (p<0.05), and then restored to normal, compared with the control.

Conclusions. Collectively, these results indicate that p120ctn may play an important role in the acute inflammatory response of airway epithelium, which may mediate by NF-κB signaling activation via IκB phosphorylation.

FR-096

Pulmonary light chain deposition disease—a case report

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Aims. A 61-year-old female with a history of chronic obstructive pulmonary disease complains about progressive dyspnoea. Diffuse nodular and cystic changes in the CT scan suggest Langerhans' cell histiocytosis. Serum level of IgG kappa is elevated.

Methods. After lateral thoracotomy a wedge resection from the right lower lobe is performed. Histological examination shows interstitial and perivascular deposits of amorphous eosinophilic material. Although the deposits resemble amyloid in routine stain, the Congo-red stain is negative and shows no birefringence. Immunohistologically there are only kappa light chains, but no lambda chains are detectable within the amorphous material. In contrast, the admixed plasma cells are polyclonal. In the bone marrow biopsy, the number of polyclonal plasma cells is only slightly increased.

Results. Since neither a plasmocytoma nor another lymphoproliferative disorder is evident, pulmonary light chain deposition disease is diagnosed. Nevertheless, following a plasmocytoma regime, the patient was treated with cyclophosphamide, bortezomib and dexamethasone. Hereafter, only a slight progression has been noticed within the last two years.

Conclusions. We report a very rare case of light chain deposition disease restricted to the lung.

FR-097

Dirofilariasis—a rare cause of a pulmonary nodule

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Aims. A 54-year-old female smoker of Mediterranean descent presents with dyspnoea. In the left upper and lower lobe of the lung the CT scan shows three well circumscribed nodules measuring less than 1 cm.

Methods. After lateral thoracotomy the nodules were removed by enucleation and wedge resection.

Results. Histopathological examination identifies the nodule in the upper lobe to be a fibrotic subpleural lymph node. The lager mass in the lower lobe represents a chondrohamartoma. The third nodule shows a thick fibrotic capsule surrounding a necrotic and calcified parasite. PAS and Grocott stains highlight the worm's internal structures. The histopathological findings meet the diagnosis of pulmonary dirofilariasis.

Conclusions. Dirofilaria immitis (dog heart worm) is thought to be transmitted by mosquitoes from dogs, cats and others. The adult worms reside in the right ventricle of the definitive host. Since humans are unsuitable hosts, the parasite dies inside the right ventricle and embolizes to the lungs. Although rare, human pulmonary dirofilariasis is endemic in all continents. No further therapy is necessary since the patient has been cured by surgical excision.

FR-098
Pulmonary dendriform ossification—an entity?

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Aims. We report on three different patients with pulmonary dendriform ossification.

Methods. Commonality among patients: All patients are male and middle aged or elderly (57, 67 and 75 years old). The CT scan shows a reticular opacity pattern. From the right lower lobe a wedge resection or lobectomy is performed. The histopathological examination reveals a dendriform ossification in all subjects.

Results. Individual conditions: Patient no 1: The 57-year-old dental technician presents with a tension pneumothorax. During thoracoscopy a perforation of the visceral pleura by ossified coral-like structures is visible. Patient no 2: The 75-year-old former smoker complains about progressive dyspnoea on exertion. A severe COPD (GOLD III) with combined restrictive and obstructive ventilation disorder is diagnosed. The temptative diagnosis of an idiopathic pulmonary fibrosis leads to a wedge resection. A few weeks after operation, the patient develops a pneumothorax. Patient no 3: The patient presents with bilateral pulmonary masses. After diagnosis of mucinous lung cancer by core needle biopsy, thoracotomy with lobectomy of the right lower lobe and wegde resection of the middle lobe is performed. A multifocal mucinous (colloid) adenocarcinoma with large mucinous bronchiolo-alveolar carcinoma is diagnosed. A palliative chemotherapy (pemetrexed and carboplatin)is given. The patient dies from neutropenic sepsis a few weeks later.

Conclusions. Dendriform ossification of the lung is a rare disease of unknown origin. It may present as main diagnosis or can accompany other conditions, i.e. lung cancer. Dendriform ossification predisposes to pneumothoraces. Clinical, radiological and functional features of the presented patients vary clearly. Pulmonary dendriform ossification is not likely to be an etiological entity.

FR-099
Pulmonary manifestations of Crohn's disease

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Aims. Crohn's disease can be associated with extraintestinal manifestations, including rheumatologic, ocular, dermatologic, biliary and pulmonary manifestations. Pulmonary manifestations include parenchymal disease, pleuritis, granulomatous stenosis of the large airways and overlap syndromes.

Methods. We present a case of a 13-year-old girl with a history of an inflammatory bowel disease for 10 years. The clinical course was severe with resistance to therapy and various courses of different immunosuppressive therapies including regular Infliximab-therapy in increasing doses since 2009. Histopathologically in intestinal biopsies a Crohn's disease had been ascertained. Several infectious complications occurred including abscesses of cervical lymph nodes and a liver abscess in 2010 and 2011. In November 2011 she was presented with a history of shortness of breath and fever since 3 days. She showed a decreased oxygen saturation and tachypnea. Pneumonia of the left lower lobe was diagnosed. After delayed clinical improvement further examinations were initiated. Lung function testing revealed a reduction of FEV 1 to 40% predicted and VC to 39% predicted. CT-Scans showed mosaic pattern with focal hyperinflation and ground-glass opacity. In the BAL a marked lymphozytosis of 69% was found. Diagnosis of obliterative bronchiolitis was suggested. The patient refused therapy with high dose steroids. After a period of 3 months under close observation and without clinical improvement or deterioration, lung biopsy was planned.

Results. Lung biopsy revealed granulomas without fibrosis. Due to histological manifestation of these granulomas, pulmonary manifestation of Crohn' disease was diagnosed and a sarcoidosis excluded.

Conclusions. Diagnosis of concurrent extraintestinal manifestations of Crohn's disease in the lung are rare in children but have to be taken into consideration as a differential diagnosis when the intestinal manifestation of Crohn's disease is proven.

FR-100
The p53 target gene desmocollin 3 acts as a novel tumor suppressor through inhibiting EGFR/ERK pathway in human lung cancer

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Aims. Desmosomes are intercellular junctions that confer strong cell-cell adhesion. Altered expression of desmocollin 3 (DSC3), a member of the desmosomal cadherin family, was found in various cancers. The aim of our research is to elucidate its functional involvement in human lung carcinogenesis.

Methods. Expression/localization of DSC3 was analyzed by real-time reverse transcription-PCR, western blotting, immunofluorescence and immunohistochemistry. Methylation status of DSC3 was examined by demethylation tests, methylation-specific PCR and bisulfite sequencing. To investigate the effect of the tumor suppressor gene p53 on DSC3, transient transfection with a wild-type p53-expression vector was performed. Furthermore, functional studies after stable transfection of a DSC3 expression vector were carried out, including proliferation assay, soft agar assay, migration/invasion assay.

Results. It turned out that downregulation of DSC3 in lung cancer cells was associated with DNA hypermethylation. In primary lung tumors, DSC3 was a potential diagnostic marker for lung squamous cell carcinoma, and DSC3 DNA hypermethylation was correlated with poor clinical outcome. Overexpression of p53 resulted in an increased expression of DSC3 in a DSC3-unmethylated lung cancer cell line H2170, but not in H1299, a DSC3-methylated cell line. However, combination of p53 transfection with demethylation agent 5-aza-2-deoxycytidine treatment led to increased expression of DSC3 in H1299 cells. Functional studies showed that ectopic expression of DSC3 inhibited cell proliferation, anchorage-independent growth, migration, as well as invasion, and most interestingly led to reduced phosphorylation levels of extracellular signal-regulated kinase 1/2.

Conclusions. Taken together, our data suggested that DSC3 acts as a novel tumor suppressor gene through inhibition of epidermal growth factor receptor/extracellular signal-regulated kinase signaling in lung cancer cells.

FR-101

Tuberous sclerosis complex (TSC)-related cell signaling in the pathogenesis of lung cancer

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Aims. Hamartin and tuberin, encoded by the tuberous sclerosis complex (TSC) genes TSC1 and TSC2, form a tumor suppressor complex which is implicated in PI3K-Akt signaling and acts as a functional inhibitor of the mammalian target of rapamycin (mTOR). We have addressed the role of hamartin (TSC1), phopsho-tuberin (p-TSC2) as well as phospho-mTOR (p-mTOR) in non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC) specimens and lung cancer cell lines.

Methods. 168 NSCLC resp. SCLC specimens were recruited using tissue microarrays (TMA) and were immunohistochemically analysed concerning the expression of TSC1, p-TSC2 and p-mTOR. Further, these data were correlated with upstream factors of EGFR- and MAP Kinase-associated signaling. Western blot analyses for TSC1, p-TSC2 and p-mTOR as well as mutational analyses for TSC1 were performed in NSCLC and SCLC cell lines.

Results. TSC1 expression was observed in more than 50% of adenocarcinomas (AC), squamous cell carcinomas (SCC) and in 14% of SCLC specimens. Staining of p-mTOR was found in a subset AC and SCC, either cytoplasmic or nuclear. p-TSC2 expression was found only in a minority of cases. In AC, TSC1 was correlated with p-EGFR (Tyr-1068) and p-EGFR (Tyr-992) expression. P-mTOR was correlated with p-EGFR Tyr-1173 in AC, and inversely correlated with p-EGFR Tyr-992 in SCC specimens (all p<0.01). There was no effect on TSC1, p-TSC2 and p-mTOR expression responsive to EGFR mutations. In SCLC and NSCLC cell lines, an inverse correlation between TSC1 and p-mTOR was found, compatible with the inhibitory role of the TSC complex.

Conclusions. Our results show TSC1, p-TSC2 and p-mTOR expression in a substantial subset of NSCLC/SCLC specimens and an inhibitory role of the tuberous sclerosis complex for m-TOR activation in lung cancer cell lines could be shown. These findings indicate a functional role of the tuberous sclerosis complex in lung cancer pathogenesis.

FR-102

The homeobox gene HOPX is methylated and exerts tumor suppressive function through inhibition of PI3K/AKT-MDM2-p53-p21 axis in human lung cancer

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Aims. Our previous studies showed that HOPX has tumor suppressor function in lung cancer. However, the regulation of HOPX in lung cancer has not yet been well elucidated. The aim of the study is to investigate the epigenetic regulation, analyse the mechanisms of HOPX as a tumor suppressor, and evaluate the clinical relevance of HOPX in lung cancer.

Methods. Real-time RT-PCR and Western blot analysis were performed to analyse the expression of HOPX in lung cancer cell lines. The protein expression of HOPX in 120 primary lung tumors was evaluated by immunohistochemistry on tissue microarry. To investigate methylation status of HOPX, demethylation test, bisulfate sequencing (BS), and methylation-specific-PCR (MSP) were carried out. For the functional analysis, stable transfection as well as MTT assay, wound healing assay, migration/invasion assay, apoptosis and cellular senescence assay was performed.

Results. HOPX was downregulated in 11 out of 12 lung cancer cell lines. Majority of primary lung tumor tissues (69 out of 120) exhibited low expression of HOPX protein. Eight lung cancer cell lines restored HOPX expression after treatment with demethylation agent 5-Aza-2-deoxycy-

tidine, and the methylation status of HOPX in exon 1 was confirmed by BS. In primary lung tumors, MSP showed that HOPX was methylated in 64 out of 88 (72.7%) samples, and lower expression of HOPX was significantly correlated to HOPX DNA hypermethylation (p<0.001). Additionally, methylation of HOPX was significantly associated with adenocarcinoma of lung (p<0.001). In line with our previous results, HOPX could suppress tumor cell growth and mobility. Furthermore, HOPX overexpression could increase tumor cell senescence possibly through inhibition of PI3K/AKT activity and upregulation of p53 and p21. On the contrary, knockdown of HOPX by siRNA reduced cellular senescence levels by activating PI3K/AKT pathway and downregulation of p53 and p21.

Conclusions. DNA hypermethylation is a critical mechanism for HOPX gene silencing in lung cancer, and methylation status of HOPX may be a diagnostic marker for patients with adenocarcinoma of lung. HOPX acts as tumor suppressor possibly through interrupting PI3K/AKT-MDM2-p53-p21 pathway.

FR-103

P53 E3 ubiquitin protein ligase homolog (mouse) (MDM-2) immuno-expression is a strong predictor of poor overall survival in malignant pleural mesothelioma

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Aims. Malignant pleural mesothelioma (MPM) is a biologically aggressive tumor harboring a dismal prognosis, for which novel and/or additional treatment modalities are needed. In contrast to other solid malignant tumors, TP53 mutations are extremely rare in MPM. However, in TP53 wild-type tumors, the function of P53 can be deleted by the E3 ubiquitin protein ligase homolog (mouse) (MDM-2), which was found to be overexpressed in several tumour types including lung, breast, colon, stomach and liver malignancies. Hence, the immunoexpression of P53 and Mdm2 in MPM was investigated and its effect on overall survival (OS) was determined.

Methods. The immunoexpression of Mdm2 and P53 was investigated by immunohistochemistry in tissue microarrays (TMA) from 63 patients with MPM of varying subtypes (epitheloid, biphasic and sarcomatoid) and statistical survival analysis was performed.

Results. 13 out of 63 (20.6%) tissue samples showed nuclear immunoexpression of Mdm2. P53 immunoexpression could not be detected. Statistical analysis of the Cox-regression showed a significant correlation between the Mdm2 immunoexpression to survival in a linear [Likelihood ration test: p=0.0477; Score (logrank) test: p=0.0242] and logarithmic scale [likelihood ration test: p=0.0420; Score (logrank) test: p=0.0226].

Conclusions. Mdm2 immunoexpression is significantly associated with poor survival in MPM of varying subtypes compared to Mdm2 negative tumors. This might be due to an amplification and/or overexpression of Mdm2, which potentially results in an inactivation of functional P53 protein.

FR-104
Expression of desmoglein 1–3 in human lung cancer

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Aims. Desmogleins (DSG1-4) are membrane spanning components of the cell-cell connecting desmosomes. Desmosomal proteins have been found to be dysregulated in different cancers. However, the role of DSGs in lung cancer seems to be ambiguous. To find out the role of DSGs and their possible diagnostic and prognostic impact, it is first of all important to get a differential picture of their expression in lung cancer.
Methods. In this study mRNA expression of DSG1-3 in lung cancer cell lines and in human bronchial epithelial cells (HBEC) was examined by real time RT-PCR. Methylation status of DSG2 was evaluated by demethylation test (treatment of cells with demethylation agent 5-aza-2'-deoxycytidine) and bisulfite sequencing (BS). In primary lung tumors, protein expression of DSG1-3 was analyzed by immunohistochemistry (IHC) on tissue micro array (TMA) containing 398 lung tumour samples representing 199 primary lung tumour patients. The protein expression level was compared to clinicopathologic features of the patients.
Results. It turned out that DSG1-3 was downregulated in most of the examined lung cancer cell lines. Re-expression of DSG3 after demethylation treatment could be detected in adenocarcinoma (AD) cell lines. Also an increased expression of DSG2 mRNA was found after demethylationin in some cell lines. Complete or partial methylation of DSG2 promoter as well as exon1/intron 1 region could be detected in five out of six cancer cell lines, while no methylation was found in HBEC. The methylation status was correlated to the mRNA expression of DSG2. By IHC it could be confirmed that a high protein expression of DSG2 and DSG3 correlated to the diagnosis of squamous cell lung cancer (SCC; p=0.015 and p=0.000, respectively), additionally a lower expression of DSG3 was linked to higher tumor grade (p=0.000) in the whole tumor settings.
Conclusions. DSG1-3 was downregulated in the majority of lung cancer cell lines compared to HBEC, which could be partially explained by DNA methylation. DSG2 and DSG3 proteins might be potential diagnostic markers for SCC, and moreover DSG3 is a potential differentiation marker for lung cancer.

FR-105
Diagnostic evaluation of the DNA methylation biomarker SHOX2 in pleural effusions

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Aims. SHOX2 DNA methylation has recently been introduced as a sensitive and specific lung cancer biomarker in bronchial aspirates. Cytological investigation is the gold standard for diagnosis of malignant pleural effusion but has a limited sensitivity of 57% according to the literature. This is to a great extent caused by a sparse amount of tumour cells in the effusion fluid, leading to a false negative or equivocal cytological diagnosis. The concern of our study was to determine the performance of a SHOX2 DNA methylation assay (SDMA) in pleural effusions and to evaluate its potential diagnostic aid for routine cytology.
Methods. From January 2010 to August 2011, pleural effusion specimens of 3203 patients were sent to the Department of Cytopathology. To ensure good quality and processing, only specimens from cooperating institutions that sent more than 20 effusions during this period were finally enclosed. A clinical follow-up was obtained for the remaining cohort of 1536 pleural effusions. SHOX2 DNA methylation was analysed in triplicates with an in vitro, methylation sensitive Real Time PCR based diagnostic test kit, the Epi proLung BL Reflex Assay (Epigenomics AG, Berlin).
Results. 724 specimens of 389 men and 331 women (4 not known, median age 72 years) had a valid result, the remaining specimens were invalid,

most probable due to DNA degradation. Cytology, SHOX2 DNA methylation and combined cytology+ SHOX2 DNA methylation achieved sensitivities of 54%, 41% and 60%, and specificities of 99%, 96% and 96%, respectively (adjusted cut-off ΔΔCT=7.5). SHOX2 DNA methylation was independent of gender and age. The SDMA detected 55% of primary lung cancers, 62% of breast cancers, 44% of gastrointestinal carcinomas, 58% of lymphoma and 45% of miscellaneous tumours. SHOX2 DNA methylation was observed in 22 patients with benign conditions, including cardiac congestion, pneumonia, sepsis, arrhythmia, renal failure and others.
Conclusions. Additional SDMA after inconclusive cytology (negative and equivocal) slightly enhances detection of malignant pleural effusion compared to cytology alone. Reported lung cancer specificity of the SDMA was not confirmed in malignant pleural effusions.

FR-106
Immunohistochemical characterization of CD24 in lung cancer

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Aims. Lung cancer is a major cause of cancer related deaths worldwide. Growing evidence supports a pathogenic role of the expression of the glycosylated cell surface protein CD24 in the pathogenesis of lung cancer. Expression of CD24 has been found to be associated with a more aggressive course of the disease. However, conventional antibodies used in previous immunohistochemical studies have raised technical concerns related to epitope specifity.
Methods. Here, we have investigated the role of CD24 in non-small cell lung cancer (NSCLC) and its clinicopathological significance in a cohort of NSCLC patients. Tissue microarrays (TMA) comprising 148 adenocarcinoma (AC) and squamous cell carcinoma (SCC) of the lung were immunostained using a new monoclonal antibody against CD24 (SWA11).
Results. Cytoplasmic expression of CD24 has been observed in 35% of AC and 25% of SCC. Membranous staining was obtained in 14% of AC resp. 25% of SCC. Membranous staining of CD24 was significantly associated with lymphonodular spread (p<0.05), but did not correlate with tumor grades. Furthermore, membranous CD24 expression was related with poorer overall survival (p<0.05). In contrast, cytoplasmic staining of CD24 revealed no influence on the survival rates. After stratification for NSCLC subtypes, membranous CD24 staining tended to correlate with lower survival rates in AC, however, without reaching significance. Membranous CD24-positive cases frequently showed a simultaneous staining of the neuroendocrine marker chromogranin (p<0.05).
Conclusions. Our results favour the monoclonal antibody SWA11 as a promising new immunomarker for a more specific detection of CD24 as a cell surface protein in NSCLC. Membranous but not cytoplasmic CD24 staining is associated with a poorer outcome, indicating the importance of a careful consideration of subcellular staining differences. Our preliminary results also point towards CD24 as new neuroendocrine immunomarker.

FR-107
The potential influence of poly(ADP-ribose)polymerase inhibition in the personalized therapy with Gefitinib in human non-small-cell lung cancer

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Aims. Given that PARP-1 was found to be overexpressed in various cancers including human lung cancer at mRNA level (Ossovskaya et al. 2010), and the protein expression of PARP-1 in human lung cancer was not well studied we wanted to evaluate the clinicopathological relevance

of PARP expression in lung cancer patients and we aimed to explore whether PARP-1 alone or combination with EGFR-TKI Gefitinib may act as a new therapeutic target in lung cancer since it was shown that EGFR inhibition by monoclonal antibody Cetuximab augments cellular susceptibility to PARP inhibition in head and neck cancer cell lines.

Methods. The expression of PARP-1 in lung cancer cell lines and in patient samples was examined on mRNA and protein levels by real-time RT PCR and western blot. The effect of Olaparib on cell proliferation was analyzed by colony forming and crystal violet assay in three NSCLC cell lines, H2170, H1650, as well as H1975 (Gefitinib reistant). Furthermore we analyzed the underlying mechanisms which could be responsible for decreased proliferation after drug treatment by western blot analysis and SA-β-Gal staining.

Results. We found that PARP-1 was overexpressed in a panel of lung cancer cell lines at both mRNA and protein levels. In primary lung tumors, 67.4% of patients (163 out of 242) had no expression of PARP-1, while the other 32.6% (79 out of 242) of patients were staining positive with PARP-1. PARP-1 protein expression did not differ significantly by clinicopathological parameters such as sex, histology, tumor size, lymph node status and survival though, there was a tendency that well-differentiated tumors showed less expression of PARP-1 (p=0.077). Proliferation assay revealed that all three cell lines had decreased proliferation rate after Olaparib alone or Olaparib in combination with Gefitinib treatment. Analysis of key proteins involved in EGFR/Akt, MAPK, and p53 pathways by western blot showed that the treatment effects on cell proliferation were in a cell-specific manner, for example, in H2170, decreased cell proliferation is accompanying cellular apoptosis, while in H1975, cellular senescence could be detected.

Conclusions. Taken together, our results suggest that PARP-1 is a potential marker for tumor differentiation, PARP-1 inhibitor might be a potential therapeutic agent for treatment of patients with NSCLC, and moreover, the combination therapy of Olaparib and Gefitinib may overcome resistance to EGFR-TKI by induction of senescence and/or apoptosis.

FR-108
11-year survival of a patient with untreated, EGFR positive pulmonary adenocarcinoma

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Aims. Lung cancer is the most common cause for cancer related death and the fourth most common cause of death in Germany. The five-year survival rate in European and North American countries is in between 5.5% and 15.7%. In the last couple of years significant progress has been made concerning personalized medicine in this disease entity. Novell targeted therapies with tyrosin kinase inhibitors (TKI) for patients with activating mutations in the EGFR gene offer a new treatment option.

Methods. A 78-year-old male patient was referred to the Thoraxklinik Heidelberg with atypical thoracic pain after cardiologic assessment. In 2001 he had been diagnosed with pulmonary adenocarcinoma of the right upper lobe in the Asklepios Hospital Munich-Gauting. Because of his overall good clinical condition the patient had declined any treatment to this point. Compared to the computer tomography of 2001 a new PET-CT revealed a significant growth of the lesion in the right upper lobe as well as new, small bipulmonar lesions suspicious of lung metastases.

Results. A new histological assessment was performed on endobronchial forceps biopsies of the now occluded right upper lobe. The diagnosis of a partial lepidic differentiated adenocarcinoma was consistent with the histopathology of the preserved specimens of 2001. We were able to perform an EGFR mutation analysis of the new as well as of the tumor specimens of 2001 through PCR-amplification and bidirectional sequencing of exons 18 to 21. In each of the probes we detected a deletion combined with an insertion c.2127_29del (p.E709_T710delinsD) in exon 18.

Conclusions. The above mentioned mutation has already been described in the literature but there is no information concerning the responsiveness of tyrosine kinase inhibitors in this particular mutation. Now that the cancer had spread we decided to start treatment with erlotinib. Due to cutaneous side effects the patient decided to discontinue this therapy. Since then he has been in regular follow-up showing a stable disease and good general state of health.

FR-109
Epigenetic regulation of CD34 in solitary fibrous tumors (SFTs)

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Aims. Apart from its role in hematopoietic differentiation, the cell surface protein CD34 is of importance for the classification of soft tissue tumors. It is particularly highly expressed in Solitary Fibrous Tumors (SFTs). SFTs are mesenchymal spindle-cell tumors with variable fibrous components, which were originally described in the pleura. However, in the course of time, SFTs were also recognized in many other extrapleural locations. Although CD34 is relevant for the diagnosis of SFTs, its function in these tumors is not known. Interestingly, it has been reported that a decrease in expression is supposed to indicate malignant progression in SFTs. Up to now, regulation of CD34 expression has not been clarified. In general, methylation of CpG loci in the promoter region of genes is known to play an important role in modification of gene expression. The aim of this study was to determine the relevance of DNA methylation for the regulation of CD34 in SFTs.

Methods. We analyzed a cohort of 34 SFT samples from 23 patients aged between 33 and 79 years. Using pyrosequencing after bisulfite conversion, we examined the methylation levels of four CpG loci within the CD34 promoter region. To interlink DNA methylation and CD34 expression, quantitative mRNA analysis was performed by means of reverse transcription real-time PCR. Finally, we identified the amount of protein present in the tumor samples using semi-quantitative immunohistochemistry.

Results. In our cohort, we observed a broad range of CD34 methylation rates for all four CpG loci covering 0 to 100% (mean 19% to 47%). Similarly, protein levels varied from absent to strong. Connecting these findings, we discovered significant inverse correlations of −0,584 to −0,719 (Pearson correlation coefficient, p<0.01) between DNA methylation and protein levels for all four CpG loci.

Conclusions. We suggest DNA methylation to play a relevant role in regulation of CD34 expression in SFTs. Additional investigations regarding functional analyses are ongoing.

FR-110
BRAF V600E mutation status in histiocytoses does not allow diagnostic distinction between pulmonary and systemic disease

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Aims. When a case of pulmonary histiocytosis is encountered, one key diagnostic question is regarding simultaneous involvement of other organ systems. Recently, BRAF V600E mutations were described in 57% of histiocytoses and it is currently unknown whether mutational testing can help in the diagnostic distinction between "lung-only" his-

tiocytoses and "systemic" cases where at least one other organ-system is affected. Here, we validated the BRAF V600E mutation frequency in a series of histiocytoses and examined association of mutation status with pulmonary and/or systemic disease.

Methods. ICD-O codes were used to retrospectively identify cases from our clinical database and the study cohort was selected by applying the following inclusion criteria: histologically confirmed diagnosis, sufficient material for molecular testing, and availability of clinical information including primary presentation and involved organ systems. Lesions were microdissected and after DNA extraction and PCR, targeted pyrosequencing was used to assess the BRAF V600 status. Statistical comparison applied the Fisher's exact test and statistical significance was defined as P<0.05.

Results. A total of 39 cases fulfilled our inclusion criteria and BRAF V600E testing revealed mutations in 19 lesions (49%); which is compatible with prior reports (e.g. n=35/61, 57%; p=0.42 Fisher's). Restricting the analysis to cases with pulmonary lesions revealed 4 mutations in 13 patient samples (33%), which is also in accord with prior reports (e.g. n=5/12; 42%; p=0.69). These two comparisons confirm the relatively high mutational frequency in histiocytosis and established our cohort as valid to address our specific question regarding mutation frequencies in pulmonary vs. systemic histiocytoses. We compared 7 "systemic" with 6 "lung-only" cases (by CT/radiology or clinicopathological information) and found 3 mutations in "systemic" cases (42%) and only one mutated case (in a smoker) in the "lung-only" lesions (17%), respectively. These data suggest a higher frequency in multi-system histiocytoses but disease extent at time of presentation is indefinite, when, for example compared with autopsy studies. Nonetheless, statistical comparison of mutation frequency by affected site did not reveal a significant difference (p=0.56, Fisher's).

Conclusions. Here, we validated the mutational frequency of BRAF V600E in histiocytoses and determined that mutation status alone does not allow diagnostic distinction between pulmonary and systemic disease.

FR-111
Nuclear size, CK19 and p63 expression as diagnostic marker for classification of WHO thymoma

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Aims. Thymoma are rare epithelial tumors of the thymus, currently classified according to histomorphological criteria of the WHO. However, still a number of problems and conflicts exist concerning inter-observer reproducibility and definition of morphologically defined subtypes of thymoma. Especially, subclassification of B-thymoma remains to be difficult, as diagnostic and functional relevant markers are rare. In this study, nuclear size as criteria for malignancy, p63 oncogene expression and epithelial marker CK19 expression have been examined in WHO thymoma subtypes.

Methods. We investigated nuclear size, the expression of p63 and CK19, as well as TdT+ lymphocyte content in 15 cases of A, AB, B1, B2 and B3 thymoma, classified according to histomorphological criteria of the WHO in comparison to normal thymus of the first decade by means of immunohistochemistry and semi-automatic quantitative morphometry.

Results. Analyzation of nuclear size of p63+ tumor epithelial cells showed that nuclear size does not differ significantly between B1, B2 and B3 thymoma. Likewise, nuclear size of p63+ tumor epithelial did not differ significantly between A, AB and B thymoma. All thymoma subtypes showed CK19 and p63 expression in tumor epithelial cells. Interestingly, from benign B1 to rather malignant B3 thymoma, the expression of CK19 and p63 increased significantly. A thymoma showed highest CK19 expression levels of all thymoma subtypes. As expected, TdT+ lympho-

cyte content decreased from AB and B1 to B3 thymoma. A thymoma showed lowest numbers of TdT+ lymphocytes.

Conclusions. Decrease of TdT+ lymphocytes and increase of CK19 and p63 expression in tumor epithelial cells could be used as diagnostic markers for subclassification of A and B thymoma. Benign B1 thymoma show low levels of p63 and CK 19, whereas rather malignant B3 thymoma and A thymoma are characterized by high p63 and CK 19 expression. Nuclear size of p63+ tumor epithelial cells is not helpful in differentiating subtypes of thymoma.

FR-163
Association of lung adenocarcinoma with diffuse idiopathic pulmonary neuroendocrine cell hyperplasia (DIPNECH)

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Aims. DIPNECH is so far regarded as precursor lesion of neuroendocrine lung tumors, specifically carcinoids. A relationship with lung adenocarcinomas has not been clearly established so far. We present a series of four cases with concomitant presence of adenocarcinoma and DIPNECH in the lung.

Methods. The cases were identified from the archives of the institutes of pathology of Jena University Hospital and the Charité. The clinical data were retrieved from the hospital information system. The microscopic findings of adenocarcinoma and DIPNECH were reviewed. A panel of neuroendocrine and epithelial markers was performed and analyzed immunohistochemically. In addition, the H&E slides of a series of 82 lung carcinomas were reevaluated for presence of DIPNECH foci and the parameters of the IASLC/ATS/ERS classification for lung adenocarcinoma.

Results. DIPNECH foci were composed of small intramucosal nests of proliferating pulmonary neuroendocrine cells alongside or at the periphery of terminal airways. All detected foci measured less than 5 mm in maximal diameter and showed a consistent reactivity against Synaptophysin. They did not express epithelial markers of squamous cell carcinoma and adenocarcinoma. The DIPNECH foci in three cases were clearly associated with the adenocarcinoma while in one they were observed in the non-neoplastic lung tissue. The adenocarcinoma with DIPNECH inside showed mainly a low grade histology while the fourth case were intermediate to high grade. The histologic evaluation of the HE slides of the other 82 lung cancer cases showed no suspected or definite foci of DIPNECH. Within this series we could confirm the prognostic significance of the IASLC/ATS/ERS classification of lung adenocarcinoma.

Conclusions. Our series suggest that in a subset of lung adenocarcinoma is characterized by the presence of DIPNECH within the tumor suggesting a causal relationship. These adenocarcinomas seem to be low grade and may have a particular tumorigenesis and clinical behaviour. This observations needs to be confirmed on larger tumor collectives. We could confirm the prognostic relevance of the new adenocarcinoma classification.

Postersession Urologische Pathologie

FR-112
Glutathione S-transferase pi protein expression in prostate tissue

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Aims. Hypermethylation of gluthatione S-transferase pi (GSTpi) is a common epigenetic alteration in prostate cancer resulting in loss of GSTpi protein expression, although a large scale analysis has not been conducted so far.
Methods. A prostatectomy cohort (n=640) in tissue microarray format was evaluated for GSTpi immunoreactivity in malignant and benign tissues. The staining characteristics were correlated with clinicopathological data.
Results. In 99% of all cases GSTpi stained prostate cancer cells with markedly less intensity than adjacent normal secretory epithelium. In the majority of cases (96.3%) no staining at all occurred in malignant cells with 3.7% mostly mild to moderate staining in cancer cells. A heterogenous staining pattern was observed in the normal secretory epithelium in 73.2%. In 25% of all cases normal secretory epithelium was negative for GSTpi. Basal cells were stained in mild to high intensity in normal tissue and prostate intraepithelial neoplasia (PIN) respectively.
Conclusions. GSTpi can be a useful discriminator between malignant and benign prostate epithelial cells. However, the heterogeneous staining pattern requires a careful comparison. Aberrant staining of tumour cells is seen more often than with p63, leaving GSTpi inferior as a basal cell marker. However, it might be a helpful auxiliary tool in the evaluation of difficult cases.

FR-113
Intraductal papilloma of the prostate as an incidental finding

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Aims. In the epididymis is already known papillary cystadenomas. Papillary polyps of the prostatic urethra have also been described. An intraductal papilloma of the prostate is rarely observed and is even far only from Zimmermann and Zeltner (1979) have been described.
Methods. We report an 80-year-old patient, who is because of an urothelial carcinoma operated. By complete dissection of the prostate, we have found prostatic hyperplasia, cribriform hyperplasia, high-grade prostatic intraepithelial neoplasia and two intraductal papillary neoplasms with a maximum extension of 3 mm. These neoplasms have been built by papillae. The papillae have been lined by double rows of epithelial cells without mitoses or Atypien. The myoepithelial cells are intact. Immunohistochemically there is positivity to the PSAP and intact myoepithelial cells with positivity for p63 and CK5/14. No proliferative activity with 0% for Ki-67.
Results. Thus, the diagnosis of intraductal papilloma of the prostate is provided.
Conclusions. The present case report describes a rare case of intraductal papilloma of the prostate. Because of its rarity, the diagnosis can be difficult. Due to lack of the proliferation activity and invasion, it can clearly be classified as a benign neoplasm and because of dense fibrovascular

stroma and benign branched epithelium, it can be differentiated from high-grade intraepithelial neoplasia of the prostate.

FR-114
The level of IGF2-expression in prostate cancer correlates with specific promoter usage

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Aims. IGF2 has recently been discussed as potential oncogene in various tumor entities. After fetal development IGF2 is maternal imprinted and only expressed from the parental allele. Aging is one of the major reasons for genomewide methylation changes and herewith the imprinting of IGF2 is partly lost (LOI). Aging is also one of the major risk factors for Prostate cancer (PCA) and an increased gene dose do to LOI makes IGF2 a potential factor in the development of PCA. LOI of IGF2 has recently been described as "field defect" in PCA, whereas IGF2 expression did not correlate with LOI. Therefore the mechanisms of IGF2 expressional regulation and the role of the insulin signaling cascade in PCA remain unclear. Nevertheless it has been shown that in several tumor entities a different promotermethylation of IGF2 is correlated with the clinical outcome. The goal of this study is to further specify the IGF2 expression in PCA and to characterize its regulation mechanisms.
Methods. Fresh frozen tissues of 56 patient samples were makrodissected for tumorous and adjacent non-tumorous areas. RNA and DNA was isolated and analyzed for LOI of IGF2 through the ApaI SNP. Subsequently IGF2, IGF2 promoters, CTCF and miR-675 (H19) were examined by qRT-PCR and their expression levels were correlated between the different groups (T/LOI, T/ROI, N/LOI, N/ROI). In addition the methylation status of the IGF2 promoters and the imprinting control region (ICR) in PCA and healthy tissue was quantified by pyro sequencing of bisulfite converted DNA.
Results. As shown in other studies we also found an overall lower expression of IGF2 in PCA in comparison to non-cancerous prostate tissue. However, the expression levels of IGF2 in PCA seem to be normally distributed and in about 20% of the cases we found increased levels of IGF2 in comparison to normal tissue. We could show that the higher expression of IGF2 in PCA is not only dependent of LOI but significantly correlates to the specific promoter methylation.
Conclusions. In a subgroup of PCA IGF2 expression tends to be increased in comparison to normal healthy epithelia. This cannot be explained with the LOI of IGF2 in the ICR. Therefore imprinting is not the primary regulatory mechanism for the expression of IGF2 in PCA. Nevertheless the diverging expression level can be explained independently of LOI/ROI by the use of different promoters of IGF2. These promoters of IGF2 show a distinct differential methylation.

FR-115
Expression of phospho-histone H3 in prostate carcinoma

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Aims. Prostate cancer is the second most common cancer in men and the sixth most common cause of death from cancer in men worldwide. While the development of androgen-independent growing prostate tumors remains incurable, a major fraction of patients is overtreated. Currently, a sufficient pathological distinction between patients in need of further treatment and those for which watchful-waiting remains an option is still lacking. Increase in cell proliferation has been suggested

to be a hallmark of prostate cancer (PCa) aggressiveness and is routinely assessed using the Ki-67 antibody. While Ki-67 staining is expressed through the G1, S, G2, and M phase, phosphorylation of the chromatin constituent histone H3 occurs during the late G2 phase and mitosis. So far, in several carcinomas, including breast and ovarian carcinoma, the expression of PHH3 has been associated with poor prognosis. To date, neither the expression rate nor its prognostic prognosis in prostate carcinoma has been reported. We undertook this study to investigate the expression of PHH3 in prostate carcinoma tissue sections of different grades in comparison to benign prostatic tissue.

Methods. Expression of PHH3 was assessed the on paraffin-embedded tissue of the Cancer of Swedish Men (COSM) cohort. Analysis included triplicate biopsy cores of total of 116 patients (n=90, prostate tumor; n=26, benign prostate tissue). Staining against PHH3 was performed with standard immunohistochemistry and analyzed using Definiens imaging software.

Results. Prostate tumor tissue exhibited a significantly higher (p<0.0001) frequency of PHH3+ cells compared to benign prostate tissue (mean 25.87 ± 2.0 vs. 2.340 ± 0.41). Consistent to this, electronic gating on tumor tissue and tumor stroma, respectively, showed higher intensity of PHH3+ cells in "gated" tumor tissue compared to tumor stroma, which appeared to be almost negative. Importantly, expression of PHH3 on prostate tumor tissue negatively correlated with PSA recurrence.

Conclusions. Our data point to a conceivable role of phospho-histone H3 as biomarker in prostate carcinoma.

FR-116
PITX2 DNA methylation predicts outcome in prostate cancer patients with Gleason 7a disease

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Aims. The prospective discrimination of aggressive and clinically insignificant prostate carcinomas still poses a significant and unsolved problem. This is particularly true for patients at intermediate risk (Gleason 7a), were additional diagnostic tests for risk stratification are desired most. PITX2 DNA methylation has previously been shown to be a powerful biomarker to predict outcome in breast, lung and prostate cancer patients. This study aimed to verify the clinical performance of a newly designed assay to measure PITX2 methylation in prostate cancer patients with Gleason 7a disease in a multicentre study.

Methods. A quantitative real-time PCR assay for the sensitive and accurate quantification of PITX2 methylation in formalin-fixed and paraffin-embedded tissues was used. A methylation cut-off for patient stratification was applied as established in a previous study. Finally, the clinical performance was analyzed in a prostatectomy cohort of n=201 men with Gleason 7a disease from four clinical centres.

Results. Univariate analyses (Kaplan Meier, Cox) of bio-chemical recurrence-free survival in patients with Gleason 7a disease stratified by the DNA methylation status of PITX2 showed a significant discrimination between patients at low and at high risk of biochemical recurrence (BCR). These results confirmed PITX2 hypermethylation as a significant predictor for BCR (p=0.014, HR=2.06 [1.16–3.68]). Importantly, PITX2 hypermethylation added significant prognostic information (p=0.046, HR=1.81 [1.01–3.24]) to pathologic T stage, and surgical margins in a multivariate analysis.

Conclusions. This assay might aid in a refined risk stratification of prostate cancer patients with Gleason 7a disease following radical prostatectomy. Future analyses of PITX2 methylation in prostate biopsies are warranted to clarify, if this prognostic value can also aid in clinical therapy planning at the biopsy stage.

FR-117
PITX2 expression is regulated by DNA methylation and strongly correlates to androgen receptor expression in prostate cancer tumor cells, normal adjacent tissues and benign hyperplasia

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Aims. PITX2 DNA methylation has extensively been validated as a strong prognostic biomarker in breast, lung and prostate cancer. In prostate cancer, PITX2 hypermethylation added significant independent prognostic information to Gleason score and clinical parameters in a multivariate analysis. However, the functional role of PITX2 during prostate cancer development and progression is poorly understood, so far. Recently it was shown in a cell model that PITX2 might act as an upstream regulator of the androgen receptor (AR) gene. This function would support the pivotal biological role of PITX2 during prostate cancer progression. However, cell lines only insufficiently reflect the biology of a heterogeneous and complex tumor. Hence, this study aimed to analyze the co-expression of PITX2 and AR in clinical samples.

Methods. Prostate carcinomas with different Gleason scores were dissected and PITX2 DNA methylation was measured by a methylation-specific high-resolution melting assay (MS-HRM). Additionally, an immunohistochemistry (IHC) assay for accurate and robust in situ quantification of PITX2 protein expression was established and its analytical performance was verified.

Results. The expression of PITX2 was strongly correlated to the expression of AR in tumor cells (p=0.028), in benign hyperplasia (p<0.001), and in normal adjacent tissues (p<0.001). Furthermore, there was an inverse correlation between PITX2 methylation and protein expression of PITX2 (p=0.028). No correlation with Gleason score was found.

Conclusions. It has been shown for the first time that decreased expression of the PITX2 gene product is directly correlated to methylation of the gene locus in prostate cancer. These findings highlight the biological relevance of PITX2 protein expression and DNA methylation in prostate cancer.

Abstracts

FR-118
Metabolite profiling as tool for the identification of differentiating and prognostic markers of prostate carcinoma

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Aims. Analysis of metabolites has emerged a promising tool for define novel biomarkers. We aimed to evaluate the diagnostic and prognostic potential of metabolites in prostate cancer (PCa) tissue after radical prostatectomy.

Methods. 107 matched-paired tissue samples collected after radical prostatectomy were subjected to the MxPTM Broad Profiling by gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (Patent WO 2010/139711 A1). The selected metabolites aminoadipic acid, cerebronic acid, gluconic acid, glycerophosphoethanolamine, 2-hydroxybehenic acid, isopentenyl pyrophosphate, maltotriose, 7-methylguanine, and tricosanoic acid were related to clinicopathological variables including prostate volume, tumor stage, Gleason score, preoperative prostate-specific antigen (PSA) and disease recurrence data. Non-parametric statistical tests, receiver-operating characteristics (ROC) and univariate and multivariate analyses (Kaplan-Meier curve; Cox regression) were performed.

Results. All analysed metabolites showed higher concentrations in malignant than in non-malignant samples except for gluconic acid and maltotriose, which had lower levels in tumors. ROC analyses showed a clear differentiation for all metabolites with a maximal area under the curve of 0.86 for tricosanoic acid. However, the metabolites were not related to tumor stage and Gleason grade. Aminoadipic acid, gluconic acid, and maltotriose levels were associated with tumor recurrence (Kaplan-Meier analysis) and were, together with tumor stage and Gleason score, a successful metabolite combination in the multivariate Cox regression model for the prediction of tumor recurrence.

Conclusions. This exemplary study performed with selected metabolites from a global metabolic profiling investigation proves that metabolites in prostate carcinoma tissue can be used, in combination with traditional pathological and histomorphological parameters, as promising diagnostic and prognostic tools.

FR-119
Comparison of p40 (DNp63) and p63 expression in prostate tissues as diagnostic basal cell markers

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Aims. p63 is one of the standard markers for basal cells of the prostatic gland. Recently, it has been suggested that the p63 isoform p40 might be more specific as a basal cell marker. Here we compare the staining characteristics of p63 and p40 in normal and malignant prostate tissue.

Methods. A prostatectomy cohort (n=640) in tissue microarray format was evaluated for p63 (clone 4A4) and for p40 (rabbit polyclonal) immunoreactivity in malignant and benign tissues. On the TMA, each case was represented by 5 tissue cores (2 mm) encompassing normal tissues of different zones and two cores of representative tumor. Immunoreactivity of basal and secretory cells was evaluated in a semiquantitative manner and compared case-wise.

Results. In benign tissues, p40 showed a highly similar immunoreactivity compared to p63. The staining patterns were identical in 88% of

cases. An additional cytoplasmic p40 staining in tumour cells occurred in 59.6% of cancer cases. For the nuclear staining, differences were seen in carcinomas: 1.4% of carcinomas were p63-positive whereas only 0.6% of cases were p40-positive.

Conclusions. p40 stains prostatic basal cells as reliably as p63 with minor and insignificant differences. However, it displays significantly less aberrant staining of tumour cells than p63, establishing the higher specificity of p40.

FR-120
The mediator complex subunit MED12 is a novel target for therapeutic intervention in castration resistant prostate cancer

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Aims. Castration-resistant prostate cancer (CRPC) is the most aggressive form of prostate cancer (PCa) and remains a significant therapeutic challenge. A multi-subunit protein complex, termed Mediator, is known to be an important co-activator for a broad range of regulatory transcriptional factors including the androgen receptor (AR), yet its role in PCa is unclear. A study using PCa cell lines has indicated that Mediator seems to play a role in cell proliferation when androgen is limited, or in the absence of the AR. Furthermore, the gene encoding the Mediator subunit MED12 has recently been reported to be mutated in primary PCa. Therefore, the aim of our study was to investigate whether MED12 may serve as novel target for therapeutic intervention for patients with CRPC.

Methods. We have assessed the amplification/deletion status of MED12 as well as its protein expression by fluorescence in situ hybridization (FISH) and immunohistochemistry (IHC), respectively, on a PCa progression cohort consisting of locally defined PCa, patients with primary PCa and corresponding lymph node metastasis, and CRPC.

Results. FISH analysis revealed no amplifications or deletions of MED12 in our PCa progression cohort. However, we found MED12 to show a strong nuclear overexpression in 30% of the CRPC cases, compared to a nuclear overexpression in only 3% of primary non-metastasized PCa, 0% of primary with lymph node metastasis PCa, and 7.5% of the lymph node metastasis. Interestingly, MED12 nuclear overexpression was highly significantly correlated with increased proliferative activity.

Conclusions. Our findings suggest that MED12 may be involved in promoting PCa cancer cell proliferation and survival in absence of androgens, and may serve as a novel target for therapeutic intervention for patients with CRPC.

FR-121
Estrogen-receptor alpha expression correlates with histopathological parameters in prostate cancer patients

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Aims. Tumor stage, Gleason score and surgical margin status are the most important prognostic factors for prostate carcinoma (PCA) patients. Targeting the gonadotropin releasing hormone receptor is a routine practice in advanced staged PCA patients. However the knowledge on alternative hormone receptor systems is rather limited. This study aimed to clarify whether PCA patients maybe sub stratified by expression of steroidal and non-steroidal nuclear hormone receptor systems (estrogen receptor alpha and beta, thyroid hormone receptor alpha 1/2,

thyroid hormone receptor beta, vitamin D, retinoid x receptor, progesterone receptor). Herein we present our first data on expression of Estrogen-receptor alpha (ERa) in relation to grade/stage in PCA cases.

Methods. Immunohistochemistry of ERa was performed on formalin fixed paraffin embedded material retrieved from 88 primary PCA cases. The immunoreactive score (IRS) was used to asses ERa positivity which was correlated to histological tumor grade and Gleason score. In this work we present the first results of a study which includes 178 patients. We are going to analyze the expression of seven different hormone receptors (see above).

Results. ERa positivity (IRS>0) was detected in neoplastic glands [22.7% (20/88)] as well in the associated stromal tissue [100% (88/88)]. Concerning tumor cells we found a nuclear ERa immunophenotype with an average expression of IRS=0.4±0.09. ERa expression was significantly higher in stromal cells as compared to the tumor itself. We detected a positive correlation between the ER alpha immunreactivity and grading and furthermore a correlation between ER alpha expression and Gleason score (grading: gamma = 0.75, p=0.003; Gleason score: gamma = 0.66, p<0.001). In 95% (19/20) of ER alpha positive samples poorly differentiated prostate carcinoma was diagnosed.

Conclusions. Within this study we identified a positive correlation between ERa expression and tumor grade as well as Gleason score in a subpopulation of PCA cases. The assessment of different hormone receptors (estrogen receptor beta, thyroid hormone receptor alpha 1/2, thyroid hormone receptor beta, vitamin D, retinoid x receptor, progesterone receptor) could lead to a better prognostic evaluation and perhaps to new predictive strategies for an individualized therapy.

FR-122

Morphologic analysis of Gleason 7 carcinoma of the prostate after biopsy and radical prostatectomy with the differentiation to low, intermediate and high risk tumor. Findings in consideration to ISUP criteria

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Aims. Carcinoma of the prostate with Gleason score (GS) 7 have different prognosis that has been known for years. After modification of grading of Gleason by the International Society of Urological Pathology (ISUP) in 2005, we attempted to analyze biopsy specimens and the corresponding specimens of radical prostatectomies (RP) by means of some new criteria of ISUP of 2010 and further supplementations of 2012.

Methods. Biopsy specimens and the corresponding specimens of RP of 530 adenocarcinomas of the prostate of the years 2009 to 2011 were graded using the modified GS with additional consideration of the state of the nucleoli. In the biopsy specimens, carcinoma with GS 7 and the pattern 4+3 were placed to the group of high grade (high risk) tumors, GS 6 and atypical nucleoli to the intermediate grade (intermediate risk) tumors, and GS 6 and only few and small atypical nucleoli to the low grade (low risk) tumors.

Results. Carcinoma with GS 7 and the pattern 3+4 were significantly different compared to the carcinoma with GS 6 with regard to only few and small nucleoli (p=0.0001), however similar to the group of GS 6 with atypical nucleoli (intermediate type, p=0.71). The values of agreement of carcinoma classified as low, intermediate and high grade were 75%, 63%, and 96%, respectively. The intermediate group showed an upgrading rate of 36% from GS 6 to GS 7, which is a clear increase. Furthermore, the correlation between organ confined and non-organ confined growth showed differences from 63% and 37% in the intermediate group (p=0.0001). The rate of metastases in the intermediate group is very low in contrast to the high risk group. Concerning the free margins the intermediate group clearly showed more favorable values with 79% compared to 57% of the high grade (high risk) group (p=0.0001).

Conclusions. The values of grading, staging, margins and metastases indicate that carcinoma of the prostate with the pattern of Gleason 3+4 correspond to an intermediate group of carcinoma in contrast to high grade (high risk) carcinoma with GS 7 and pattern 4+3. The conclusion for the diagnostic practice is the determination of the percentage of pattern 4 in GS 7 and of the extension of the tumor infiltration in biopsy specimens to differ between intermediate and high risk carcinoma.

FR-123

Two major pathways of penile carcinogenesis: HPV-induced penile cancers overexpress p16ink4a, HPV-negative cancers associated with dermatoses express p53, but lack p16ink4a-overexpression

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Aims. Penile squamous cell carcinomas (SCC) arise either through transforming infections with human papilloma virus (HPV) or independent of HPV, often in the background of lichen sclerosus (LS) and lichen planus (LP). Despite impact on therapy and prognosis, etiologic stratifications are missing in most histological diagnoses and publications about penile cancers/precursors. Classification of penile lesions into HPV-induced or HPV-negative via immunohistochemical demonstration p16ink4a-overexpression, a surrogate marker for transforming HPV-high-risk infections, and p53-expression in the absence of p16ink4a-overexpression.

Methods. Archival formalin-fixed material of 123 invasive penile cancers and 43 pre-invasive lesions was evaluated for the presence of LS, LP, 28 HPV-genotypes, and expression of p53 and p16ink4a.

Results. 72/123 SCC and 33/43 pre-invasive lesion showed p16ink4a-overexpression independent of HPV-HR genotypes involved: 66/72 SCC and 29/43 precursor lesions revealed a single HPV-high-risk-genotype (HPV-HR16 in 75%>>33,31,45,18,56), 5/72 SCC and 4/43 precursor lesions multiple HPV-HR-genotypes. 1 SCC revealed HPV-LR and HR-DNA. 51/123 SCC and 10 precursor lesions were p16ink4a-negative, but showed nuclear p53 expression in tumour cells and basal keratinocytes. 49/51 SCC and 10/10 precursor lesions lacked HPV-DNA. 2/51 SCC contained HPV18 and HPV45 DNA resp., but p16ink4a-negativity classified them as non-HPV-induced. 27/51 SCC showed peritumoral LS, 13/51 SCC peritumoral LP and 11SCC revealed no peritumoral tissue. Histologically, HPV-negative precursors showed hyperkeratotic, verrucous, atrophic and basaloid differentiation.

Conclusions. p16ink4a-overexpression identifies HPV-HR-induced penile carcinogenesis independent of HPV-HR-genotype. p53-expression along with p16ink4a -negativity identifies HPV-negative cancers. Correct etiologic classification of penile lesions during diagnostic work-up allows optimal therapy decisions.

FR-124

Gene copy number alterations and expression of FGFR 1 and 2 in primary squamous cell carcinomas of the penis

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Aims. To date no accepted therapy in patients with metastatic penile cancer is established, which is individually adapted to specific molecular features. Chemotherapy and radiation is the treatment of choice, if systemic disease is present, but not individually targeted and with some severe side effects. FGFR1 amplification was recently reported to be frequent in squamous cell carcinomas of the lung and head and neck and could be a potential specific treatment target in penile cancer.

Methods. 80 primary penile squamous cell carcinomas were evaluated on a tissue microarray to screen for FGFR 1/2 gene copy number alterations by FISH (Zytovysion) and the protein expression status was evaluated by immunohistochemistry.

Results. Amplification in FGFR 1 was found in 4 out of 80 tumors. No amplification was found in FGFR 2. Polysomies were more often present in FGFR1 and FGFR2, occurring in 18 and 15 cases, respectively. Protein overexpression of FGFRs was rare. Neither amplification nor polysomies with a strong FGFR protein expression were detectable. The association of FGFR 1/2 alterations with clinicopathologic features was not significant.

Conclusions. Although FGFR1/2 alterations are rare in penile squamous cell carcinomas, a small subgroup of patients could be candidates for inclusion in ongoing phase 1 studies treating patients with FGFR amplification with targeted therapies against FGFR.

FR-125
Sertoliform cystadenoma: a rare tumour of the rete testis

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Aims. We analysed a sertoliform cystadenoma of the rete testis representing a rare benign tumour arising in the rete testis.

Methods. A 66-year-old man was presented in the urological clinic because of slightly increased PSA blood level. The investigation of the prostate revealed a benign hyperplasia. Further urological examinations showed an approximately 2 cm in diameter mass in the area of the epididymal head. On ultrasound examination this mass proved to be cystic and irregularly bounded.

Results. Macroscopical findings: The cutting surface of the testis showed a homogenous brown colour. Within the area of the tunica albuginea/rete testis a 2.1×1.8×1.4 cm tumour was detectable. The cut surface of this tumour was of grey/white color and showed small cysts of about 5 mm in diameter. Histological findings: A tumour of apparently two cell compartments was recognizable. Epithelial like tumour cells showed cystic dilatations. In addition a uniform tumour cell population was ordered in tubules and acini showing a sertoliform growth pattern. The cytoplasm of the uniform tumour cells was eosinophilic, the nuclei showed prominent nucleoli. Immunohistochemical findings: the uniform tumour cells revealed positivity for inhibin, S-100, and CD 99. The germ cell markers as well as synaptophysin, EMA and BCL-2 were negative. Keratin expression could be seen in the cystic areas representing altered rete testis invaded by the tumour.

Conclusions. Because of the sertoliform growth pattern, the cystic areas and the anatomical location of the tumour a sertoliform cystadenoma of the rete testis was diagnosed. So far only five cases of a sertoliform cystadenoma of the rete testis have been reported. This case presentation should help for further diagnosis.

FR-126
Pseudoglandular seminoma

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Aims. The incidence of testicular tumors has increased in recent decades, with 8480 new detected cases in 2010 in the United States. In Germany 3970 new cases during 2008 were reported. Almost half of all intrascrotal tumors are seminomas. WHO defines 2 different types: classic and spermatocytic seminoma. Additional histological variants

have been described (intratubular, interstitial, tubular, pseudogladular, micricystic, cribriform), but these are controversial. Some authors believe that they represent artifacts, e.g. being caused by small biopsy samples or poor fixation.

Methods. A detailed clinical and histopathological review of a clinical case and review of the literature were performed.

Results. We report pseudoglandular structure of a seminoma in the left testis of a 24-year-old man. Sonographically a left testicular tumor was suspected. After intraoperative confirmation of the diagnosis, a left testis ablation was performed. The maximal tumor diameter was 1.2 cm. Histologically the tumor was composed of epithelial cells with a pseudoglandular arrangement and small cleft-like luminal spaces. Immunohistochemical reactivity of tumor cells against OCT4, D2-40, CD 117, MNF and PLAP were observed while no reaction against inhibin, beta HCG, AFP, EMA and CD 30 was appreciable.

Conclusions. We feel that our case represents a seminoma with pseudoglandular structures. Fixations artefacts could be ruled out. Yolk sac tumor represents an important differential diagnosis that needs to be excluded because of the different therapeutic regimen. This can usually be done by immunohistochemistry.

FR-127
CD138 (Syndecan1) in urothelial carcinoma of the bladder

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Aims. CD138 is a transmembrane glycoprotein involved in cell-cell- and cell-matrix-interactions. Its membranous presence is regulated by the balance between its expression and proteolytic ectodomain shedding. The role of CD138 in the progression of urothelial carcinoma of the bladder (UCB) is poorly understood.

Methods. CD138 levels were analysed by immunohistochemistry and ELISA in 119 paraffin-embedded tissue and 79 serum samples. Correlations with clinicopathological and follow-up data as well as with levels of circulating angiogenic and proteolytic factors were analysed.

Results. Membranous CD138 protein expression was detectable in normal urothelium and superficial UCB in contrast to lower rates in muscle-invasive UCB (p<0.001). However, in muscle-invasive UCB stromal expression was significantly enhanced (epithelial-mesenchymal transition; p=0.001) and serum concentrations of CD138 ectodomain were elevated compared to controls or non-muscle invasive carcinoma (p<0.001). Highest serum concentrations were detected in lymph node positive cases (p<0.001). In univariate analysis increase of stromal CD138-expression, loss of membranous expression in tumor cells and high serum concentrations were associated with poor prognosis, while only stromal CD138-expression retained significance in multivariate analysis.

Conclusions. Loss of membranous CD138 protein expression in cancer cells and the parallel increase of serum CD138 ectodomain levels in advanced UCB suggest the involvement of CD138 shedding in UCB progression. MMP-7 might play a significant role in this process as our data additionally suggests. Stromal CD138 protein expression was identified as an independent risk factor for disease-specific survival and might therefore be used for more precise risk stratification. Finally, high preoperative CD138 serum levels might help to identify lymph node positive cases and thus optimize therapy decisions.

FR-128
Primary mucinous adenocarcinoma of the renal pelvis

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Aims. Primary epithelial tumors of the renal pelvis are rare. The most common is the transitional cell tumor. Adenocarcinomas account for less than 1% of tumors of the renal pelvis.
Methods. We describe the case of a 51-year-old patient who presented with a persistent abdominal pain for a year. The diagnosis of hydronephrosis in the upper portion and shrinkage of the lower part of the left kidney was performed based on CT scans. Primary carcinoma in the gastrointestinal tract, biliary tract, lung, pancreas and other organs in CT examinations are excluded. Macroscopy: 14×9×8 cm nephrectomy specimen with multiple cysts up to 3.5 cm size and cystic mass in the renal pelvis of 3.5 cm size.
Results. Histological examination showed that the tumor is formed of glands and papillae lined by pseudostratified epithelium with hyperchromatic nuclei. Scattered signet ring cells were found in pools of extracellular mucin. Sections from the ureter to show a part of an adenocarcinoma in situ. Immunohistochemistry, the cells show positivity for CK7, CK20 and CK8/18 with negativity for TTF-1 and CDX-2.
Conclusions. The diagnosis of primary mucinous adenocarcinoma of the renal pelvis with adenocarcinoma in situ is made of the ureter. Based on the histology should endourological clarification of the lower and upper urinary tract are performed to rule out other findings. This rare case exemplifies the morphology and the immune profile of a primary adenocarcinoma of the renal pelvis. The immunohistochemical marker profile and the in situ component here allow the safe exclusion of metastasis ans secondaries from other organs.

FR-129
Clinicopathologic and molecular genetic study of MiTF/TFE family renal translocation carcinomas

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Aims. To further delineate clinicopathologic and molecular genetic features of MiTF/TFE family RCC.
Methods. We assessed the utility of TFE3 FISH in establishing the diagnosis for suspected or unclassified cases with negative or equivocal TFE3 immunostaining by analyzing 24 RCCs with TFE3 FISH and comparing the molecular findings to the results of TFE3 and cathepsin K immunostaining in the same tumors. We also examined seven additional cases of TFEB RCCs by clinicopathologic, immunohistochemical, molecular, and ultrastructural analyses.
Results. 10 tumors were originally diagnosed as Xp11.2 RCC based upon positive TFE3 immunostaining and 14 were originally considered unclassified RCCs with negative or equivocal TFE3 staining, but with a range of features suspicious for Xp11.2 RCC. 17 cases showed TFE3 rearrangement associated with Xp11.2 translocation by FISH, including all tumors with moderate or strong TFE3 (n=10) or cathepsin K (n=7) immunoreactivity. FISH-positive cases showed negative or equivocal immunoreactivity for TFE3 or cathepsin K in seven and ten tumors, respectively. Morphologic features were typical for Xp11.2 RCC in 10/17 tumors. Unusual features included one melanotic Xp11.2 RCC, one tumor with mixed features of Xp11.2 RCC and clear cell RCC, and other tumors mimicking clear cell RCC, multilocular cystic RCC, or high grade urothelial carcinoma. Of the seven additional cases of TFEB RCC, four tumors had the typical morphologic features of TFEB RCC,

whereas three cases demonstrated uncommon morphologic features, mimicking epithelioid angiomyolipoma, chromophobe cell RCC, and clear cell RCC, respectively. Immunohistochemically, aside from TFEB and cathepsin K, KSP-Cad was another sensitive and relatively specific marker for TFEB RCC, supporting a distal nephron origin for these renal tumors. We also observed different ultrastructures including mitochondrion with areas of lipofuscin pigment in the smaller cells in these cases. In addition to PCR method, we also developed a TFEB FISH assay to serve as an effective diagnostic tool.
Conclusions. We identified 24 new molecularly confirmed MiTF/TFE RCC using the TFE3 and TFEB FISH assay and expanded the clinicopathologic spectrum of these genotypes neoplasm. Our results suggest a combination of these markers TFE3, cathepsin K, TFEB, and KSP-Cad to improve the accuracy of diagnosis of MiTF/TFE RCC and show that the TFE3 and TFEB FISH assay is a useful complementary method for confirming the diagnosis of these tumors.

FR-130
Prognostic significance of tumor thrombus consistency in patients with renal cell carcinoma

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Aims. Locally-advanced renal cell carcinoma (RCC) is often presenting with a venous tumor thrombus (VTT). Although some prognostic parameters (e.g. pathological stage and grade) have been identified, the clinical course of patients with VTT often differs despite of similar clinical-pathological characteristics. In this respect, the histological VTT consistency in the nephrectomy specimen was recently suggested as novel prognostic parameter.
Methods. The VTT consistency (solid vs. friable) was determined based on H&E morphology in a retrospective cohort of 200 RCC patients nephrectomized between 1994 and 2011. The VTT consistency was correlated to clinical-pathological parameters, and its predictive value for overall survival was assessed using Cox regression models.
Results. Among the 200 RCC patients, 65% had a solid and 35% a friable VTT. A friable VTT was correlated with advanced pT-stage, higher VTT level, papillary RCC subtype, and a lower age. The median overall survival of patients with a friable VTT was significantly shorter than in patients with a solid VTT (29 vs. 89 months), but the multivariate Cox analysis failed to support a VTT consistency as independent predictor of patients' survival. However, VTT consistency was an independent significant predictor of overall survival in patients without evidence of distant metastasis ($p < 0.01$).
Conclusions. We present evidence that in patients with RCC and VTT, VTT consistency is an important prognostic predictor of overall survival. In particular, in patients with non-metastatic RCC, VTT consistency is the second-most important predictor for patients' outcome after pathologic stage. Hereby, we suggest that RCC patients, suffering from what we call a "high risk" disease, might profit from a more aggressive therapeutic regime, e.g. with adjuvant therapy.

Abstracts

FR-131
No association of a certain rs3242 SNP genotype and renal cell carcinoma

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Aims. Renal cell carcinoma, mainly of the clear cell subtype, was demonstrated to be associated with SFRP1 loss. Micro-RNAs are known to regulate gene expression by binding to complementary sequences of mRNA molecules and thereby targeting them for degradation. SFRP1 mRNA yields a single nucleotide polymorphism (SNP) called rs3242 in its 3' untranslated region (UTR). If a T-allele is present at the SNP-position, two miRNAs, hsa-miR-603 and hsa-miR-3646, can bind to SFRP1 mRNA. To date, there is no data on rs3242 SNP genotype distribution in renal cell carcinoma (RCC).

Methods. We prepared DNA from 377 formalin fixed paraffin embedded (FFPE) normal tissues of RCC patients and FFPE normal tissues or blood from 332 control patients. The rs3242 SNP region was amplified and analyzed by restriction fragment length polymorphism analysis (RFLP), as the C allele creates a restriction site for RsaI. RsaI cleaves the 137 bp PCR product into a 44 bp and a 93 bp fragment. Evaluation of restriction fragments was carried out on 2.5% agarose gels. χ^2 test was applied for testing the significance of genotype distribution differences between cases and controls, between histological subtypes and TNM stages of the corresponding tumors and between patient groups with different age of onset. Nine DNAs were randomly selected to perform Sanger sequencing of the rs3242 region to verify RFLP results.

Results. Sanger sequencing and RFLP analysis showed identical results in the nine selected DNAs. Genotype distribution of cases and controls was in Hardy Weinberg equilibrium. Genotype distribution among RCC patients was T/T in 13.8% (52/377), C/T in 47.5% (179/377) and C/C in 38.7% (146/377), whereas it was T/T in 14.2% (47/332), C/T in 41.6% (138/332) and C/C in 44.3% (147/332) among control patients, but was not significant (p>0.05). We also could not find significant associations of genotype and age of onset, TNM stage or subtype of the corresponding tumors.

Conclusions. There is no evidence of an influence of rs3242 genotype on risk for RCC, onset of RCC or tumor characteristics.

FR-132
Expression profile of EPH receptors and ephrin ligands in clear-cell renal cell carcinoma (ccRCC)

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Aims. The EPH receptors tyrosine kinases and their cell-bound ligands, the ephrins, play a key role in the regulation of migration and cell adhesion. An imbalance in the receptor-ligand ratio is associated with cancer development and progression. In this study, detailed protein and mRNA analyses of EPHA1 and EPHA2 as well as of their ligand ephrin-A1 were performed in ccRCC.

Methods. EPHA1, EPHA2 and ephrin-A1 protein expression was analyzed by immunohistochemistry on tissue microarrays comprising 119 primary ccRCC and corresponding non-malignant tissues. Relative gene expression was measured on 76 cryo-preserved primary ccRCC and corresponding non-malignant tissues by quantitative PCR. Associations of protein and mRNA levels with clinicopathological and follow-up data were then investigated.

Results. Immunohistochemical staining of both EPHA1 and EPHA2 was generally lower in tumors compared to normal renal tissue, whereas that of ephrin-A1 was generally higher. The median gene expression of EPHA1 was significantly down-regulated (6.7-fold) and that of ephrin-A1 was significantly up-regulated (1.6-fold) in ccRCC tissue specimens, while the median gene expression of EPHA2 was not significantly altered. The lack of EPHA1 protein expression was significantly associated with a longer progression-free, tumor-specific and overall survival (p<0.05). ccRCC cases missing EPHA2 protein expression had by trend a longer tumor-specific and overall survival (p<0.10). For ephrin-A1 neither protein level nor mRNA levels of all three genes showed prognostic relevance.

Conclusions. EPHA1, EPHA2 and ephrin-A1 are differentially expressed in ccRCC. This imbalance in the receptor-ligand ratio could result in an impaired EPH-ephrin signaling and thus contribute to ccRCC pathogenesis.

FR-133
Functional characterization of CD70, a biomarker specific for clear cell renal cell carcinoma

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Aims. To improve prognosis and treatment of clear cell renal cell carcinoma (ccRCC) patients, biomarkers suitable for the detection of ccRCC metastases and treatment response would be of utmost importance. CD70, a member of the tumor necrosis factor ligand superfamily, was previously identified as a specific biomarker for ccRCC and novel therapeutic agents targeting CD70 are already in clinical trials. However, functional studies characterizing CD70 in renal cell cancer are lacking.

Methods. We analyzed the expression of CD70 and its receptor CD27 using a tissue micro array (TMA) with more than 300 RCC cases and microarray analysis. Correlation to the von-Hippel-Lindau tumor suppressor (VHL) mutation status and reconstitution of VHL in a ccRCC cell line was done. To analyze the methylation status of the CD70 promoter in ccRCC, we performed bisulfite sequencing. The effect of inhibition of DNA methyltransferases in ccRCC cell lines was tested.

Results. In the TMA high CD70 expression was observed in 78% of ccRCC. Microarray analysis of 79 ccRCC cases confirmed the TMA result. Notably, CD70 is expressed in all analyzed brain metastases originating from CD70 positive primary ccRCC. By analyzing VHL and VHL target protein expression patterns as well as the VHL mutation status, we found that CD70 is linked to the inactivation of VHL. Re-expression of VHL in the VHL-deficient cell line A498 leads to attenuated CD70 expression. It was reported that demethylation of CD70 promotor contributes to CD70 overexpression in CD4+ T cells in various CD4+ T affecting immune diseases. Interestingly, hypomethylation of the CD70 promotor was linked to overexpression of CD70 in ccRCC cell lines and tumor tissue. By inhibiting DNA methyltransferases CD70 expression was increased in the ccRCC cell lines A498 and 786-O. We identified ccRCC which co-expressed both the receptor of CD70, CD27 and CD70 or expressed only one of the two proteins or were negative. Only CD27 positive but CD70 negative ccRCC were associated with early tumor stage, low grade and better overall survival.

Conclusions. Our data suggests that the upregulation of CD70 is characteristic feature for ccRCC. The expression of CD70 seems to be promoted by both the loss of VHL and by promoter hypomethylation. Notably, the expression of the ligand CD70 or of its receptor CD27 or of both in subsets of ccRCC suggests its importance for the biology of this tumor subtype. Further experiments will show if CD70 and CD27 will exert effects on cell proliferation.

FR-134
Cancer-related inflammation in papillary renal cell carcinoma: a key role for tumor-associated macrophages?

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Aims. Numerous tumors show inflammation in the microenvironment inflammation including macrophages. These tumor-associated macrophages (TAM) are known to play a key role and are partially able to promote cancer development. Especially the immunosuppressive M2 TAM phenotype is associated with an increased tumor growth, invasiveness, and metastases. The differentiation of macrophages into the alternative phenotype M2 is mediated, inter alia, by macrophage colony-stimulating factor (M-CSF). Papillary renal cell carcinoma (RCC) represents a rare tumor, which is divided, based on histological criteria, into two subtypes, of which type II papillary RCC shows a worse prognosis. Both subtypes typically show a dense infiltrate of macrophages.
Methods. In the present study the expression of CD68, CD163, CD3, CD20, M-CSF, CD31, and Ki67 were examined in 34 papillary RCC of histological type I and 30 papillary RCC of histological type II (n=64). The expression of M-CSF in four different RCC cell lines (Caki1/Caki2/786.0/A498) was evaluated by PCR and immunocytochemistry.
Results. All papillary RCCs showed a dense infiltration of CD68 positive macrophages with no significant differences between both subtypes. Nearly all macrophages in papillary RCC type II displayed a CD163 expression, which is typical for M2 macrophages. The type I papillary RCC showed only in up to 30% of macrophages positivity for CD163. Furthermore, papillary RCC type II demonstrated a significant higher expression of M-CSF within the tumor cells as well as a higher proliferation rate (Ki67), and capillary density (CD31). The number of B- and T-lymphocytes showed no significant differences between both subtypes. The PCR as well as the immunocytochemistry confirmed the expression of M-CSF in all investigated RCC cell lines.
Conclusions. The cancer related inflammation in papillary RCCs showed no significant differences with regard to overall macrophages as well as the B- and T-lymphocytes. In papillary RCC types II nearly all macrophages demonstrate the alternatively activated M2 phenotype. In addition papillary RCC types II as well as RCC cell lines induce a differentiation of macrophages towards the M2 phenotype by secreting M-CSF. The high number of M2 macrophages in papillary RCC type II could be a reason for the worse prognosis as compared to the subtype I.

Postersession Paidopathologie

FR-135
Can the lung floating test still be regarded as a valuable tool for identification of still births?

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Aims. In Germany, in contrast to other countries the lung floating test is still an obligatory measure to distinguish whether a newborn was born dead or alive. Questioning if the test is appropriate for the changed birth collective of our days, lungs of newborns were tested with the floating test.
Methods. Lungs from 209 induced abortions and stillbirths (n=195) as well as neonates (n=4) and babies (n=10) that had died within 2 days to 10 months post partum were tested by lung floating test.

Results. The test was negative for the lungs of all stillborn children. Of the 14 live births, test was positive in only 10 cases.
Conclusions. The lung floating test was correctly negative in all those cases, where the children definitively had not breathed resp. were still born. It was wrongly negative in 4 of 14 cases in which the children had definitively been breathing. Hence, reliability of lung floating test in children that (might) have been breathing is only 71%.

FR-136
Fetal autopsy rates in a German perinatal center—have they changed over the years?

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Aims. Autopsy is still the gold standard in clarifying the diagnosis of unsuspected death in fetuses and remains an indispensible part of quality management in perinatal centers.
Methods. A review of our database enclosed 517 fetal autopsy cases (242 male, 258 female, 17 not clarified) in a German tertiary perinatal center between January 2006 and October 2012. The clinical profile of patients receiving an autopsy at our institution was compared to those that were denied an autopsy by their next of kin. The following parameters were included in the analysis: maternal and gestational age, cause of the abortion (induced versus spontaneous) and the number of induced abortions because of genetically proofed chromosomal aberrations. In the cases with denied autopsy an external description of the fetuses was performed for the recognition of external malformations and the weight and different lengths was noted.
Results. The overall autopsy rate was 68% (352/517) and significantly declined from 95.5% in 2006 to 51.9% in 2012. Chromosomal aberrations occurred in 30% of the cases that were denied an autopsy vs. 17% of the cases in where an autopsy was performed (p=0.01). The autopsy rate was 66% of the fetuses after induced and 69% after a spontaneous abortion (p=0.64). There was no difference in maternal age in cases with and without autopsy (30.7±6 vs. 30.5±6 years, p=0.93), but gestational age was significantly lower (18.7±3.9 weeks vs. 19.4±4.1, p=0.03).
Conclusions. There is an obvious decrease of post-mortem examination at our institution. The causes for that might be multifactorial but unacceptable. A decrease of the autopsy rates interfere with the educational impact of pathology and might provoke a declining quality of sonographic examinations because of missing quality control by autopsy.

FR-137
Melanotic neuroectodermal tumor of infancy

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Aims. Retinaler anlage tumor, also called ameloblastoma, melanotic adamantinoma or melanotic progronoma is a rare, benign melanotic tumor, occurring mainly in infants under 12 months of age in the maxilla and mandible.
Methods. We report this tumor in a boy of 9 years. The specimen, from the nasal septum was resected under the clinical diagnosis of a hemangioma. It measured 2×1×1 cm.
Results. Conventional light microscopy showed a submucosal lesion measuring 1.4 cm with large adrenal structure with a broad fibrous seam and adjacent mucous salivary glands. The tumour cells were small, monomorphous, with an eosinophil cytoplasm and small, round, centrally located nuclei, arranged trabecularly and glandularly and surrounded by a mucopolysaccharid-rich matrix and numerous macrophages with

a small-grained dark brown to black pigment. Gordon-staining showed a prominent fibrovascular network. There were no mitosis and no necrosis. Immunohistochemically the cells showed a staining with the antibodies against S 100, cytokeratines, the larger, peripherally located cells CD34, Vimentin, CD56. The pigmented cells displayed HMB45 and Melan A. Electronmicroscopy showed premelanosomes and melanosomes of various electron densities due to varying melanin contents.
Conclusions. Electronmicroscopy helped supporting the diagnosis of this rare tumor.

FR-138
Papillary fibroelastoma in a neonate

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Aims. Papillary fibroelastoma (fibroelastic papilloma), an endocardial tumor, is the second most common benign heart tumor. Most common location is at the aortic valve. Up to now they have been described mainly in elderly patients with an age peak—according to the literature—in the eight decade. In children they are very rare. We describe a papillary fibroelastoma in a neonate, already visible prenatally.
Methods. A male neonate (6 d) was operated on a small subaortic tumor located on the left coronary leaflet of the aortic valve and obstructing the left ventricular outflow tract, clinically simulating a critical aortic stenosis. Prenatal echocardiography displayed a thickening of a part of the aortic valve.
Results. Two small whitish specimens, measuring 0.5×0.3×0.4 cm altogether. Microscopically there were finger-like papillary structures of a myxoid mesenchymal tissue covered by CD34-positive endothelial cells. In alcian staining acid mucopolysaccharides could be proven. There were no mitoses. Postoperatively, double ultrasound showed no longer a significant obstruction of the outflow tract.
Conclusions. The histological findings together with the localisation allowed the diagnoses of a papillary fibroelastoma of the left-coronary aortic valve leaflet. To the best of our knowledge this is the first case of a papillary fibroelastoma that had already been demonstrated by ultrasound prenatally. Like in about half of the reported cases the tumour was located at its predilective location at the aortic valve leaflet. Apart from problems resulting from the obstruction of the outflow tract this tumour has to be resected because of the risk of systemic embolization.

FR-139
13-year-old tuberous sclerosis patient with renal cell carcinoma associated with multiple renal angiomyolipomas developing multifocal micronodular pneumocyte hyperplasia

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Aims. The autosomal dominant tumor syndrome tuberous sclerosis complex (TSC) is based on the mutated TSC1 (harmatin) and TSC2 (tuberin) gene. These patients are developing typical cutaneus signs like peau chagrin, angiofibromas of the skin, astrocytomas of the brain, lymphangioleiomyomatosis of the lung, and angiomyolipoma (AML) of the kidney. Only a few cases of TSC patients with a multifocal micronodular pneumocyte hyperplasia (MMPH) of the lung are described.

Furthermore an increased incidence of renal cell carcinoma connected with TSC could be proved.
Methods. The patient was a 13-year-old white woman who presented in the age of 6 months salaam seizure and hypopigmented skin lesions which were classified as peau chagrin. In addition radiological studies demonstrated the typical cortical tubers leading to the diagnosis of TSC. In the following examinations a large number of AMLs were found in both kidneys. One lesion showed an increasing size and tumor-like aspects in magnetic resonance imaging (MRI). Four months postoperatively, a follow-up CT scan revealed multiple bilateral pulmonary nodules. To exclude lung metastases of the RCC, multiple open-lung biopsies were performed.
Results. The tumor of the kidney showed a solid, sometimes papillary architecture, and was composed of clear cells with voluminous cytoplasm, discrete cell borders, and vesicular chromatin. Immunohistochemical examinations revealed a weak to strong staining for vimentin, CD10 and P504s. CK7 or HMB-45 could not be shown in the carcinoma cells. Based on morphological and immunohistochemical aspects it was hypothesized that it could be a RCC of XP 11.2 translocation type. This hypothesis was confirmed by three independent consultant pathologists, but a typically expression of TFE3 and TFEB could not be demonstrated. The nodules of the lung were composed of proliferated and enlarged type II pneumocytes which had abundant eosinophilic cytoplasm and prominent nucleoli. Significant atypia or mitosis as well as an increased proliferation rate could not be observed. Immunohistochemically, the hyperplastic epithelial cells showed an expression of pan-CK, EMA, and TTF-1, whereas stains for HMB-45, actin, vimentin and ER were uniformly negative.
Conclusions. The here described case shows a rare case of a young TSC patient with multiple AMLs of the kidney developing a RCC as well as MMPH. The combination with MMPH is very rare and leads to a diagnostic challenge especially in case of RCC.

FR-140
An unusual case of follicular tumor in a child: a potential diagnostic challenge

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Aims. Follicular lesions and papillary carcinomas are both uncommon in the early childhood, especially in male patients. Tumor with a follicular morphology and questionable or cytological characteristics can be a diagnostic problem. Herein, we presented a case follicular tumor of uncertain malignant potential (FTUMP) in a child, focusing on the differential diagnosis.
Methods. An 8-year-old child, with a large (3.5 cm) nodule of a hot nodule in the right lobe of thyroid was referred to surgery (2007). The lesion was clinically worrisome, because of its very fast growth. Formalin fixed paraffin embedded blocks were stained with routine Haematoxylin and Eosin and representative blocks were selected for immunohistochemistry and PCR analysis.
Results. Macroscopically, a 3.5 cm large, well-demarcated, brown tumor occupied the great part of the thyroid lobe. Microscopically, the lesion had a thick capsule, with a diffuse follicular architecture. The nuclei were clear, enlarged, oval, sometimes showing grooves, nuclear overlapping and having centrally located nucleoli. Moreover the tumor showed multiple "incomplete" capsular interruptions, without a clear cut capsular penetration. There was no lymphatic or blood vessel's invasion. Mitoses or necrosis were also absent. The immunohistochemical stains with Galectin-3 and HBME-1 were negative. The lesion did not show BRAF (V600E) point mutation. Because the tumor had questionable

capsular invasion, in absence of vascular invasion, we concluded for the diagnosis of FTUMP. The follow up of the patient (5 years) is negative.
Conclusions. In this case we presented an unusual case of follicular lesion in child, with a morphology that can mimic both a follicular carcinoma and a follicular variant of a papillary carcinoma. Beside a carefully histological examination of the entire lesion, immunohistochemistry and molecular analysis of such lesions are of paramount importance to avoid misdiagnosis and patient's distress.

FR-141
An unusual case of toxic adenoma of the thyroid in young woman: a potential diagnostic pitfall

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Aims. Hyperfunctioning adenomas are very uncommon, representing about 1% of all follicular thyroid adenomas. These lesions, often "hot" on radionuclide scans, usually show a follicular architecture, with focally delicate papillary features. Herein, we presented a case of toxic adenoma with diffuse papillary morphology and we discuss the differential diagnosis.
Methods. A 16-year-old woman, with the clinical history of a hot nodule in the left lobe of thyroid was referred to surgery (2007). Formalin fixed paraffin embedded blocks were stained with routine Haematoxylin and Eosin and t representative blocks were selected for immunohistochemistry and PCR analysis.
Results. Macroscopically, a 2 cm large, well demarcated, grey-tan tumor occupied the great part of the surgical specimen. Microscopically, the lesion was well encapsulated, with a diffuse papillary and micro-papillary architecture. The nuclei were slight enlarged, oval, sometimes showing grooves and nuclear overlapping, miming a papillary carcinoma. The immunohistochemical stains with Galectin-3 and HBME-1 were completely negative. Moreover there was no BRAF(V600E) point mutation. All these findings confirmed the diagnosis of a toxic adenoma. In a follow up of 5 years there is no evidence of recurrence or metastasis.
Conclusions. In this case we presented an unusual case of toxic (hot) follicular adenoma in very young woman, with an uncommon diffuse papillary architecture. The clinical history and the macroscopic aspect of the lesion may be suggestive, among others, for a "pediatric solitary papillary hyperplastic thyroid nodule" (sec LiVolsi), wherever the microscopic features of the tumor can be confused with a papillary thyroid carcinoma. A carefully combination of histological, immunohistochemical and molecular features is crucial for a correct diagnosis.

FR-142
Primary ciliary dyskinesia and polysplenia

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Aims. Primary ciliary dyskinesia is a rare disease, transmitted autosomal recessively, with an incidence of 1:15000–30000. It is associated with (feverish) infections of the upper and lower respiratory tract, caused by the reduced or defective function of respiratory ciliae. Findings often associated PCD are situs inversus, bronchiectasia, rhinosinusitis, otitis media, polycystic liver- and kidney diseases, biliary atresia, retinopathy and—less frequently—polysplenia.
Methods. We report about a girl of 8 years with recurrent obstructive respiratory ailments resp. pneumonia and chronic coughing starting at toddler's age. Despite of these findings the girl subjectively was free

symptoms. Negative were a sweat-test, CFTR-mutation screening and alpha-1-antitrypsin-, total IgE- and tuberculosis-test. Nevertheless, the right basal lung radiologically displayed a persistent reduced transparency and in HRCT of the thorax a partial atelectasis of the right middle lobe paracardially and slight signs of bronchiektases were noted. Furthermore a polysplenia was found. At that nasal brush biopsy was taken and studied by electronmicroscopy.
Results. In the first instance electronmicroscopy of the nasal ciliae showed no groundbreaking findings but a new brush biopsy displayed findings of a primary ciliary dyskinesia.
Conclusions. Combination of clinical data plus electronmicroscopy of nasal ciliae allowed the diagnosis of PCD. The case demonstrates that signs of recurring obstructive respiratory ailments in association with polysplenia should awake the pathologist to the differential diagnosis of PCD, even when a first electronmicroscopical diagnosis of ciliae has been inconclusive.

FR-143
Congenital portosystemic shunt (so called "Abernethy malformation")—experiences with a rare entity

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Aims. Malformations of the portal vein (so called "Abernethy malformations") are rare entities, which may show clinical relevance in childhood. In "type 1" the intrahepatic portal vein is missing. In fact the "portal blood" is drained in the inferior caval vein exclusively. "type 2" shows a hypoplastic portal vein which is connected with the vena cava inferior. In consequence "intestinal blood" perfuses the liver as well as the inferior caval vein.
Methods. Three patients with "Abernethy malformations" were observed at the University Hospital of Innsbruck within the last 10 years. Patient 1, a 7-year-old boy, was suffering from a severe intestinal infection showing a 24 hours lasting period of somnolence referable to a sonographic diagnosed "Abernethy malformation type 2". Patient 2, a female newborn with multiple malformations, developed a cholestatic hepatopathy originating from a sonographic verified "Abernethy malformation type 1" within the first month of life. Patient 3, an 18-year-old woman suffering from idiopathic pulmonary arterial hypertension, was taken to the hospital due to a sudden cardiopulmonary decompensation which had to be treated by ECMO. By further investigations an "Abernethy malformation type 2" was found by abdominal CT.
Results. In the follow up of 5 respectively 4 years, the first two patients showed no further shunt-associated complaints. In Patient 3 bilateral sequential lung transplantation had to be carried out due to her severe respiratory problems. Unfortunately she died the postoperative course. The diagnosis of an "Abernethy malformation type 2" was confirmed by autopsy.
Conclusions. Abernethy malformations are rare entities. In most cases they are diagnosed incidentally by sonography. Symptoms range from inconspicuous to severe clinical courses with hepatic encephalopathy and/or hepatopulmonary syndrome. In "type 1" overlapping associated anomalies respectively benign or malignant liver tumours may be observed. Therapy depends on the type of malformation and severity of symptoms. In type 1 conservative therapy with temporary medication may be sufficient; in severe cases liver transplantation may become necessary. In type 2 interventions on the shunt may be useful.

FR-144
Unilateral renal angiodysplasia in a pediatric case of severe arterial hypertension

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Aims. Renal parenchymal and vascular disorders are the leading cause of secondary hypertension in childhood. Here we report on a pediatric case of severe hypertension cured by nephrectomy, presenting an uncommon renovascular pathology.

Methods. The clinical data and pathomorphological findings are presented.

Results. A 4-year-old girl presented with severe arterial hypertension. She was eutrophic, afebrile, alert and had no symptoms of illness, no edema, and no history of prior renal, urogenital or other relevant disease. Echocardiography showed myocardial hypertrophy consistent with chronic hypertension. Abdominal ultrasound revealed a dystrophic left and a normal right kidney but no tumor mass. Endocrine workup detected increased plasma renin activity but was otherwise normal. Serum creatinine and cystatin C were normal, indicating no impairment of renal function. Urinalysis showed mild proteinuria, but no hematuria or nephritic sediment. Hematological and immunological parameters were inconspicuous. DMSA scintigraphy revealed a non-functioning left kidney. Unilateral obstructive nephropathy was suspected. The kidney was surgically removed. Subsequently, blood pressure gradually subsided. On macroscopic examination, the kidney had a typical shape but was too small with 16 g in weight and 5.6×3.5×2 cm in size and reduced overall parenchymal thickness. There were no signs of hydronephrosis and no foci of necrosis, hemorrhage, tumor or stones. The ureter was slightly dilated. Histology revealed areas of largely normal, mature renal cortical and medullary parenchyma with only mild focal global glomerulosclerosis and regular vasculature, alternating with well-delineated areas of strongly dilated and irregularly shaped peritubular capillaries and veins accompanied by total glomerulosclerosis, complete tubular atrophy and interstitial fibrosis with mild chronic lymphoplasmacellular inflammation and moderate arterio-arteriolosclerosis. The renal sinus contained many malformed veins with strands and nodules of collagen-rich paucicellular fibrous tissue thickening the vein walls and causing luminal obliteration or splitting. These findings led to the histopathological diagnosis of renal angiodysplasia.

Conclusions. Unilateral renal angiodysplasia may be a rare cause of severe hypertension in children, which may be cured by nephrectomy.

FR-145
Case report: stereomicroscopic examination is a helpful procedure to diagnose primary congenital pulmonary lymphangiectasia as a rare cause for non-immune hydrops fetalis

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Aims. Non-immune hydrops fetalis (NIHF) often leads to intrauterine or postpartal death of newborn while reasons are often unclear. Primary congenital pulmonary lymphangiectasia (CPL) can be a rare cause for NIHF.

Methods. We describe a recurrent case of familial prenatally diagnosed NIHF. In 2011, during the first pregnancy hydrops fetalis was diagnosed in the 17th gestational week and an abortion induced three weeks later. The subsequently performed autopsy of the fetus did initially not reveal any plausible cause for the hydrops fetalis. In 2012, NIHF was diagnosed in the 21st gestational week during the second pregnancy. In the 29th week a male infant was prematurely born and died eight hours after birth because of respiratory failure.

Results. Beside effusions in serous cavities (pleural, pericardial, peritoneal), anasarca and an incomplete separation of the right upper and middle pulmonary lobe, conventional macroscopic examination did not reveal any conspicuous diagnostic findings. Only by stereomicroscopic examination of the lung and the main thoracic vessels an abnormal spiderweb-like tissue surrounding both lung hila could be detected. Corresponding histological findings indicated perivascular and -bronchial dilatation of lymphatic vessels. Taken together the results lead to the diagnosis of CPL confirmed by a consulting reference paidopathologist. According to the Noonan classification of CPL (Noonan et al. 1970) the case was assigned group three and a primary CPL according to the Bellini classification (Bellini et al. 2006), respectively. Reexamination of the stored autopsy formalin fixed tissue from the fetus with the NIHF from 2011 indicated similar but less distinctive histological findings according to the lymphatic vessel abnormalities of the lung.

Conclusions. In recent literature seven similar cases were published between 1970 and 2009, three of them siblings. This and our cases suggest a genetic background for primary CPL which has to be investigated in the future. The diagnosis of CPL does not always succeed, but stereomicroscopic examination is a helpful procedure to diagnose CPL as a cause of NIHF.

Postersession Herz-, Gefäß-, Nieren- und Transplantationspathologie

FR-146
Post-mortem CT-angiography in clinical autopsy

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Aims. Although clinical autopsy rates are declining in Germany and worldwide, pathologists are not taking advantage of modern imaging techniques to increase diagnostic yield. After evaluation of native post-mortem CT (PMCT), post-mortem CT-angiography (MPMCTA) was established. We performed native PMCT and PMCTA before autopsy with a focus on deaths due to cardiovascular disease.

Methods. Contrast solution calibrated to 300 HU was applied via the femoral arteries. Full-body native PMCT and PMCTA were performed prior to autopsy. During autopsy, all three body cavities (head, thorax, abdomen) were opened and organs were inspected macroscopically. Histological specimens of internal organs (heart, lungs, kidneys, adrenal glands, liver, pancreas, spleen) and conspicuous findings were collected.

Results. PMCTA markedly improved radiological evaluation of the cardiovascular system compared to native PMCT. Contrast solution perfused the arterial system including cerebral, thoracal, coronary and abdominal arteries, upper and lower extremities. Histologically, contrast medium was found in arteries as small as the glomerular arteries. No artefacts complicating macroscopical or microscopical examination were produced. Vital thrombosis was not affected by contrast application. Postmortal blood clots (cruor mortis) were no obstacle to contrast perfusion. No dilution of contrast solution was observed.

Conclusions. PMCTA is a feasible method to complete the macroscopic and microscopic examination of the cardiovascular system during clinical autopsy. Arteries can be evaluated in situ without opening arterial lumina or risk of creating artefacts. The data presented indicate that PMCTA is a precious supplement to clinical autopsy, enhancing the insights of radiologists and pathologists alike.

FR-147
Postmortale multiphasische CT-Angiographie zur Klärung von Blutungsquellen und Gefäßverschlüssen

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Ziele. Die Klärung von Todesfällen bei der Beurteilung von unklaren Blutungsquellen und Gefäßverschlüssen oder bei der klinischen Qualitätssicherung nach potenziell iatrogen verursachten Komplikationen kann durch die neu entwickelte Methode der multiphasischen postmortalen CT-Angiographie (MPMCTA) sinnvoll unterstützt werden.
Methoden. Auf Basis eines Standardprotokolls wird mit einem speziellen Perfusor ein lipophiles Kontrastmittel in das arterielle sowie venöse Gefäßsystem eingebracht und anschließend eine Teilzirkulation erzeugt.
Ergebnisse. Die MPMCTA stellt gegenüber der Obduktion einen entscheidenden Mehrwert beim Nachweis von Blutungsquellen dar. Sie kann bei diffusen Blutungen z. B. im Abdomen bei der statischen Befunderhebung im Rahmen der Obduktion mitunter nicht darstellbare Leckagen demonstrieren, eine Abgrenzung zwischen Blutungen venösen und arteriellen Ursprungs klären sowie grundsätzlich die Dokumentation von Perfusionsverhältnissen nach vaskulären Eingriffen ermöglichen. Die anatomische Nähe unbeabsichtigt verletzter Strukturen kann in der Rekonstruktion hervorragend herausgearbeitet werden.
Schlussfolgerung. Es werden Beispiele für Komplikationen nach neuro- und abdominalchirurgischen Eingriffen gezeigt, bei der die MPMCTA die entscheidenden Hinweise erbrachte.

FR-148
Qualitätssicherung nach Komplikationen bei kardiovaskulären Interventionen: die Rolle der postmortalen Bildgebung

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Ziele. Bei der Begutachtung von Komplikationen ärztlicher Behandlungsmaßnahmen können Methoden der Bildgebung je nach Fragestellung der Dokumentation entscheidender morphologischer Befunde für oder gegen die Feststellung einer iatrogen verursachten Schädigung dienen. Die Klärung von Todesfällen kann in diesem Zusammenhang durch postmortale Bildgebung (z. B. native Computertomographie, CT-gestützte Angiographie, Magnetresonanztomographie) sinnvoll unterstützt werden.
Methoden. Es werden typische Fallkonstellationen nach Komplikationen minimal-invasiver angioplastischer Interventionen demonstriert, bei denen autoptische Befunde durch Ergebnisse postmortaler Bildgebung unterstützt werden können. Dazu zählen Koronarrupturen bei perkutaner transluminaler Koronarangiographie und hämorrhagische Komplikationen nach Gefäßverletzungen bei transapikalem oder transfemoralem Aortenklappenersatz (TAVI) sowie nach endoluminalem Aortenstenting in Kombination mit Debranching-Operationen.
Ergebnisse. Bildgebung bietet gegenüber der Obduktion aber auch einen entscheidenden Mehrwert beim Nachweis von iatrogener Luftembolie. Die Dokumentation von Fehllagen eingebrachter medizinischer Hilfsmittel vor autoptischer Eröffnung mit der Gefahr der Verlagerung ist

möglich. Entscheidende Kriterien für die Indikationsstellung einer postmortalen Bildgebung lassen sich aus der notwendigen vorausgehenden Einsichtnahme in klinische Dokumentationen gewinnen.
Schlussfolgerung. Postmortale Bildgebung bietet sich auch an, um für Zwecke im Sinne klinischer Qualitätssicherung u. U. auch ohne Autopsie entscheidende Befunde beizutragen.

FR-149
Comparison of the biocompatibility of a surface-modified vascular prosthesis with higher flexibility and a clinically used protein coated prosthesis

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Aims. Today, one of the most important problems in implant medicine is the combination of a perfect implant-design for clinical application combined with ideal biointegration of implant materials. The optimal and fast fixation of the vascular prosthesis reduces the risk of infection as well as of thrombosis, two major events which lead to rapid functional loss. Today, protein-coated implants for clinical use show a high stiffness compared to gelatin-coated prosthesis. To improve the flexibility of the prosthesis a modified protein coating was developed.
Methods. Modified protein-coated vascular prosthesis (Uni-Graft Synthetic Soft, Aesculap) based on expanded polytetrafluorethylen (ePTFE, expanded Teflon) were used in a sheep model to compare the biocompatibility to a standard Proteograft CV 1900. The tissue integration of the implants had been analyzed after three months of implantation by morphologic evaluation of qualitative parameters. Furthermore, semiquantitative analysis of inflammation, periimplant fibrous reaction and giant cell induction was performed.
Results. After an implantation period of 3 months the Sythetic Soft-prosthesis show a mild acceleration of neointima development without alteration of neoendothelialization. The inflammatory response was slightly reduced compared to the standard prosthesis. The number of foreign body giant cells was reduced. The vascularization of the outer implant zone was reduced insignificantly at the Sythetic Soft-Implants. Other histological parameters—granulocytic infiltration, periimplant fibrosis as well as thickness of periimplant capsule—did not show any alteration after implant surface modification.
Conclusions. The change of implant surface using a modified protein impregnation procedure, which leads to a higher flexibility of the vascular prosthesis, shows a better tissue integration of the new prosthetic device without any thrombotic effects. The new implant will be used in clinical studies to prove the results of the animal experiments.

FR-150
Nestin-positive cells within patients with chronic thromboembolic pulmonary hypertension

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Aims. Chronic thromboembolic pulmonary hypertension (CTEPH) is a life-threatening disease of the pulmonary vessels and is caused by single or recurrent thromboembolisms in the larger proximal pulmonary arteries (PA) as well as by arteriopathy in the smaller distal PA. Both processes lead to the obliteration of the vessels, resulting in an increase of the pulmonary arterial resistance and the induction of the development of pulmonary heart disease. The precise pathophysiology is not

yet clarified, and a potential therapy is only possible by a pulmonary endarterectomy (PEA) of the thrombotic vessels. Nestin is an intermediate filament that is expressed mainly during embryonic development, but also in the adult organism under certain circumstances. It can be used as a marker for neuronal stem and precursor cells and for activated endothelial cells during neovascularization. In this study the expression of nestin was analysed in thrombotic tissue from endarterectomized patients with CTEPH.

Methods. PEA tissue samples from 20 CTEPH patients (10 women, 10 men, average age: 53.25 years) were treated according to standardized processes for immunohistochemical evaluation. The biopsies were examined immunohistologically for their Nestinexpression by the use of monoclonal antibodies against nestin. In each case, the proximal and distal lesions were evaluated.

Results. Nestin-positive cells were detected in both proximal and distal lesions of the pulmonary arteries. They were found as single cells in fibrotic thromboembolic material (proximal and distal). Furthermore, in endothelial cells in the neointima (proximal and distal) and in recanalized vessels (distal) Nestin-expression was detectable.

Conclusions. For the first time nestin-expressing cells were detected in samples of PEA from CTEPH patients. These cells may represent precursor cells of endothelial or mesenchymal origin, such as premyofibroblast-like progenitor cells, which play a potential role in the pathophysiology of CTEPH. In further studies should be investigated whether nestin-positive cells differentiate into other cells types and weather they lose their Nestin-positivity. Furthermore, it is important to clarify whether these cells originate from the bone marrow, or from the reactive vessel wall.

FR-151
KIT and CSFR tyrosine kinases are overexpressed in angiosarcomas

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Aims. Angiosarcomas are a very aggressive group of tumors, most often arising in deep soft tissue, skin and visceral organs. They are characterized by a high metastatic rate and an overall 5-year survival of 10–15% at most. Clinical management consists primarily of surgery and radiotherapy or cytotoxic chemotherapy and no effective targeted therapies are currently available. The aim of our study was to evaluate the expression and mutational status of potential molecular therapeutic targets in angiosarcomas.

Methods. 88 vascular tumor cases, comprising conventional angiosarcoma (n=17), post-radiation angiosarcoma of breast (n=4), hemangioendotheliomas (n=17), Kaposi sarcomas (n=15), and hemangiomas (n=35) were included in this study. Expression of EGF-receptor tyrosine kinase family (EGF-R, HER3), type III receptor tyrosine kinase receptors c-kit (CD117), CSFR and its ligand CSF1 as well as signaling molecules implicated in the mTOR pathway (mTOR, PTEN) was analyzed by immunohistochemistry. In addition, selected angiosarcoma cases were screened for mutations in CSF-R (coding region), EGFR (exon 19 and 21), c-kit (exon 9, 11, 13 and 17), PIK3C, B-RAF, K-RAS, N-RAS genes.

Results. CSFR and c-kit expression is seen in a significant number of angiosarcoma cases as compared to the other vascular tumors studied, accompanied by coexpression of CSF-L in only a few cases. In contrast, angiosarcomas are negative for EGFR and HER3. Activation of signaling molecules of the mTOR pathway is frequent. No significant loss of PTEN immunoreactivity was detected. Mutations of CSFR, EGFR, PIK3C, B-RAF, K-RAS, N-RAS genes are rare.

Conclusions. CSFR and c-kit expression is found in the majority of angiosarcomas and may reflect "oncofetal" expression, however, activating mutations in the juxtamembrane or tyrosine kinase domain or downstream molecules of the receptors are rare. Coexpession of CSF-L

is not necessarily present. Activation of mTOR pathway is seen as well, whereas loss of PTEN does not play a role. The benefit of targeted therapy against these RTKs in angiosarcoma remains to be determined.

FR-152
miRNAs regulate cancer stem-like cells differentiation into tumor cells in angiosarcoma

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Aims. Increasing evidence shows that miRNAs can act as oncogenes or tumor suppressors. miRNA expression patterns in tumors may have substantial value for diagnostic and prognostic determinations as well as for molecular targeted therapy. Recently studies suggest that cancer stem cells (CSC) are proposed to be a distinct population and cause relapse and metastasis by giving rise to new tumors. Therefore, development of specific therapies targeted at CSCs holds hope for the treatment of malignant tumors, especially for sufferers of metastatic disease. Angiosarcoma is a rare malignant vascular tumor with significant metastatic characteristics. In our study, we aim to unravel key miRNA(s) involved in driving CSC differentiation of angiosarcoma to generate new tumors. This can be of rather interest in order to design new therapies against the angiosarcoma relapse or metastasis since the key miRNA(s) can be targeted to inhibit CSC differentiation.

Methods. To achieve our aim, techniques such as flow cytometry and cell sorting, RT-PCR, immunofluorescence staining, tumorigenicity assays, sphere assays and microarrays et al were applied.

Results. Using flow cytometry we isolated CD133+ cells from human angiosarcoma cell line (ISO-HAS) and fresh human angiosarcoma tissues. In stem cell media, those CD133+ cells formed sphere clusters and were characterized by upregulated expression of stem cell genes such as oct4, nanog, c-myc and sox2 et al. We also found that CD133 positive population was more resistant to commonly used chemotherapeutics and was able to grow a tumor with a smaller number of cells inoculated into a mouse than CD133 negative cells population. Taken together, our present findings lead to the thought that CD133+ cells are potentially cancer stem—like cell in angiosarcoma. Next, we are going to profile miRNA expression of CD133+ and CD133– cells in ISO-HAS cell line and angiosarcoma tissues and identify subsets of miRNA that are differentially expressed. Among them, the key miRNA(s) involved in regulating CSC differentiation into tumor cells will be identified with differentiation assays of CD133+ cells by knock-down or over-expression of candidate miRNAs.

Conclusions. In conclusion, for the first time, our study identified cancer stem-like cells/CD133+ cells in angiosarcoma. The possible identification of the key miRNA(s) regulating CD133+ cell differentiation into tumor cells may provide us new approaches to prevent tumor relapse and metastasis by targeting the miRNA(s)in CSCs.

FR-153
Diagnostic accuracy of conventional cytopathology in pericardial effusions

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Aims. The aim of our investigation was to report on the diagnostic accuracy of conventional pericardial effusion cytology. Cytological diagnoses of 425 pericardial effusions, performed on MGG- and Pap-stained smears were compared with clinical and/or histological follow-up of the respective patients. In 194 cases, a final follow-up diagnosis of malignancy was made; 187 cases revealed metastatic tumor to the pericardium (96.4%), seven cases (3.6%) were diagnosed as malignant me-

sothelioma. In 231 cases, the follow-up revealed benign causes for the pericardial effusion.

Methods. The specimens contained 10–50 ml of effusion fluid on average. After centrifugation and decantation of the supernatant, six slides per specimen were made from the sediment and stained according to May-Grünwald-Giemsa (n=3, after air-drying) or Papanicolaou (n=3, after alcohol fixation), respectively.

Results. The sensitivity of our cytological diagnoses on pericardial effusions was 84.5% (164/194); the specificity was 97.0% (224/231). The positive predictive value was calculated as 95.9% and the negative predictive value as 91.3%. The most frequent primary tumors were lung cancer (59.8%, n=116), breast cancer (14.4%, n=28) and leukemia/lymphoma (6.2%, n=12). In the negative group, inflammation was the most common cause of effusion (27.7%, n=64), followed by post-surgical changes (20.4%, n=47) and cardiac insufficiency (11.7%, n=27). Several cases remained without clearly defined etiology.

Conclusions. In contrast to pleural effusions or ascites, data about pericardial effusion cytology are comparably sparse. Our investigation showed higher diagnostic accuracy of conventional cytopathology compared with recent data for pleural effusions and ascites, possibly due to a higher cell/fluid ratio in pericardial effusion which might result in a lower rate of sampling errors. Adjuvant methods applied on this large series of cases (immunocytochemistry, DNA image cytometry, fluorescence in situ hybridization) helped to further improve the diagnostic accuracy; the respective data, however, are still under analysis and will be reported separately.

FR-154
Histomorphometric analysis of myocardial fibrosis after heart transplantation

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Ziele. Hauptkomponenten der myokardialen extrazellulären Matrix sind Kollagen Typ I und Kollagen Typ III. Darüber hinaus ist Kollagen Typ IV in der Basalmembran vorhanden. Das strukturelle Remodeling der Kollagenmatrix führt zu einer überschießenden Akkumulation kollagener Fibrillen und so zur pathologischen Myokardfibrose. Funktionelle Konsequenzen ergeben sich in einer Zunahme der Myokardsteifigkeit. Das Phänomen des Kollagen-Turnover bei herztransplantierten Pat. ist bisher noch nicht evaluiert. Ziel dieser Studie war es, den Verlauf von Kollagen Typ III und IV (Koll. III bzw. Koll. IV) in Endomyokardbiopsien (Bx) nach Herztransplantation (HTx) darzustellen und die Aussagekraft ihrer immunhistochemischer Färbungen mit in der Routine angewendeten Fibrosedarstellungen mittels Sirius-Rot-Färbung zu vergleichen.

Methoden. Insgesamt wurden 274 Biopsien zum Zeitpunkt Null-Bx = Tag der HTx, FU1 = 4 Wochen, FU2 = 1 Jahr, FU3 = 3 Jahre, FU4 = 5 Jahre post-HTx von 77 Pat. (66 Männer, HTx 2003–2006, Durchschnittsalter: 49 Jahre bei HTx) computerbasiert histomorphometrisch untersucht. Der Anteil an Koll. III und IV sowie an Myokardfibrose wurde für jede Bx anhand von 12 Messungen (1 mm2) ermittelt.

Ergebnisse. Im Mittel nahm der Anteil an Koll. III kontinuierlich zu, während sich der Anteil der Fibrose eher schwankend darstellt(Null-Bx: 13,0 vs. 8,59%, FU1: 11,94 vs. 7,87%, FU2: 12,0 vs. 8,47%, FU3: 12,19 vs. 6,95%, FU4: 16,17 vs. 7,9%; p<0,01). Das Verhältnis der Mittelwerte von Koll. III zur gemessenen Fibrose steigert sich somit ausgehend von 1,52 (FU1) auf über das 2-fache im 5. Jahr post-HTx (FU4: 2,05 vs. Null-Bx: 1,51, FU2: 1,42, FU3: 1,75). Koll. IV stieg im Mittel noch deutlicher an (Null-Bx: 12,24%, FU1: 12,43%, FU2: 14,26%, FU3: 19,76%, FU4: 22,27%). Im Verlauf post-HTx ist ein höherer Anteil Koll. III von >20% auch mit einem höheren Anteil an Fibrose (10–19,9%) assoziiert (FU2: p=0,02; FU4: p<0,02).

Schlussfolgerung. Im langfristigen Verlauf post-HTX steigt der Anteil an Koll. III und IV im Vergleich zur Fibrose in der Bx signifikant an. Die Auswirkung des kollagenen Remodelings auf die kontraktile Funktion des Myokards herztransplantierter Patienten ist offen und bedarf weiterer Untersuchungen.

FR-155
Validation of a rapid protocol for mRNA isolation and quantification suitable for pre-transplant evaluation of donor biopsies

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Aims. Currently, histopathological donor organ evaluation is only performed on frozen sections or rapidly processed paraffin-embedded tissue. The advent of pre-transplant therapy calls for advanced pre-transplant histopathological diagnostics. mRNA expression analysis has been shown to complement conventional histological examination of tissue samples, however standard protocols for the isolation and quantification of mRNA are too time-consuming and thus not suitable for donor biopsy evaluation. In order to meet the increasing demand for refined pre-transplant tissue diagnostics we evaluated a more rapid protocol.

Methods. Ten cryostored liver samples were retrieved from the archive of the Institute of Pathology. Each 3 frozen sections were cut and mRNA isolation and quantification by RT PCR was performed with two different protocols. The novel, rapid protocol included RNA isolation with the ZR RNA mini Prep column (Zymo Research) followed by a one-step RT PCR (Express One-Step SuperScript qRT-PCR, Invitrogen). Our standard protocol included RNA isolation with trizol, ethanol and isopropanol, followed by cDNA synthesis and TaqMan RT PCR. The time to complete each protocol was recorded. For each sample, ADAMTS13 mRNA was quantified with the two different protocols and relative expression levels were compared.

Results. The rapid protocol was completed within 138 min while the standard protocol required overnight isolation of mRNAs. Mean CT-values were significantly lower when determined with the rapid protocol than with the standard protocol for all transcripts and samples measured. Pairwise correlation of relative ADAMTS13 expression as determined by the two protocols was 0.66 (p=0.0396).

Conclusions. The novel rapid protocol can deliver mRNA quantification within less than 150 min with good accuracy compared to our standard protocol. It makes mRNA expression studies suitable for an application in pre-transplant diagnostics not only for liver, but also for heart, kidney and lung biopsies. Further research projects based on this novel rapid protocol could define predictive and prognostic mRNA transcripts helpful in directing pre-transplant therapy of donor organs.

FR-156
A case of severe rhinocerebral mucormycosis with rhizopus oryzae on prosaconazole prophylaxis following allogeneic stem cell transplantation

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Aims. Rhizopus oryzae, the most common Mucorales species, is responsible for more than 70% of mucormycosis cases. It predominantly occurs as an opportunistic infection in immunocompromised risk groups, such as patients with diabetes mellitus, neutropenia, malignant hematologic disorders and patients receiving immunosuppressive drugs such as cyclosporin A (CyA) or corticosteroids. Although it represents a rare infectious disease, mucormycosis is a rapidly progressive and potentially fatal infection with an overall mortality rate greater than 50%. It is well-known that patients with diabetes are predisposed to primary si-

nonasal and rhinocerebral infections, whereas patients' sufferings from haematological malignancies, as well as those who have undergone solid organ or hematopoietic stem cell transplantation are significantly more susceptible to invasive pulmonary mucormyosis.

Methods. Here we describe a 45-year-old male patient who was diagnosed with bcl-abl-positive ALL. He therefore underwent allogeneic hematopoietic stem cell transplantation from a HLA-compatible unrelated donor. Sustained morphological and molecular complete remission was confirmed at day +58.

Results. Post transplant complications included a severe acute intestinal GvHD grade IV. A second-line salvage therapy containing corticosteroids, Alemtuzumab and Infliximab could be successfully. Five month later the patient presented a miosis, ptosis and painful swollen eyelid on the right side. Initial cerebral CT-Scan which showed a sinusitis maxillaris, ethmoidalis et sphenoidalis. The prophylaxis with prosaconazol was switched to parenteral amphotericin B. Expansion of the periorbital edema and progressive loss of vision led to surgical interventions of the right maxillary region and ethmoidectomy. Biopsy samples showed invasive zygomycosis with angioinvasive character causing thrombosis and widespread necrosis. Microbiological cultures identified rhizopus oryzae. After a short-term clinical stabilization mucormycosis rapidly worsened with radiological evidence on MRT for cerebral involvement, including right-sided frontobasal white matter edema.

Conclusions. Mucormycosis is a rare but devastating and life-threatening infection. However, in immunocompromised patients, such as patients after allogenic stem cell transplantation the incidence has been increasing recently. In this regard, attention should be paid to the occurrence of breakthrough zygomycosis despite early prophylactic antifungal treatment with prosaconazole.

FR-157
A rat renal allograft model for transplant glomerulitis and glomerulopathy

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Aims. The investigation of chronic humoral rejection in renal allografts is currently limited by the lack of suitable experimental models. Therefore we set out to establish such a model in the rat that should closely resemble the glomerular capillary inflammation and features of transplant glomerulopathy commonly observed in human renal transplants deteriorating due to humoral rejection.

Methods. Kidneys from Fischer 344 rats were transplanted into Lewis rats (allografts). As controls served isografts from Lewis rats into Lewis rats. Three rats each were sacrificed at 6 weeks, 12 weeks and 26 weeks after transplantation. Renal transplant tissue was embedded in paraffin and evaluated by light microscopy of Jones stains for changes indicating acute and chronic glomerular capillary damage.

Results. No evidence of acute or chronic glomerular capillary damage was seen by light microscopy in isografts or allografts 6 weeks after transplantation. 12 weeks after transplantation transplant glomerulitis, focal and segmental duplication of glomerular basement membranes consistent with human transplant glomerulopathy and focal and segmental glomerulosclerosis were present in allografts and absent in isografts. 26 weeks after transplantation these changes were further advanced in allografts and still not detectable in isografts.

Conclusions. Light microscopic changes in our Lewis 344 to Fischer model renal allograft model closely recapitulate glomerular changes observed in acute and chronic humoral rejection in human renal transplants. Further comparative histological, serological and miRNA and mRNA expression analyses will show, if this promising model will indeed be suitable to investigate the mechanisms of acute and chronic humoral rejection and to develop novel therapeutic interventions.

FR-158
Oral carnosine supplementation prevents diabetes induced microvascular damage in the retina and the glomerulus in a rat model of diabetes

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Aims. Diabetic retinopathy and nephropathy are microvascular complications of diabetes characterized by damage of endothelial cells and perivascular cells such as pericytes and podocytes mediated by diabetes induced biochemical alterations. Carnosine, a naturally occurring dipeptide, supposedly is a protective factor regarding diabetic complications. The aim of this study was to assess the effect of orally administered carnosine on diabetes induced biochemical alterations and the development of diabetic retinopathy and nephropathy in a rat model of diabetes.

Methods. Retinas and kidneys of streptozotoxin-induced diabetic rats treated or not treated with carnosine (1 g/kg/day) and non-diabetic control rats were analyzed by western blotting for markers of diabetes induced biochemical changes: ROS-, AGE-production and activation of hexosamine pathway. Furthermore, expression levels of angiogenic growth factors, such as Ang-1, Ang-2 and VEGF were analysed in the retina and apoptosis related factors including bax and cytochrom c were assed in the kidney cortex. Besides, retinal vascular damage, retinal glia activation and retinal expression of heat-shock proteins as well as glomerular apoptosis and podocyte loss were assessed by immunohistochemistry, immonufluorescence and quantitative morphometry.

Results. Oral carnosine treatment prevented retinal vasoregression and retinal pericyte loss associated with increased Hsp27 expression and normalization of Ang-2. In the Kidney, carnosine inhibited proapototic signalling and thereby prevented glomerular apoptosis and podocyte loss. Vascular protection in the retina and the kidney was independent of biochemical changes, as all diabetic groups showed elevated levels of ROS and AGE as well as hexosamine pathway activation.

Conclusions. Carnosine treatment protects the diabetic rat retina and kidney from diabetes induced damage, independent of diabetes induced biochemical changes. The vasoprotective effect of carnosine might be mediated indirectly by induction of survival factors, anti-apoptotic pathways and normalization of angiogenic growth factors.

FR-159
Hypoxia-induced mesenchymal alterations in renal proximal tubule epithelial cells: evidence for miR-124/MMP2-regulated cell migration

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Aims. Interstitial fibrosis and tubular atrophy (IFTA) is associated with chronic renal transplant failure. Ischemia and hypoxia directly or indirectly contributes to IFTA. Atrophic tubular epithelial cells acquire a mesenchymal phenotype through a process called epithelial mesenchymal transition (EMT). The aim of this study was to investigate regulation of EMT-related genes and microRNAs in an in vitro hypoxia model in renal proximal tubulus cells.

Methods. Renal proximal tubule epithelial cells (RPTEC) were cultured under hypoxic conditions and a migration assay was performed. Expression of 45 EMT-related protein-coding genes and 384 microRNAs was analysed by real-time qPCR. Cells were transfected with artificial

pre-miR-124 precursor molecules or scrambled control and western-blot was used for protein detection.

Results. RPTEC cells showed an increased expression of matrix modulation factor metaoproteinase 2 (MMP2). This was correlated with decreased expression of miR-124, a microRNA which can bind to the MMP2 transcript and inhibits protein translation. Hypoxia-induced up-regulation of MMP2/down-regulation of miR-124 was associated with increased migratory ability. The inverse MMP2/miR-124 regulation was confirmed by artificial miR-124 over-expression in RPTEC cells and resulted in decreased MMP2 levels and decreased migratory ability.

Conclusions. MMP2 is involved in basement membrane degradation and fibrosis. These results show that proximal tubular epithelial cells show a hypoxia-sensitive regulation of MMP2 via miR-124. This molecular mechanism is associated with cell migration and might contribute to EMT in renal transplants. The suppression of MMP2 by miR-124 may be a therapeutic option to EMT in transplants.

FR-160
Sulfatides are required for renal adaptation to chronic metabolic acidosis

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Aims. Low blood pH, as it occurs in metabolic acidosis, affects cellular functions and can lead to increased morbidity and mortality. Urinary ammonium excretion by the renal collecting duct into the urine is essential to maintain stable blood pH. Ammonium is concentrated in the interstitium of medulla and papilla, those parts of the kidney in which the highest concentrations of sulfatides, highly charged anionic glycosphingolipids, can be detected. Yet, the basic physiological function of renal sulfatides in vivo has not been elucidated.

Methods. Sulfatide synthesis was inhibited cell-specifically by Pax8-driven genetic disruption of cerebroside sulfotransferase (Cst) along the renal tubule using newly generated Cst-flox mice. Compensatory synthesis of other negatively charged glycosphingolipids was inhibited by a concomitant deletion of glucosylsceramide synthase (Ugcg).

Results. Renal sulfatide-deficient mice had lower urinary pH accompanied by lower ammonium excretion. Upon acid diet, they showed impaired ammonuria, decreased ammonium accumulation in the papilla, and chronic hyperchloremic metabolic acidosis. Expression levels of ammoniagenic enzymes and the NKCC2-cotransporters were higher, and transepithelial NH_3 transport, examined by in vitro microperfusion of cortical and outer medullary collecting ducts, was unaffected in mutant mice.

Conclusions. Based on our results we suggest that sulfatides act as counterions for interstitial ammonium facilitating its retention in the papilla. This study points to a seminal role of sulfatides in renal ammonium handling, urinary acidification, and acid-base homeostasis.

FR-161
Lipid nephropathy in patients enrolled in a methadone substitution program

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Aims. Kidney biopsies show often accumulation of lipids or lipid-like material in different cell types. The distribution pattern and the ultrastructural characteristics of this material are a valuable source of information in the differential diagnosis. We describe a distinct novel lipid nephropathy occurring in patients with a positive history of narcotic abuse, who were enrolled in a methadone substitution program and presented with proteinuria (2.5–20 g/day) and impaired renal function.

Methods. Light-microscopical, immunhistochemical and ultrastructural investigations of kidney biopsies and correlation of the findings with the current literature.

Results. In all three cases, renal biopsy revealed pronounced lipid deposition in glomerular, interstitial and tubular cells and a focal segmental glomerulosclerosis. By immunohistochemistry, part of the glomerular and interstitial foam cells could be identified as CD68 positive lipid storing macrophages. Focal segmental deposition of immune complexes was present only in one of the three patients; this patient had also an active HCV infection, however mesangioproliferative glomerulonephritis was excluded by immunohistochemical and ultrastructural studies. A HIV infection was ruled out in all 3 patients. Also none of the patients suffered from a prior dyslipidemia or hyperlipidemia.

Conclusions. We consider this to be a distinct renal pathology observed in patients with a history of drug addiction and current enrolment in a methadone substitution program. It is characterized by prominent lipid deposits in glomerular, interstitial and tubular cells. When this form of renal pathology is diagnosed, an association with narcotic abuse, methadone intake or intravenous methadone abuse should be included in the differential diagnosis.

FR-162
Quantitative real-time PCR allows miRNA and mRNA expression analysis of parietal epithelial cells

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Aims. Glomerular crescents develop from parietal epithelial cells (ParEpCs). The presence and the extent of crescents correlate with a dismal prognosis. So far, studies on ParEpCs were limited because they could not be analyzed in human biopsies by quantitative real-time PCR (qRT-PCR) for mRNAs and miRNAs. This proof-of-principle study shows that our improved laser microdissection and RT-PCR techniques can overcome this limitation and enable mRNA and miRNA expression analysis of ParEpCs and the crescents derived thereof.

Methods. In a pilot study we isolated three compartments glomerulus (G), ParEpCs and periglomerular tissue (PGT) from a paraffin embedded renal biopsy with normal glomeruli. After preamplification we quantified the compartment markers WT1 (podocytes), PAX-2 (ParEpCs) and CD45 (leukocytes contained in the PGT) and small nuclear RNA reference transcripts (RNU48 and snRNU6) by qRT-PCR. Results are given as relative expression levels.

Results. Relative expression levels were as follows:
– WT1 in G: 2.3, in ParEpCs: 1.9, in PGT: not detected,
– PAX-2 in G: 0.1, in ParEpCs: 2.4, in PGT: 1.5,
– CD45 in G: 0.0, in ParEpCs: 0.0, in PGT: 0.2,

– RNU48 and snRNU6 were expressed at a quantifiable level in all three compartments.

Conclusions. Our results show that it is possible to isolate ParEpCs from other kidney compartments. The improved laser microdissection and RT-PCR techniques allow quantification of mRNAs and miRNAs in ParEpCs. This enables advances in the research on ParEpCs pathology and crescent formation.

Postersession Gynäko- und Mammapathologie

SA-054
Differences and similarities between malignant melanoma of the vulva and the vagina

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Aims. Malignant melanoma of the vulva and vagina is relatively uncommon and accounts for <5% of all melanomas in women. The aim of our study was to establish the biological properties and evaluate potential therapeutic targets in these tumors.

Methods. We collected a series of 66 cases from 3 centres and re-evaluated the tumour tissue for predominant growth pattern (superficial spreading/SSM, nodular/NM and mucosal-lentiginous/MLM) and tumour thickness. KIT (CD117) expression was detected immunohistochemically. In addition, tumours were screened for BRAF, NRAS and KIT mutations by PCR and DNA sequencing as well as for KIT amplifications by fluorescence in situ hybridisation (FISH).

Results. None of the cases contained BRAF mutations, NRAS and KIT mutations were detected in 5 and 8 cases, respectively, all of which were vulvar melanomas. Moderate or strong KIT expression was detected in 29 cases, 6 of which (all located on the vulva) contained KIT amplifications.

Conclusions. While malignant melanoma of the vulva contains NRAS and KIT mutations as well as KIT amplifications in up to 20% of cases, these molecular alterations are virtually absent in tumours originating from the vagina. This finding suggests that in spite of the anatomic proximity, the development of vulvar and vaginal melanomas involves different pathways that may be targeted by novel treatment approaches.

SA-055
Immunocytochemical detection of HPV L1 capsid protein and p16 in the ThinPrep Pap Test of the cervix uteri

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Aims. Since the Pap test was introduced in the 1940s, there has been an approximately 70% reduction in the incidence of squamous cell cervical cancers in many developed countries by the application of organised and opportunistic screening programs. The efficiency of the Pap test, however, is bound by high interobserver variability and high false-negative and false-positive rates. The use of biomarkers has demonstrated the ability to overcome these issues, leading to improved positive predictive value of cervical screening results. In addition, the molecular HPV analysis will necessitate the usage of a follow-up test with high specificity to triage of conspicuous Pap smear test. Here we report our data about detection of HPV L1 capsid protein and p16 in Thinprep Pap Test of the cervix uteri.

Methods. We investigated 44 ThinPrep Pap tests after a conspicuous result in the Pap smear test and a positive detection of HPV high-risk DNA in the HPV hybrid capture test II immunocytochemically against HPV L1-antibody (Cytoimmun, Pirmasens) and p16 antibody (Roche) by using the Roche benchmark GX.

Results. 44 cases of ThinPrep Pap tests with a positive detection of HPV high risk using HC II test were immunocytochemically investigated. In 70% of the cases we could detect mild dysplasia, in 10% moderate dysplasia and in 4% severe dysplasia. The L1 positive cases were split the following way: 32% mild dysplasia, 0% moderate dysplasia, 0% severe dysplasia. In contrast p16 positive cases showed 55% mild dysplasia, 100% moderate dysplasia and 100% severe dysplasia.

Conclusions. HPV L1 is a capsidic protein that is expressed in the early, productive phase of HPV infection, but progressively lost during cervical carcinogenesis. An analysis of ThinPrep Pap test showed that the L1 capsid protein is produced in about 32% of mild dysplasias, whereas it could only be detected in about 0% of moderate and severe dysplasia using immunological methods, due, in part, to HPV integration that accompanies the development of cervical neoplasia. The detection of the HPV L1 capsid protein to confirm the association of the lesion with HPV, has been reported to serve as a prognostic marker that can differentiate between patients who will undergo a transition from a precursor lesion to cancer and those whose lesions will regress. While the data is still preliminary, in cases where the grade of lesion is morphologically difficult to assess, the L1 pattern may be helpful for deciding the appropriate management of women.

SA-056
Tumor width is of more prognostic impact than tumor length in carcinoma of the uterine cervix staged pT1b

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Aims. Tumor size is a well recognized prognostic factor in early stage cervical carcinoma (CX). However, limited knowledge exists about the value of tumor width (i.e. horizontal tumor extension) when it was compared to tumor length.

Methods. Tumor size was determined on 537 formalin fixed CX with upfront surgery, staged pT1b and was recognized as (1) tumor length i.e. vertical tumor extension and (2) tumor width i.e. horizontal tumor extension. Different measurement of tumor size was correlated with recurrence free and overall survival during a median follow-up time of 94 months.

Results. Tumors with bulky disease (>4 cm) represented significant reduced recurrent free and overall survival. Patients with small tumors (<4cm; pT1b1) represented a significant reduced recurrence free (191.0 vs. 178.5 months) and overall survival (193.4 vs. 179.4 months) if the largest tumor diameter was seen at horizontal measurement. In stage pT1b2 (tumors >4 cm) there was a significant increased risk for tumor associated death if largest dimension was seen in horizontal tumor growth [RR 3.5 (95% CI: 1.7–7,2), vs. 0.8 (95% CI: 0,4–1,1)].

Conclusions. Large horizontal tumor extension (i.e. tumor width) is of more prognostic impact than tumor length (cranio-caudal extension) and associated with reduced recurrence free and overall survival in CX, post-operatively staged pT1b. These findings may explained by the fact that tumors with large horizontal extension might be capable of involving lymphatic vessels and the parametrial tissue much earlier than tumors with large vertical dimension (i.e. tumor length). So, extensive sampling is recommended in large horizontal tumor extension.

SA-057
Absence of MED12 mutations in uterine leiomyosarcomas

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Aims. Uterine smooth muscle tumors are classified according to the presence or absence of nuclear atypia, hyaline necrosis and increased mitotic counts into either leiomyomas or leiomyosarcomas. However, cases not fulfilling the criteria for the full diagnosis of leiomyosarcoma exist, and these cases remain a diagnostic challenge. Only recently, mutations in the mediator complex subunit 12 (MED12) have been identified to represent to most common genetic aberration in uterine leiomyomas. MED12 is a chromatin modulator regulating the interaction between nuclear hormone receptors (e.g. estrogen receptor) with the DNA, thus enhancing the transcriptional function of the respective hormones.
Methods. For the current study, we analysed the MED12 mutation status in 24 uterine leiomyosarcomas, and compared these to 20 uterine leiomyomas. Estrogen receptor positivity was conducted using immunohistochemical stainings on tissue microarrays. Clinical follow-up was gathered from the clinical reports.
Results. Fourteen (70%) of the leiomyomas harboured mutations in the MED12 gene. In contrast, only two (8%) of the leiomyosarcomas displayed a mutation. All leiomyomas had a homogenous and strong estrogen receptor expression, while the leiomyosarcomas had a moderate to high expression which was more heterogeneous.
Conclusions. In contrast to leiomyomas, uterine leiomyosarcmas only rarely harbor MED12 mutations. We suggest that this observation reflects a different genetic background, with probably different dependency on estrogen levels. Mutation analysis of MED12 may be a valuable diagnostic tool specifically in atypical uterine smooth muscle neoplasms not fulfilling the criteria for high-grade leiomyosarcoma.

SA-058
Human peritoneal mesothelium—a mechanical and potentially paracrine barrier

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Aims. Ovarian carcinoma spreads by implantation of tumor cells onto the peritoneal mesothelium. We established a three-dimensional co-culture model to simulate the interactions of ovarian carcinoma cell aggregates with human peritoneal mesothelial cells (HPMC).
Methods. Multicellular tumor spheroids (MCTS) of the human ovarian cancer cell line SK-OV-3 were directly inoculated onto either confluent HPMC monolayers or their submesothelial matrix or were co-cultured with mesothelium without direct cellular contact.
Results. Inoculation of MCTS onto submesothelial matrix resulted in rapid attachment (within 30 min) of the tumor cell aggregates followed by rapid emigration (within 12 h) and growth of tumor cells. Intact mesothelium increased the time required for MCTS attachment (up to 180 min) and led to almost complete inhibition of tumor cell emigration and to 47% tumor growth suppression. Bromodesoxyuridine-incorporation into tumor cell nuclei was almost completely abolished in co-cultured MCTS. Growth also was inhibited in MCTS treated with supernatants of HPMC. Analysis of co-culture supernatants revealed that HPMC-derived TGF-beta was almost completely bound by MCTS. Addition of a function-blocking anti-TGF-beta antibody (30 μg/ml) to the co-cultures abrogated the growth inhibitory effect of mesothelium by 50%.
Conclusions. The present model provides a dynamic system to study the complex interactions of ovarian carcinoma cells with HPMC over extended time periods and suggests that the mesothelium constitutes a mechanical and partly TGF-beta-mediated paracrine barrier to the progression of ovarian cancer.

SA-059
Correlation of histomorphology and macroscopic appearance of peritoneal endometriosis

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Aims. Endometriosis is one of the most common benign gynecological diseases.It affects 4–30% of women of childbearing age. Macroscopically, peritoneal endometriotic lesions show a broad color spectrum. In past studies, the different lesion colors have been correlated to disease activity and lesion age. However, up to now, no study exists in which endometriotic lesions of all the main color categories (red, black, brown, white) were analyzed according to a standardized scheme in a single patient collective. Our aim was to verify whether the color concept of endometriosis could be corroborated by a comprehensive histomorphological study.
Methods. 64 peritoneal endometriotic lesions (31 black lesions, 15 white lesions, 11 brown lesions, 7 red lesions) were resected from 41 premenopausal nulliparous women. The histomorphology of each lesion was evaluated on Hematoxylin-Eosin, Elastica van Gieson and Prussian Blue stains according to a standardized scheme. The main categories assessed were gland size, gland content, gland epithelium morphology, amount of endometriotic stroma and adjacent tissue reaction. Proliferation and endocrine activity in endometriotic glands and stroma were analyzed immunohistochemically (ER, PR, MIB1). For all categories a predetermined scoring system was used.
Results. Our analysis showed a wide overlap in all of the color categories for most of the histomorphological and immunohistochemical characteristics. In particular, no clear-cut differences could be demonstrated regarding epithelial and stromal proliferation and endocrine activity. The degree of the adjacent stromal reaction increased from red to black and brown to white lesions. This may be interpreted as evidence that the lesion color may, indeed, reflect the lesion age. The color of black lesions was mainly determined by dilated endometriotic glands with bloody content. White endometriotic lesions showed a strong, scar-like stromal reaction but no loss of endometriotic glands.
Conclusions. Our results show that the color concept of endometriosis is not as straightforward as it is often made out to be. Our data demonstrate that the different color categories show no significant differences regarding proliferation and endocrine activity. This raises the question whether the colors of endometriotic lesions do, indeed, reflect disease activity. More meaningful markers are needed to gauge disease activity in endometriosis. Molecular profiling may constitute a fairly new and promising venue in this matter.

SA-060

Frequency of KRAS mutation in serous ovarian borderline tumors and their peritoneal and lymph node implants

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Aims. Genes of the RAF family, which mediate cellular responses to growth signals, encode kinases that are regulated by RAS and participate in the RAS/RAF/MEK/ERK/MAP-kinase pathway. Activating mutations in BRAF and KRAS have been identified to play a major role in the pathogenesis of low-grade serous ovarian carcinomas via serous borderline tumors. We showed already that there is a possible clonal relation comparing s-BLT and its peritoneal implants illuminated by BRAF p.V600X analysis.
Methods. Completing our data in frequency of KRAS mutation in peritoneal and lymph node implants of thirteen cases of s-BLT we performed subsequent macro- or microdissection followed by DNA- extraction of the adequate tissue. To reveal the activating mutation of KRAS codons 12/13 and 61 we performed pyrosequencing of 14 samples with a sensitivity of at least 5% mutated alleles. Molecular analysis was performed from the ovarian tumor as well as within three to five peritoneal implants and one to five lymph node implants.
Results. In 5 of 13 patients with s-BLT we showed already KRAS mutation in the primary tumor. In two of five patients with lymph node implants and in three of five with peritoneal implants we could confirm the particular KRAS mutation suggesting a clonal origin in terms of abdominal tumor spread.
Conclusions. The frequency of KRAS and BRAF mutation in s-BLT is concordant with the reported frequency within LG-OCA. Supporting the theory of clonal origin of implants in serous ovarian borderline tumors we could confirm the particular mutation in lymph node implants and peritoneal implants.

SA-061

Ovarian metastases and their difficult differentiation from primary ovarian carcinomas—an autopsy study

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Aims. The ovary is a common site of metastases from other primary malignancies. In the literature, metastases account for 5–30% of ovarian cancers. The differentiation of primary ovarian cancer from distant metastases is crucial for therapy and prognosis but is difficult in clinical praxis as metastases to the ovary can closely simulate clinical behavior and even histomorphologicy of primary ovarian carcinomas. The objective of this study was to determine the incidence of ovarian metastases with respect to relevant clinicopathological and immunohistochemical characteristics for discrimination from primary ovarian carcinoma.
Methods. Autopsy reports, histologic slides, and clinical files from 233 patients who died of ovarian cancer between 1975 and 2005 at the University Hospital Basel, Switzerland were studied. Patients which showed an unusual clinical and metastatic behaviour were reviewed by a gynecopathologist with particular attention to histomorphological and immunohistochemical characteristics. For immunohistochemical studies antibodies against CDX2, CK7, CK8/18, CK20, CEA, estrogen receptor (ER), progesterone receptor (PR), and p53 were used.

Results. From the 233 patients originally diagnosed with primary ovarian carcinoma at autopsy, in 25 patients (11%) distant metastases to the ovary from other sites could be identified. The median age of patients with ovarian metastases was 71 years (range 47–88 years). Bilateral involvement of the ovaries was seen in 52%. In 76% of cases the tumors were <10 cm. Carcinomas of the gastrointestinal tract (72%) were the most common sources of ovarian metastases, mainly the colon (40%) followed by the pancreas (20%), the stomach (8%), and the biliary tract (4%). The remaining cases were metastases from carcinomas of the genital tract (24%) and the breast (4%). CDX2, CK7, CK20 and ER were the most effective antibodies to discriminate primary ovarian carcinoma from distant metastases to the ovary.
Conclusions. Autopsy data may yield important information concerning the correct identification of malignancies and may assist physicians in making clinical management decisions. The present study shows that identification of ovarian metastases can be objectively and definitively accomplished by histopathological and immunohistochemical analyses which is crucial for prognosis and adequate therapy. In addition, our data shows a rather high incidence of metastases to the ovary in cancer patients.

SA-062

A current perspective on the pathological assessment of adult-type granulosa cell tumors of the ovary

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Aims. The diagnosis of adult-type granulosa cell tumors of the ovary (aGCT) is based on histomorphology aided by immunohistochemical stains of sex cord markers in cases equivocal by conventional histological stains. If positive, these markers are able to support a diagnosis of sex cord-stromal tumor, however, they are not specific for tumor subtypes within this tumor category. Recently, molecular analysis for the somatic 402 C/G missense point mutation in the Forkhead box protein L2 (FOXL2) has emerged as a potential diagnostic test for aGCT. We have investigated the impact of FOXL2 mutation testing in a large cohort of aGCT diagnosed by conventional histology and immunohistochemistry.
Methods. FFPE-tissue cores from a cohort of 52 ovarian tumors previously diagnosed as aGCT by expert gynecopathologists were immunohistologically analysed for inhibin, calretinin, CD10, CD56, CG99, CD117, synaptophysin, chromogranin, WT1, EMA, PR, ER, MelanA, p53, pan-keratin, and FOXL2. FOXL2 mutation status was determined by Sanger sequencing and high sensitivity digital TaqMan allelic discrimination assay. Histomorphology was reassessed by two expert gynecopathologists.
Results. FOXL2 mutation analyses could be successfully performed in 46 cases, of which 40 were positive for the 402C/G mutation, confirming a diagnosis of aGCT. The remaining 6 cases negative for the 402C/G mutation were re-examined histologically and by immunohistochemistry. One case was re-classified as endometrioid carcinoma with sex cord-like differentiation, in keeping with a FOXL2 wild-type status. A second case was confirmed as a FOXL2 wild-type aGCT. In the remaining 4 cases a final diagnosis of sex cord stromal tumor, unclassified, non-specific for aGCT was made.
Conclusions. In cases where a diagnosis of aGCT is a consideration but unequivocal diagnosis is not possible based on routine histological and immunohistochemical staining, FOXL2 mutation testing is helpful to clarify diagnosis.

SA-063

Second opinion pathology in clinical trials of ovarian carcinomas: the use of a new internet-based pathology platform makes rapid case review prior to patient enrolment possible

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Aims. It has been shown that a considerable number of patients in clinical trials of ovarian carcinoma have diagnoses in conflict with inclusion criteria. Central case review prior to randomization will therefore become a standard procedure in study protocols of ovarian carcinoma trials. To meet study inclusion schedules, the process of pathological case review has to be completed in a timely manner. We hypothesize that our new internet-based high throughput infrastructure will be capable of providing specialized second opinion pathology within 10 working days.

Methods. In order to be eligible for trial enrolment, patients scheduled for the currently recruiting AGO OVAR17 trial have to be registered for a central pathology review using our new infrastructure. Original slides are requested from local pathologists in order to be scanned and uploaded to a secured internet server. A network of internationally recognized gynecological pathologists is connected to the server through a custom-designed software platform. If necessary, immunohistochemistry is available through a collaborating pathology laboratory. Discrepant cases are discussed between local and network pathologists, before a final diagnosis is made.

Results. Our new internet-based high throughput infrastructure was set up successfully. Five gynecopathologists from Austria, Switzerland and Germany provide specialized review of all cases scheduled for inclusion in the AGO OVAR17 trial for all study centers of the AGO study group. Overnight courier shipment and a centralized study office play a key role in the logistics of the complex course of action in central pathology review. Interim analyses show the average time to completion of case review to be less than 10 working days.

Conclusions. Preliminary data suggest that the use of our new internet-based infrastructure may allow for specialized case review prior to patient randomization in clinical trials. The current approach might help to avoid violation of clinicopathologic inclusion criteria in clinical trials.

SA-064

Re-expression of GABARAP by p38 inhibitor induces autophagy and inhibits growth in breast cancer cells

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Aims. p38 inhibitors were recently described to induce autophagic vacuoles and cell death in colon and ovarian cancer cells lines and, therefore, this effect was supposed to be specific for transformed cells and to open therapeutic options. GABARAP (gamma-aminobutyric acid receptor associated protein) belongs to a protein family which is involved in processes of cellular migration and phagocytosis. Our previous study revealed that GABARAP functions as a putative class II tumor suppressor gene in breast cancer cells. The aim of this project was to elucidate the impact of pharmacological blockade of p38 kinase on the expression and function of GABARAP in different breast cancer cell lines and study the mechanism by which this blockade inhibits the proliferation of cancer cells.

Methods. In an effort to evaluate the impact of p38 signalling on breast cancer cell fate, we treated MCF7, BT20, CAL51, MDA-MB231, MCF10A and HBL-100 cell lines with SB202190 inhibitor (specific for p38 alpha/beta kinases).

Results. We found that p38 is required for breast cancer cell homeostasis as the inhibition of its kinase function by pharmacological blockade causes cell cycle arrest, autophagy and cell death in cells and induces upregulation of the GABARAP gene.

Conclusions. GABARAP is an essential component of autophagic vacuoles and autophagosomes. Our previous study revealed that GABARAP functions as a putative class II tumor suppressor gene in breast cancer cells. Interestingly, re-expression of GABARAP is achieved by MAP kinase inhibitor and this leads to induce autophagy and inhibit breast cancer cells growth. The study supports a role of GABARAP in tumorigenesis. The data may lead to development of new cancer therapies that manipulate p38 pathway and autophagy.

SA-065

Oncogenic PIK3CA mutations in lobular breast cancer progression

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Aims. Infiltrating lobular breast cancer (ILBC) is a tumor biologically distinct breast cancer subtype. A high frequency of oncogenic PIK3CA mutations has been reported in ILBC, which may allow for targeted therapy with newly developed PI3K inhibitors. This is of particular clinical relevance for ILBC patients, who have failed to respond to current treatment regimes and suffer from tumor recurrence or dissemination. In anticipation of this therapeutic strategy, we investigated PIK3CA mutations in ILBC with special reference to late stage tumor progression.

Methods. A total of 88 ILBCs from 73 patients, including primary tumors (PTs, n=43), ipsilateral locally recurrent tumors (LRTs, n=15), and distant organ metastases (DOMs, n=30), were compiled on tissue microarrays. Established ILBC marker proteins were evaluated by immunohistochemistry and PIK3CA hot spot mutations in exons 9 and 20 by direct sequencing. Matched PT/LRT, PT/DOM, and DOM/DOM cases were characterized on a patient by patient basis. Following correction for redundant patient representations, mutation frequencies were compared in PTs versus LRTs or DOMs.

Results. Nearly all specimens were E-cadherin negative (99%), estrogen receptor (ER) positive (91%), and lacked basal epithelial markers (100%), demonstrating correct ILBC classification. PIK3CA mutations were detected in 32/88 (36%) specimens. The mutation rate was similar in PTs (33%) and DOMs (26%, p=0.769), but approximately two fold increased in LRTs (69%, p=0.022). Consistently, matched PT/LRT and LRT/DOM cases showed additional PIK3CA mutations in LRTs.

Conclusions. Intriguingly, these findings imply that PIK3CA mutations are positively selected for during ILBC progression to local recurrence but not distant metastasis, which may have clinical implications for PI3K inhibitor-based therapy.

SA-066

PTP1B expression is an independent positive prognostic factor in human breast cancer

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Aims. Protein tyrosine phosphatase 1B (PTP1B) is a non-transmembrane protein tyrosine phosphatase that has come into focus as a critical regulator of multiple signaling pathways. The role of PTP1B in breast cancer remains unclear, with evidence suggesting that PTP1B can exert

both tumor-suppressing and tumor-promoting effects. Our aim was to better define the role of PTP1B in human breast cancer, and its relationship with HER2.

Methods. We conducted a immunohistochemical study on a large cohort of functionally annotated primary breast cancer specimens. 683 of 1402 (49%) evaluable primary breast cancers are positive for PTP1B.

Results. There is no statistically significant association between PTP1B expression and age, tumor size, T stage, histologic grade, lymph node status, or histological subtype. Of note, there is no significant association between PTP1B and HER2 expression (PTP1B expression 53.1% in HER2+ cancers vs. 47.5% in HER2- cancers, p value=0.0985). However, PTP1B expression is significantly associated with estrogen receptor expression (PTP1B expression 50.7% in ER+ cancers vs. 43.1% in ER– cancers, p= 0.0137) and intrinsic molecular subtype (PTP1B expression 53.9% in the luminal B HER2+ subtype, and 37.9% in the basal-like subtype). Of note, multivariate analyses demonstrate that PTP1B is an independent predictor of improved survival in breast cancer (HR 0.779, p=0.006).

Conclusions. Taken together, we demonstrate in the largest study to date that (1) PTP1B is commonly expressed in breast cancer, (2) there is no association or functional impact of PTP1B expression in HER2+ breast cancer, and (3) PTP1B expression in breast cancer is associated with significantly improved clinical outcome. Until additional studies are performed, caution should be exercised in using PTP1B inhibitors in human breast cancer.

SA-067

Intrinsic breast cancer subtypes defined by estrogen receptor signalling—prognostic relevance of progesterone receptor loss

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Aims. The majority of luminal type breast carcinomas are slowly-growing tumors with an overall favorable prognosis. However, a proportion of cases (luminal B tumors) are characterized by co-activation of growth factor receptors or non-canonical ER signaling and a poorer clinical outcome. Currently, classification of breast carcinomas into these groups typically is based on tumor proliferation as detected by Ki-67-immunostains. However, interobserver variability and the heterogeneous distribution of proliferating cells within tumors limit the use of Ki-67 under prospective, clinical circumstances. The aim of or study was to evaluate whether the expression of proteins that are part of the ER signaling network may be used to distinguish low-risk from high-risk ER-positive breast carcinomas.

Methods. We performed unsupervised hierarchical clustering of a series of 443 postmenopausal breast carcinomas using a combination of immunohistochemically-detected, established breast cancer-related proteins and a set of proteins either involved in estrogen receptor signaling or associated with resistance to endocrine therapy.

Results. Using this approach, we were able to reproduce the classical, biological classification with two distinct groups of luminal (estrogen receptor positive) tumors, one group of HER2-associated tumors and a group of triple-negative tumors. However, we were surprised to find that not proliferation or the expression of one or more of the ER-co-factors or resistance-associated factors, but PgR expression was identified as the most important stratifyer between both luminal groups. In fact, not only the four identified clusters were shown to be significantly associated with patient outcome, PgR expression alone or in combination with Ki-67 stains could be used to stratify ER-positive tumors into a low-risk and a high-risk group. ROC curve analysis revealed optimum cut-off-values for PgR expression at an immunoreactive score of 2 or 10% of positive nuclei, for proliferation, a Ki-67 score of 20% was identified.

Conclusions. Our data indicate that defining luminal B tumors by the presence of high risk criteria (loss of PgR expression or increased proliferation, i.e. Ki-67>20%) provides a robust and highly significant stratification of ER-positive breast carcinomas into luminal A and B.

SA-068

Immunohistochemical expression of neuroendocrine markers in different molecular subtypes of breast carcinoma

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Aims. The current WHO classification subdivides breast carcinomas with neuroendocrine features into well differentiated neuroendocrine tumors (NET), poorly differentiated neuroendocrine carcinomas/small cell carcinomas and invasive breast carcinomas with neuroendocrine differentiation with an overall incidence of 1% of all breast carcinomas. Well differentiated NET and small cell carcinomas of the breast are very rare. However in our experience the frequency of invasive breast cancers with neuroendocrine differentiation seems higher than indicated in the WHO. We evaluated the frequency of carcinomas with neuroendocrine differentiation in a cohort of different molecular subtypes of breast cancer.

Methods. Using tissue microarrays (TMA) we analyzed 16 G1 tubular and 9 invasive lobular (ILC) Luminal A carcinomas, 18 G3 Luminal B carcinomas, 15 Her2 amplified carcinomas and 28 triple-negative (TN) carcinomas. Additionally 9 low- and 10 high-grade DCIS and 73 normal tissues were stained with antibodies against Chromogranin A, Synaptophysin, CD56, CD117 and NSE.

Results. Luminal B carcinomas showed the highest frequency of at least partial neuroendocrine differentiation, 4 of 18 (22.2%) were diffusely positive for Synaptophysin and/or Chromogranin A in at least 70% of tumor cells. Of the high-grade DCIS 2 of 10 were positive for Synaptophysin and 1 of 10 was positive for Chromogranin A while all of the low-grade DCIS were negative for these markers. Only 1 of 24 luminal A carcinomas (ILC) and 1 of 28 TN carcinomas showed partial neuroendocrine differentiation in the Synaptophysin staining. CD56 was expressed in the majority of normal luminal cells (72.6% of cases) and showed diffuse expression in 16.7% of Luminal A carcinomas. NSE was also commonly expressed in luminal cells of normal tissue (78.1%) and was expressed in carcinomas irrespective of molecular subtype (40–100%). CD117 was expressed in virtually all normal luminal cells but only in 7.5% of luminal carcinomas and 41% of triple-negative carcinomas.

Conclusions. Synaptophysin and Chromogranin A are the most specific markers for neuroendocrine differentiation. Breast carcinomas with neuroendocrine differentiation might be underdiagnosed due to a lack of clear cut morphologic features. Regarding the molecular subtypes, Luminal B carcinomas show the highest frequency of diffuse neuroendocrine differentiation. These carcinomas might benefit from targeted therapy using Somatostatin-analoga.

SA-069

Expression profile of granulocyte colony-stimulating factor (G-CSF) correlates with unfavorable prognostic subgroups in breast cancer particularly with triple negative and G3 phenotype

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Aims. Tumor microenvironment can contribute to tumor growth resulting in aggressive clinical follow-up in breast cancer. Previous reports have demonstrated that tumor/stroma secreted G-CSF (granulocyte colony-stimulating factor) facilitates neoplastic growth and refractoriness to anti-VEGF treatment via a mobilization of myeloid-derived suppressor cells in mice. The aim of this study was to address the prognostic role of G-CSF expression in a series of primary breast cancer and to correlate the expression profile to clinicopathological parameters and molecular subgroups.

Methods. We analyzed G-CSF expression by immunohistochemistry on a large series of breast cancer including whole sections of mixed 110 primary breast cancers and a tissue micro array of 80 triple negative breast cancer samples. G-CSF expression was evaluated in the cytoplasm of invasive tumor cells and scored as 0, 1+, 2+, and 3+. The expression profile was correlated to predictive factors (ER/PR/Her2 status) and to established clinicopathological parameters.

Results. Preliminary results on G-CSF expression indicate that strong expression (3+) is associated with G3 tumors (p=0.047) and tended to be more highly expressed in triple negative breast cancer cases in a rather homogenous staining pattern. Weak or negative G-CSF expression was heterogenous and occurred in the prognostically favorable subgroups (luminal A/B). Correlation to other parameters as stage could not be established in this study.

Conclusions. Strong G-CSF was preferentially found in poorly differentiated and/or triple negative breast cancers indicating unfavorable prognostic information. This observation is in accordance with some other reports demonstrating that high G-CSF producing tumors are associated with aggressive disease and poor prognosis. This effect might be potentially considered in therapy options in triple negative breast cancers.

SA-070

Diagnostic reproducibility of imprint cytology of core biopsies of breast tumors

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Aims. Imprint cytology of ultrasound-guided core biopsies of breast tumors may allow same-day diagnosis. Aim of this study is the evaluation of the diagnostic accuracy of cytology in comparison to histology as a gold standard and the interobserver agreement of the cytology results.

Methods. 158 consecutive core needle biopsies were obtained by ultrasound-guided core biopsy of breast tumors. The biopsies were rolled unto two microscopy slides per case, the cells were fixed and stained with a quick staining method. The cytology slides were examined by two experienced cytologists independently, blinded to each other and to the histological result. The cytology results were reported using NHSBSP guidelines. They were compared to each other and to the final histology result as the gold standard to calculate diagnostic accuracy and interobserver agreement.

Results. Preliminary data clearly show a low interobserver variability of cytology results. The overall concordance between cytology and histology was high. Results will be completed in the next weeks and will be presented in full at the congress.

Conclusions. Imprint cytology of core needle biopsies of breast tumors shows a good interobserver reproducibility and a good correlation to the histological result of the specimen. Therefore imprint cytology was found to be an accurate and reliable way of diagnosing breast tumors and can be used to shorten the time to diagnosis.

SA-071

Pretherapeutic Ki67 levels as predictive and prognostic parameter in the neoadjuvant GeparTrio trial

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Aims. In this study, we evaluated a large cohort of neoadjuvant core biopsies from the GeparTrio trial to investigate the impact of pretherapeutic Ki67 levels as a predictive marker for response to neoadjuvant chemotherapy as well as a prognostic marker for progression-free and overall survival. The analysis was stratified for hormone-receptor positive and negative tumors as well as HER2 status. Ki67 has been suggested as a marker for definition of luminal A and luminal B tumors. However, the cut-offs for Ki67 are still under debate.

Methods. A total of 1166 pretherapeutic core biopsies from the neoadjuvant Gepartrio trial were evaluated for Ki67 by immunohistochemistry, a total of 200 cells were counted in each sample. Ki67 cutoffs were evaluated using web-based software Cut-off Finder (http://molpath.charite.de/cutoff/). We compared pathological complete remission rate (pCR rate) as well as the overall and disease free survival in the complete cohort as well as subgroups of patients based on hormone receptor and HER2 expression.

Results. A wide range of Ki67 cut points between 3% and 94% (for pCR) and 4–46% (for DFS and OS) were significant. The three groups of Ki67 less than 15% vs. 15.1–35% vs. >35% had increasing pCR rates of 4.2%, 12.8% and 29.0% (p<0.0005), this effect was present in 6 of 8 molecular subgroups. Ki67 was significantly linked to prognosis (DFS and OS) in uni- and multivariate analysis in the complete cohort and in hormone receptor positive, but not triple-negative tumors. Ki67 was a negative prognostic factor in non-pCR group, but not in the pCR group.

Conclusions. Ki67 is a valid predictive and prognostic marker in breast cancer which is significant over a wide range of different cut-offs. This could explain explains the different results of Ki67 cut-offs in previous studies. Distinction of three subgroups for Ki67 (0–15% vs. 15.1–35 vs. >35%) is suggested as a reasonable approach for further standardization of this marker.

SA-072

Differential response to oxoaldehydes in wildtype and Tamoxifen resistant MCF-7 breast cancer cells

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Aims. Tamoxifen resistance occurs frequently in hormone-dependent breast cancer and is a significant problem in anti-estrogenic therapy. In this study we were looking for differences in wildtype and Tamoxifen-resistant MCF-7 breast cancer cells to identify novel approaches for treatment. alpha-oxo-aldehydes such as glyoxal and methylglyoxal are by products of fatty acid oxidation and glycolysis and their concentration varies with metabolic state. They can also be formed by oxidative stress from primary Maillard products, which occur especially under hyperglycaemic conditions. As a result in increased accumulation of advanced glycation end products (AGEs) can be observed. AGEs represent a chemically divers family of non-enzymatic protein modifications that are associated with ageing and degenerative diseases. AGEs can either inhibit organ function, enzymes and signalling molecules or they activate signalling cascades via specific receptors such as the receptor for AGEs, called RAGE.
Methods. Cell viability was analysed by resazurin. Gene expression was analyzed by RT-PCR and proteins, kinase phosphorylation and AGE-modifications were determined by Western blotting. Apoptosis was analysed by measuring caspase activity using luminescent enzyme assays.
Results. Tamoxifene resistant MCF-7 cells were less tolerant to the alpha-oxo-aldehydes glyoxal and methylglyoxal. Cells responded differentially to these aldehydes by MAP-kinase phosphorylation, especially p38 MAPK. However, the expression of the aldehyde defence enzymes glyoxalase-1 and -2 and fructoseamine 3-kinase (FN3K) appeared similar in both cell lines on the mRNA level. Increased AGE-accumulation was only observed under toxic concentration of glyoxal and methylglyoxal.
Conclusions. The tamoxifene resistance phenotype resulted in an increased vulnerability towards aldehydes which are side products of glycolysis or result from oxidative stress. Further studies will therefore focus on the possible use of AGEs, glyoxalases and FN3K as prognostic markers and the effect of anti-glyoxalase therapeutics in treating tamoxifene resistant breast cancer.

SA-073

Primary neuroendocrine carcinoma of the breast

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Aims. Primary neuroendocrine tumor (NT) of the breast is a very rare tumor. The WHO recognized this entity in 2003. NT account for less than 0.1% of all breast cancer and less than 1% of all NT. The aim of this study is the presentation of two such cases.
Methods. Clinical-pathological analysis and conventional workup of two own cases and review of the literature.
Results. 1. The 72-year-old woman presented with a right breast mass found on MRI. Six years before, she had right a mastectomy and a breast implant due to intraductal carcinoma. The new tumor measured 2,6×1,5×2,5 cm. 2. The 96-year-old woman presented with a painless lump in her left breast. A diagnosis of NT was made after biopsy. The tumour measured 12×10×10 cm, was poorly circumscribed and harboured focal areas of necrosis. Both tumors were well-differentiated neuroendocrine carcinoma with expression of, Synaptophysin, NSE, E-catherin, Estrogen and Progesterone receptors while HER2 was negative similar

to stains for S-100 and Chromogranin. Ki67 labelling index exceeded 15% and 5%, respectively. Metastases to the breast were excluded.
Conclusions. Our cases of primary neuroendocrine mammary carcinoma affected elderly women. Metastatic NT needs to be excluded before making a definite diagnosis since aggressive metastatic neuroendocrine carcinomas does frequently develop in other organ system like the lung or the gastrointestinal tract. The cases suggest that NT of the breast may develop over a long period of time and may follow an indolent course. More data needs to be gathered on this entity.

Postersession Hepatopankreatobiliäre Pathologie

SA-074

HER2 gene amplification and protein expression in pancreatic ductal adenocarcinomas

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Aims. Despite advances in combination therapies, the prognosis of pancreatic ductal adenocarcinoma (PDAC) remains extremely poor. Blocking of overexpressed HER2 oncogene improves survival in breast and gastroesophageal cancer and might be also a therapeutic option in PDAC. The purpose of this study was to evaluate HER2 gene amplification and protein expression in PDAC.
Methods. HER2 protein expression was investigated using a FDA approved antibody in 87 formalin-fixed and paraffin-embedded cases of PDAC's with complete follow up. HER2 gene amplification was assessed on tissue microarrays using dual color silver in-situ hybridization (DISH).
Results. Generally, HER2 immunostaining showed considerable heterogenity. In 19 cases, >10 of tumor cells showed some positive reaction. In no case, complete membranous staining was observed. Using the scoring system developed for assessment of HER2 status in gastroesophageal cancer, 9 cases showed positive immunohistological staining (score 2+ to 3+). After performing DISH, 6 (7%) immunohistochemically 2+ or 3+ cases were found to have HER2 gene amplification, while none of these cases showed polyploidy. No association of HER2 status and clinicopathological parameters or survival was observed (p>0.05)
Conclusions. HER2 is overexpressed in a subset of PDACs, identifying them as possible candidates for a targeted therapy. For assessment of HER2 status in PDAC, the scoring system originally developed for gastric cancer is recommended.

SA-075

Diagnostic accuracy of fine needle aspiration cytology of pancreatic lesions and additional impact of DNA ploidy analysis: a retrospective clinicopathological study

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Aims. In patients with pancreatic masses fine needle aspiration cytology (FNAC) is the diagnostic method of choice. A positive cytology leads to administration of chemotherapy in patients with locally advanced or metastatic disease and avoids unneeded surgery. The accuracy of FNAC of the pancreas was investigated and compared with the results of following surgical interventions or biopsies or clinical-radiological data.

Methods. Retrospective data from the years 2005 to 2012 were retrieved from the institutional files. Sensitivity, specificity, as well as positive and negative predictive value was determined. All FNAC cases were re-evaluated using major or minor cytological criterias of malignancy as nuclear crowding and overlapping, nuclear contour irregularity, irregular chromatin distribution and others. Additionally, computer-assisted ploidy analysis in a small cohort of cases was performed.

Results. 133 diagnostic cases were retrieved from the institutional files. As compared to histology or clinical-radiological data the sensitivity and specifity of cytology was 96.3% and 88.9%, respectively. The positive and negative predictive value was 96.3% and 88.9%, respectively. There were 3 false positive results; all of them cytologically suspicious. A false positive case showed only single atypical cells with mild nuclear enlargement (minor criterion of malignancy) and a cell line of about 3.6c. One of 3 false negative cases showed nuclear crowding and overlapping as major criterion of malignancy and aneuploid single cells >5c.

Conclusions. The stringent application of major and minor cytologic criterias of malignancy is an indispensable aid in the accurate diagnosis of ductal adenocarcinoma of the pancreas. Computed-assisted ploidy analyses may provide additional valuable information to confirm the diagnosis of consequence.

SA-076
Multiprobe FISH for the elucidation of equivocal pancreatobiliary cytology

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Aims. Endoscopic fine-needle aspiration (FNA) and brush cytology are standard methods for the diagnosis of tumors of the pancreatobiliary tract. Although the majority of cytological diagnoses are straightforward, there remains a difficult category of equivocal cytological atypia. Here, we explored the utility of FISH-improved diagnostic stratification between reactive and malignant cells.

Methods. The multiprobe FISH assay UroVysion was used for copy number enumeration of chromosomes 3, 7, 17, and the 9p21 locus and applied to Papanicolaou-stained specimens with a diagnosis of equivocal atypia (n=48), adenocarcinoma (n=31), or no evidence of malignancy (n=14). We captured images of the atypical cells and saved the coordinates on an automated stage prior to hybridization. A positive test was defined as increased copy number (>2) of at least 2 chromosomes (3, 7, or 17), or homo-/heterozygous loss of 9p21.

Results. FISH confirmed all 31 cytological diagnoses of pancreatobiliary adenocarcinomas and was negative in all 14 patients with no clinical evidence of malignancy. Among the 48 cases with equivocal atypia FISH detected 17/29 cases with a final diagnosis of adenocarcinoma and was negative in all 19 cases with no final evidence of malignancy (sensitivity 59%, specificity 100%, PPV 100%, NPV 61%). Loss of 9p21 was found in 42 (88%) of all 48 FISH positive cases.

Conclusions. Multiprobe FISH combined with automated relocation of atypical cells is a powerful technique to clarify equivocal cytological atypia in pancreatobiliary cytology, allowing for a better distinction between reactive atypia and malignancy.

SA-077
Expression of the transcription factor Islet-1 in pancreatic and extrapancreatic well and poorly differentiated neuroendocrine neoplasms: a tumor type relationship

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Aims. The human insulin gene enhancer binding protein ISL-1 (islet-1) is a transcription factor involved in the differentiation of neuroendocrine pancreatic cells. Recent studies identified islet-1 as a good marker for pancreatic neuroendocrine tumors (NETs). However, little is known about islet-1 expression in pancreatic and extrapancreatic poorly differentiated neuroendocrine carcinomas (NECs) and in extrapancreatic NETs.

Methods. We studied the immunohistochemical expression of islet-1 in 124 neuroendocrine neoplasms (NENs).

Results. Among 13 pancreatic NECs 12 scored negative, whereas 5/7 pancreatic NENs with Ki67>20% but NET histology were strongly positive. In extrapancreatic NENs, strong positivity was found in Merkel cell carcinomas (25/25), pulmonary small cell NECs (21/23), medullary thyroid carcinomas (9/9), paragangliomas/phaeochromocytomas (6/6), adrenal neuroblastomas (8/8) and head and neck NECs (4/5), while no or only weak staining was recorded in pulmonary carcinoids (3/15), olfactory neuroblastomas (1/4) and NEC-like basaloid head and neck squamous cell carcinomas (0/15). Islet-1 stained the NEN component of 5/8 composite carcinomas and normal cells in the thyroid, adrenal medulla, stomach and colorectum. Expression of p53 was inversely correlated to that of islet-1 in pancreatic NECs, but paralleled the expression of islet-1 in most extrapancreatic NECs.

Conclusions. Our results demonstrate the ubiquitous expression of islet-1 in extrapancreatic NECs and neuroblastic malignancies and its common loss in pancreatic NECs. These findings suggest a different role for islet-1 in NECs of various extrapancreatic origins compared to pancreatic NECs. While loss of islet-1 in pancreatic NECs is in line with its role as a differentiation marker and confirms the distinctness of NET and NEC in the pancreas, the significance of islet-1 expression in poorly differentiated extrapancreatic NENs remains unclear.

SA-078
Similar genomic alterations in the development of cholangiocarcinoma and ductal adenocarcinoma of the pankreas detected by FISH analysis

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Aims. Cholangiocarcinoma (CCC) and ductal adenocarcinoma of the pancreas (DAC) are very aggressive tumors, with a 5-year survival less than 10%. Not surprisingly their morphology and immunophenotype are very similar. Liver and pancreas indeed share their embriological origin, both deriving from the primitive gut. FISH has been shown to play a potential role in the diagnosis of pancreatic and biliary neoplasia. The aim of the current study was to assess the genomic alterations harboring in both CCC and DAC by fluorescence in situ hybridization

(FISH) using formalin-fixed, paraffin-embedded (FFPE) tissue specimens, in tissue microarray (TMA) format.

Methods. FISH analysis was performed with the UroVysion probe set (Abbott Molecular, Des Plaines, IL), containing three centromere enumeration probes (CEP) for the chromosomes 3, 7, and 17, and one locus specific probe (9p21), on two different TMA, including 168 CCC and 25 normal liver specimens (NL), and 234 DAC, 40 PanIN 3 and 40 normal pancreas specimens (NP) respectively.

Results. CCC: We found the CEP3 monosomy in 46% of CCC and in no NL (p=0.0008), CEP7 polysomy in 45% of CCC and 13% of NL (p=0.0229) and the deletion of 9p21 locus in 66% CCC and 13% of NL (p=0.0001) respectively as compared with normal epithelium. Deletion of CEP17 was associated with poorer prognosis (p=0.0063). DAC: CEP7 polysomy was found in 37% DAC, 9% PanIN and 14% NP (p=0.0015); deletion of 9p21 locus was found in 54% DAC, in 9% of PanIN and in 14% of NP. (p=0.0001). No significant difference was found between PanIN and NP. CEP 7 polysomy was also associated with poorer survival in DAC (p=0.0173).

Conclusions. CEP7 polysomy and deletion of 9p21 are related to the development of malignancy in CCC and DAC and could therefore be used for the differential diagnosis of pancreatobiliary strictures. In CCC deletion of CEP17 is associated with shorter survival, pointing out the possible role of tumor suppressor genes in the determination of prognosis.

SA-079

Up-regulated hedgehog pathway as a potential novel drug target in biliary tract cancer

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Aims. Hedgehog (Hh) signalling contributes to tumour formation or progression through several mechanisms including increased cell proliferation and migration, whereby its role in biliary tract cancer (BTC) remains still unclear.

Methods. We analysed the expression pattern of Hh components in biliary tract cancer (BTC) using cell lines, xenograft tumour tissue and a human tissue microarray (TMA). Expression of Hh pathway components was performed using real-time RT-PCR (Taqman) and immunohistochemistry for mRNA or protein expression, respectively. The effects of the two Hh inhibitors (cyclopamine and Gant-61) were dose-, time- and cell line-dependently analysed for survival, apoptosis and cell cycle distribution in vitro.

Results. Both in vitro and in vivo the expression of Hh components is heterogeneous and is linked to a more dedifferentiated phenotype, higher grading BTC and in BTC tissue of lymph node positive patients. Both inhibitors showed clear dose-dependent cytotoxicity above 1 μm with a stronger effect on cell survival for Gant-61. Accordingly, the effects of Gant-61 was more associated to apoptosis induction, whereas cyclopamine was linked to an inhibition of proliferation. Interestingly, no clear relationship with the Hh components and drug efficiency could be found comparing drug cytotoxicity and Hh component's expression.

Conclusions. In conclusion, Hh is activated in BTC tissue compared to normal adjacent tissue, yet shows a heterogeneous expression pattern. Provided that subsequent studies can identify a reliable predictor of drug response towards Hh inhibitors, the current data demonstrate an effective anticancer activity of these drugs in BTC.

SA-080

Intraductal tubulo-papillary neoplasms of the bile ducts—clinical, morphological, immunohistochemical and molecular characterization of 4 cases

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Aims. Intraductal tubulo-papillary neoplasms (ITPNs) are very rare tumors that were recently described in the pancreas. Whether ITPNs also occur in the bile duct system is unclear. In this study, four patients with biliary tumors that resemble pancreatic ITPN were analysed with focus on (1) clinicopathological and (2) immunhistochemical profiles and (3) genotypes.

Methods. Surgical resection material with invasive and intraductal areas of all four patients was available. Conventional histomorphological analyses and immunohistochemistry for mucin core proteins (MUC1, 2, 5AC and 6), transcription marker CDX2 and protein products of common oncogenic pathways (p53, β-catenin, SMAD4, HER2, EGFR) were performed. Mutation analyses of KRAS (exon 2 and 3), GNAS (exon 8) and BRAF (exon 15) were performed by combination of real-time polymerase chain reaction and direct sequencing.

Results. The ITPNs affected two men and two women (mean age 62 years, range 42–80). There were three extrahepatic tumors and one intrahepatic tumor. All tumors were associated with an invasive adenocarcinoma. Histologically, the ITPNs showed a typical tubulo-papillary architecture and focal necrosis. Immunohistochemically, positivity was found for MUC1 (4/4), MUC5AC (3/4), MUC6 (3/4), MUC2 (1/4) and CDX2 (1/4). Focal overexpression of p53 was present in half of the tumors in the intraductal component. Slight differences were observed in the associated invasive carcinoma of all cases compared to the intraductal component. All tumor components (intraductal and invasive) showed membranous β-catenin expression and intact SMAD4 expression. Likewise, HER2 and EGFR were not overexpressed. Molecular analysis of hot spot regions of KRAS, BRAF and GNAS revealed wild type sequences in all cases.

Conclusions. Adenocarcinomas with prominent intraductal growth and predominantly tubular architecture resembling pancreatic ITPNs occur also in the bile duct system. These tumors exhibit a distinct mucin profile and wild type status of KRAS, BRAF and GNAS.

SA-081

Immunohistochemical detection of BRAFV600E mutation in biliary tract cancers and correlation with clinicopathological data

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Aims. Mutations of BRAF have been reported in various cancer types. In biliary tract cancers (BTCs) several studies on BRAF mutations have been conducted. However, studies so far showed divergent results showing BRAFV600E mutations in 1.5 to 16% of analysed patients. Moreover, these studies mostly used poorly characterized cohorts with limited patient numbers and therefore, available data could not discriminate well between different BTC subtypes. With new therapeutic options for patients harbouring BRAFV600E mutation at hand, we aimed to transfer a novel immunohistochemical screening approach for BRAFV600E mutations to a large and well characterized cohort of BTCs. Results were correlated with comprehensive clinicopathological data including patient survival.

Methods. Tissue microarrays (TMAs) containing samples of 375 BTCs [157 intrahepatic (ICCs), 149 extrahepatic cholangiocarcinomas (ECCs),

and 69 adenocarcinomas of the gallbladder (GBACs)] were stained with a specific antibody detecting BRAFV600E mutant protein (clone VE1). Positive immunohistochemical results were confirmed by Sanger sequencing. Additionally, we examined 30 randomly chosen negative cases by sequencing. Clinicopathological data of positive cases were correlated and compared to negative cases.

Results. Our screen of 375 BTCs revealed 5 cases harbouring a BRAFV600E mutation (1.3%). This mutation was detected both by immunohistochemistry (IHC) and by Sanger sequencing. All 5 cases harbouring a BRAFV600E mutation were ICCs (5/157; 3.2%). None of the ECCs and GBACs were antibody-positive. 30 randomly chosen IHC-negative control cases were BRAF wt in sequencing. Apart from the subtype-restriction of BRAFV600E mutations to ICC and a female predominance (4 female, 1 male), no significant correlation of cases harbouring BRAFV600E mutation with clinicopathological data was detected.

Conclusions. The presented approach shows that the immunohistochemical analysis using TMAs is a highly feasible and valid approach of screening for BRAFV600E mutations in BTCs. This data reveals that BRAFV600E mutation is a rare event in BTC accounting for only 1.3% of all cases (n=375). In this cohort of BTCs, BRAFV600E mutation is restricted to ICC, accounting for 3.2% in the ICC subgroup. In conclusion, BRAFV600E mutation seems to be less frequent than previously reported.

SA-082

Diffuse eosinophilic dysplasia of the gallbladder: is it a way to cancer?

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Aims. Adenocarcinoma of the gall bladder is regarded as a final stage of a process which develops through a multistep sequence (adenoma-carcinoma sequence). Eosinophilic dysplasia was first described in a case report in 2006. Such flat dysplastic changes in the gallbladder epithelium are regarded as precursor lesions of gallbladder cancer. There are many morphologic variants of dysplasia: micropapillary, cribriform and papillary. Eosinophilic dysplasia is characterised by strong eosinophilic cytoplasm, nuclear enlargement with prominent nuclei and nuclear pleomorphism. However, eosinophilic changes exist in the literature also as oncocytic metaplasia. It would be elucidated if these morphologic events represent a dysplasia as precursor lesion of gallbladder cancer or just a benign lesion of the gallbladder mucosa.

Methods. We found a gallbladder (operated for cholecystolithiasis) with extent eosinophilic changes in papillary pattern in our daily routine. Therefore a formalin-fixed gallbladder of a 63-year-old man was examined. For the evaluation, tissue sections were stained with hematoxylin and eosin, for immunohistochemistry with antibodies to CK-7, CK-20, CK19, CA19-9, Mib-1, EMA, CEA and for microsatellite instability (MLH1, MSH2, MSH6), chromosomal instability (p53), for CpG-island-methylation-phenotype (MGMT) and with p16.

Results. We found no changes in expression, neither in cytokeratins nor in MSI-markers. MGMT expressed nuclearly and p53 showed also only a restricted overexpression. CEA was negative. EMA expressed in a marked cytoplasmatic manner. Proliferative activity of the epithel (Mib-1) is about 20–30%. In p16-immunohistochemistry a diffuse marked cytoplasmatic positivity was detected throughout of the gallbladder mucosa.

Conclusions. On the basis of morphological signs of dysplasia and of immunohistochemical data we think that such eosinophilic or oncocytic changes would be interpreted as a possible precursor of gallbladder cancers. In the diagnostic decision between dysplasia and metaplastic changes the Mib-1 proliferation activity and p16 immunohistochemistry can be helpful. Regarding the molecular level, our data suggest that the eosinophilic dysplasia could be associated with p16/Cyclin D1/

CDK4 pathway in the carcinogenesis but this should be cleared with further studies.

SA-083

Concepts for the periampullary carcinoma enigma from clinico-pathologic analysis of 198 patients

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Aims. Periampullary adenocarcinomas comprise pancreatic ductal (PDAC), distal bile duct (DBDAC), ampullary (AMPAC) and duodenal (DUOAC) adenocarcinoma. The epithelia of these anatomical structures share a common embryologic origin from the foregut. While there seem to be significant differences regarding tumor biology, the classification, grading, staging and treatment of these entities remains a matter of substantial debate. Due to the anatomical complexity of the periampullary region, there is still considerable debate on how carcinomas and their precursor lesions arising in this region should be classified. Our study aimed at a detailed analysis of clinical, pathological and immunohistochemical parameters for assessment of tumor biology and identification of prognostic factors after resection of periampullary carcinomas.

Methods. 198 patients who had resection of periampullary adenocarcinoma from 2001 to 2011 were identified. All tissue samples were processed by a standardized protocol for pathological workup of pancreatoduodenectomy specimen. Archived Hematoxylin-Eosin stained slides were re-evaluated by two experienced pathologists (PB, IK). For the growth pattern, three typical subtypes were defined: intestinal, pancreatobiliary, mixed intestinal-pancreatobiliary and Poorly-differentiated carcinoma. Additionally, immunohistochemical subtyping of the growth pattern CK, CK7, CK20 and CDX2 staining were performed for each slide. Furthermore we established a Cytokeratin based tumor grading system.

Results. 127 patients had PDAC, 39 had AMPAC, 23 had DBDAC and nine had a DUOAC. The distribution of subtypes was significantly different among the carcinoma groups. Tumor location, histological subtype and grading were highly significant predictors of survival (p<0.001). In accordance, a high CK7 expression and a low CDX2 expression, which characterize PB differentiation, were significant predictors of poor survival. Only histological subtype, grading and lymph node ratio were found to represent independent predictors of survival by multivariate analysis.

Conclusions. Our results show, that there should be a change in the pathological management of making diagnoses in periampullary carcinomas. By multivariate analysis, traditional parameters as tumor location and TNM classification lost their prominence as a source of prognosticating survival of periampullary carcinoma. Therefore, we recommend comprising the histological subtype and our adjusted histological grading for a better valuation of survival.

Abstracts

SA-084

The contemporary use of electron microscopy in the diagnosis of juvenile liver diseases

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Aims. This contribution focuses on the use of electron microscopy (EM) as adjunct tool in diagnostic hepatology of mainly congenital, infectious, and storage diseases of juvenile patients (often candidates for LTX) examined in the recent 6 years in our departments.

Methods. We receive mostly small primary Karnovsky-fixed parts of liver biopsies (an important methodical prerequisite for a reliable results interpretation) and peripheral blood samples (cases of suspected storage disease). Samples were automated processed and embedded in EPON-resin (in urgent cases microwave-assisted), tissue blocks were semithin (for LM orientation) and ultrathin sectioned, and EM-examined. We demonstrate EM findings of selected cases of above mentioned diseases.

Results. In the heterogeneous group of progressive familial intrahepatic cholestasis (PFIC) diseases EM shows distinct bile characteristics (coarsely granular = "Byler bile", amorphous/filamentous, presence of cholesterol crystals) and bile canaliculus alterations pathognomonic for FIC1-, BSEP-, and MDR3-deficiency, respectively. In an Alagille syndrome patient EM revealed numerous bile inclusions in the dilated intercellular space, in an alpha-1-antytrypsin deficient patient typical amorphous accumulations in distended RER-cisternae. Two children with congenital glycogenosis condition displayed by EM in the hepatocytes non-membrane bound intracytoplasmic deposits of abnormal filamentous glycogen (d=8 nm) with sparse normal glycogen rosettes in their periphery (Andersen disease). Additionally, in the peripheral blood a fraction of leucocytes showed multiple vacuoles containing similar abnormal glycogen particles. PCR analyses identified one boy to be compound heterozygous carrier of two mutations in exon 12 of the chromosome 3p14. In the other child two mutations were found in exon 6 and 13 encoding of the GBE-1 gene, respectively. In a fatal virus hepatitis of a transplanted child multiple adenovirus particles were detected (DD: in early disease stages > CMV-hepatitis, GvHD, drug intoxication). Another not trivial LM-diagnosis seems to be the Gaucher and Nieman-Pick disease—EM reveals the pathognomonic inclusions.

Conclusions. Ultrastructural examination of liver biopsies reveals significant information for the final diagnosis, in case of storage diseases EM of peripheral blood leukocytes provides the rationale for the enzymatic and genomic mutation analysis. We conclude that EM is still a very valuable tool for diagnosis in rare diseases of the liver.

SA-085

Perilipin differentiates chronic from acute hepatocellular steatosis

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Aims. Hepatocellular steatosis is the most frequent liver disease in western countries and may develop further to steatohepatitis, liver cirrhosis, and HCC. It is caused by a broad range of disorders such as alcohol abuse and/or metabolic syndrome and is characterized by intracellular lipid droplet (LD) accumulation. We have previously shown that LD-associated proteins of the perilipin/PAT-family are differentially localized in hepatocyte steatosis and that perilipin is de novo expressed. In this study, we analyzed the conditions of PAT-, especially perilipin-expression in vitro and in vivo.

Methods. Immunohistochemical, -fluorescent, protein biochemical and molecular biological methods were used.

Results. Analysis of about 80 human liver specimens revealed that perilipin and adipophilin were abundant in chronic steatotic liver disease irrespective of the underlying etiology. Interestingly, in acute steatosis such as in previously non-steatotic transplant livers, perilipin was virtually absent while adipophilin, TIP47, and MLDP were induced. In accordance, shortterm incubation with lipids, steatogenic substances or DMSO, as well as in experimental hypoxia, TIP47, adipophilin, and MLDP were gradually induced in cells of the lines HepG2, Huh7, PLC, and Hep3B, whereas perilipin was virtually absent. Thus, longterm models in which cells were treated for up to 40 days with different combinations of compounds were established. After gradual induction of TIP47, MLDP, and adipophilin, finally, starting at about 10 days, perilipin was detectable and colocalized with adipophilin at LDs. Perilipin and associated LDs were intricately regulated on transcriptional (PPARs, C/EBPs, SREBP), post-transcriptional (alternative splicing), and post-translational level (TAG amount, proteasomal degradation, LD-fusion). In longterm steatosis models under stable downregulation of adipophilin and/or TIP47, MLDP substituted for TIP47, and perilipin for adipophilin. In contrast to TIP47 downregulation, the absence of adipophilin alone or in combination with TIP47 resulted in decreased triacylglyceride levels.

Conclusions. LD-maturation in hepatocytes in vivo and in vitro involves the sequential expression of TIP47, MLDP, adipophilin and finally perilipin. Thus, perilipin may be used for the differential diagnosis of chronic versus acute steatosis. In our models, adipophilin was the major determinant of triacylglyceride content of hepatocytes, opening up possibilities for more targeted therapeutic approaches of steatosis.

SA-086

Control of hepatic stellate cells of liver health and disease

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Aims. Endosialin has been identified as a marker of tumor endothelial cells (Science 289:1197, 2000). Yet, detailed expression profiling analyses by our and other laboratories revealed that it is not expressed by endothelial cells. Instead, Endosialin is expressed by tumor-associated pericytes and stromal myofibroblasts, identifying it as a marker of the activated mesenchymal lineage (Am J Pathol 172:486, 2008).

Methods. We consequently hypothesized that Endosialin may be functionally involved in organ fibrosis, a process critically dependent on the recruitment and proliferation of activated mesenchymal cells.

Results. In line with this hypothesis, expression profiling experiments of human liver tissue samples revealed that Endosialin expression was significantly upregulated during liver fibrosis and cirrhosis. Yet, expression did not correlate with the severity of disease suggesting that Endosialin may be an early marker of liver fibrosis. Endosialin expression was most pronounced in portal fibroblasts and hepatic stellate cells (HSC), which localized along the sinusoids in the space of Dissé and also forming scar tissue of fibrotic septa and cirrhotic nodules. To mechanistically study the role of Endosialin during liver fibrogenesis, we pursued CCl4-induced liver fibrosis experiments in wildtype and Endosialin-deficient mice. CCl4-mediated liver damage (apoptosis of hepatocytes) was similar in both genotypes. Likewise, the pattern and intensity of fibrogenesis was not different. Surprisingly though, hepatocyte proliferation during early stages of liver fibrosis (2 and 4 weeks) was significantly elevated in the absence of Endosialin. To study proliferative liver regeneration more directly, we pursued partial hepatectomy

experiments and traced hepatocyte proliferation during the rapid phase of liver regeneration. Proliferation of hepatocytes during early liver regeneration was dramatically enhanced in Endosialin-deficient mice. Collectively, the experiments identified a paracrine growth regulatory interaction between HSC and hepatocytes.

Conclusions. Ongoing transcriptomic analyses of HSC isolated from the different genotypes under physiological and pathological conditions and in combination with detailed analysis of other non-parenchymal cell populations are aimed at unraveling the molecular nature of this regulatory circuit. Definite genetic experiments showed that the activated HSC marker Endosialin plays a causal role in this interaction.

SA-087
Di George syndrome as cause of granulomatous hepatitis—case report

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Aims. Granulomas in the liver can be caused by a great variety of different etiologies like infections or infestations, hypersensitivity to drugs, foreign bodies or granulomatous liver diseases like PBC, or systemic diseases like sarcoidosis or Cohn's disease. Di George syndrome is a congenital malformation characterized by variable defects of the thymus, heart and parathyroid glands. It belongs to the Chromosome 22q11.2 deletion syndromes. Known liver changes in patients with this syndrome are unspecific and include hepatitis due to opportunistic infections. A granulomatous hepatitis sui generis has not been described.

Methods. Liver biopsy was performed in a 12-year-old girl with proven Di Georg syndrome due to elevated liver enzymes (ASAT 131 U/l, ALAT 213 U/l). Histological and molecular pathological examination was performed. Histology revealed granulomatous hepatitis with epitheloid cell granulomas without necrosis and slight cholangitis reaction. In the liver tissue, DNA from EBV was found by PCR. DNA from CMV, Mycobacteriae, Yersiniae, Parvovirus or Bartonellae was negative. A repeated biopsy after 4 years showed identical histological changes with granulomatous hepatitis

Results. Histology revealed granulomatous hepatitis with epitheloid cell granulomas without necrosis and slight cholangitis reaction. In the liver tissue, DNA from EBV was found by PCR. DNA from CMV, Mycobacteriae, Yersiniae, Parvovirus or Bartonellae was negative. A repeated biopsy after 4 years showed identical histological changes with granulomatous hepatitis.

Conclusions. Until now, unspecific changes are documented in the liver in patients with Di Georg syndrome. For the first time, we describe a persistent granulomatous hepatitis in this disease. A typical infection is excluded. The EBV DNA is regarded as epiphenomenon. Di George syndrome has to be included in the differential diagnosis of granulomatous hepatitis

SA-088
Sudden death duo to acute hepatic failure induced by *Dioscorea bulbifera L. rhizome*

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Aims. *Dioscorea bulbifera L. rhizome* is a medicinal plant which can be dietary supplements, heat-clearing, detoxicating, detumescence and odynolysis. Sometimes it may cause liver and kidney injury but unusual death. There are some enzyme markers which may help predict this injury. We report a rare case about death caused by acute hepatic failure after treated by medical herbs contain *Dioscorea bulbifera L. rhizome*.

Methods. We describe a 17-year-old girl suffered from a "breast lobule hyperplasia" for two months. After treated by a low dose of medical herbs contain *Dioscorea bulbifera L. rhizome* for 5 months, the girl showed severe jaundice and died. The serum BUN and ALT were higher but no other clinical checking. In order to identify the cause of death, autopsy examination was performed.

Results. The post-mortem examination revealed that skin all over the body was orange yellow and there was 550 ml ascites in the abdominal cavity. The liver was smaller (size: 22×15×4 cm) and slighter (weight: 722 g), it could be curled being soft. Histologically, the structure of hepatic lobules disappeared caused by mass hepatocytes necrosis with inflammatory cells infiltration and hemorrhage. Otherwise, we had not found any lethal disease in other organs. According to the literature, based on the changes of BUN/ALT and our findings, the cause of death was acute hepatic failure induced by acute hepatic necrosis after treated by medical herbs contain *Dioscorea bulbifera L. rhizome*.

Conclusions. This case report suggests that use of *Dioscorea bulbifera L. rhizome* even at a low dose is associated with a heightened risk of acute hepatic failure and death. Therefore, clinicians should always be aware of such awkward effect.

SA-089
Histomorphological features of acute autochthonous hepatitis E infection (HEV)

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Aims. Hepatitis E is caused by infection with hepatitis E virus (HEV). Infections may be asymptomatic or may cause acute (sometimes fulminante) hepatitis. In immunocompetent patients HEV infection normally induces an acute, self-limiting hepatitis. In immunosuppressed patients however chronic infections are possible, including the risk to develop liver cirrhosis. HEV is endemic in tropical and subtropical regions. In Germany only sporadic cases of autochthonous HEV infections have been reported. The aim of this study is to document the histological appearance of liver biopsies in autochthonous HEV infection. The HEV infection was confirmed serologically by the Institute of microbiology at the university hospital Hamburg (UKE). HEV infection was confirmed in 21 patients (17 men und 4 women) over the course of the last two years at the UKE.

Methods. Seven of these patients underwent liver biopsies. These tissue samples were formalin-fixed and paraffin-embedded and stained with haematoxylin-eosin, diastase-PAS, elastica van-Gieson and immunohistochemically against the cytokeratin 7 (CK7).

Results. All seven patients were male. Five were immunocompetent and presented with clinical signs of an acute (icteric) hepatitis with marked elevation of transaminases. Two patients were immunosuppressed due to prior liver transplantation. They presented with only discrete elevation of transaminases. We observed three main morphological presentations: 1) virus-like acute lobular hepatitis 2) acute cholestatic hepatitis 3) uncharacteristic findings (in the two immunosuppressed patients). One of the two post-LTX-patients developed a chronic HEV-infection with serologic persistence of viral RNA.

Conclusions. Hepatitis E is an important differential diagnosis of acute hepatitis in Germany. The main morphological features in immunocompetent patients are of acute lobular hepatitis (virus-like) or acute cholestatic hepatitis, constituting an important differential diagnosis to drug-induced liver injury. By contrast, only very discrete and non-pathbreaking changes were observed in the two immunosuppressed patients. It is especially important to recognise the possibility of an autochthonous HEV infection in this group of patients in the setting of all unexplained elevated liver enzymes, as chronic, progressive courses of HEV are possible in immunosuppressed patients.

Abstracts

SA-090
Histologically atypical manifestations of autoimmune hepatitis

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Aims. Autoimmune hepatitis (AIH) is a chronic-inflammatory liver disease leading to liver cirrhosis if untreated. An early detection is essential to initiate adequate treatment. Histology is an integral part of the scoring systems available for the diagnosis of AIH. The classical histologic findings of an AIH are portal-based inflammation with interface activity. We describe a small set of patients with subsequently confirmed AIH, who presented without the typical histomorphological picture of an AIH and displayed isolated pericentrovenulitis or postinfantile giant-cell hepatitis instead.

Methods. Liver biopsies of nine patients, who presented with signs of an acute hepatitis and fulfilled clinical criteria of an AIH, were obtained and discussed interdisciplinarily. Eight of the patients were female and the median age was 43 years (span 27–75). Two patients had a history of recent analgesics intake; the others did not report drug-intake.

Results. All patients presented with signs of an acute hepatitis and elevated transaminases. Further clinical work-up revealed elevated gammaglobulins and auto-antibodies. The liver biopsy showed centrovenous parenchymal damage in eight of the patients with hepatocellular group and bridging necroses as well as a marked pericentrovenous inflammatory activity. Only one of the patients displayed a moderate collateral portal inflammatory infiltrate. A focal interface activity around few portal tracts was found in four patients. Emperiopolesis was only found in two patients; a distinct plasmacellular inflammatory component was not detected. One patient presented with postinfantile giant-cell hepatitis, displaying panlobular, confluent hepatoycytes with up to 15 nuclei and accompanying severe cholestasis. The portal tracts showed only discrete mixed inflammatory infiltrate. Five of the patients were rebiopsied after an interval of 3 to 36 months. Four of the patients showed the typical histological morphology of AIH with portal-based inflammation and interface-activity then. The fifth patient again relapsed in the form of postinfantile giant-cell hepatitis.

Conclusions. An AIH can manifest itself with atypical histological changes in form of an isolated pericentrovenulitis or postinfantile giant-cell hepatitis, potentially leading to false interpretations as drug-/toxic-induced parenchymal damage. The knowledge of histologically atypical initial presentations of AIH is important, as untreated AIH may progress to cirrhosis within short time if left untreated.

SA-091
Multiple inflammatory hepatocellular adenomas in twins with glycogenosis type Ia: immunohistochemical, molecular-pathologic findings and methodical aspects

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Aims. Glycogen storage disease (GSD) type I und III are a well-known risk factors for hepatocellular adenomas (HCA). Based on WHO classification 2010, HCA are categorized by four entities with different genotype, phenotype and prognosis. In two recent studies, iHCA were particularly found in the context of GSD.

Methods. 22-year-old male twins with GSD type Ia underwent liver tumor exstirpation. One patient suffered from two liver tumors, the larger measuring 13 cm, the twin-brother presented with 5 tumors, the largest with a diameter of 4 cm. Tumors were tested by Sanger-sequencing for mutations in the gp130-gene (IL6ST-gene), Exon 6. Tissues were further analysed by an extensive panel of immunohistochemical antibodies including CRP, β-Catenin, Glutaminsynthetase, and Glypican 3-anti-

bodies. Molecular and immunohistologic analysis was performed on formalin-fixed, paraffin-embedded tumoral and non-tumoral tissue.

Results. All lesions revealed histologic features of iHCA with immunohistochemical overexpression of CRP. Some showed an overlap to non-classical FNH. In particular iHCAs bore massive steatosis, and cellular hydrops with steatohepatitic changes. The tumors showed a homogeneous or heterogeneous overexpression of CRP compared to the non-tumorous liver tissue, which also revealed a significantly higher CRP-expression compared to normal liver tissue of non-GSD patients. The largest tumor showed moderate cellular atypias. Staining for Glypican3 and β-Catenin (nuclear) was negative in all tumors. Four out of 9 lesions revealed a mutation in gp130-gene, Exon 6. We identified yet unpublished gp130-mutations in GSD patients. Two of the twin-brothers' tumors showed an identical deletion (Y190_N193del;c.567_578del). Remarkably, a FNH-like nodule revealed a point-mutation in gp130-gene, exon 6 (V207I;c.619G>A).

Conclusions. 1) In formalin-fixed, paraffin-embedded material, the molecular analysis of IL6ST-gene can be performed successfully. This procedure might be a helpful tool in the differential-diagnosis of iHCA in block-material for reference-pathologic evaluation. 2) Multiple iHCAs in GSD type Ia show heterogeneous mutations in gp130-gene. 3) In twins iHCA can bear the same gp130-gene mutation. 4) iHCA as well as FNH in GSD type Ia can be characterized by remarkable steatohepatitic changes.

SA-092
The expression of the long non-coding RNA HOTTIP correlates with HOXA13 deregulation in hepatocellular carcinoma and predicts patients' survival

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Aims. The epigenetic mechanisms controlled by the polycombs, trithoraxs and the HOX genes have been linked to the genesis and evolution of liver cancer. We observed that among the HOX genes, HOXA13 is highly deregulated in hepatocellular carcinoma (HCC). Recently, a lincRNA located at the 5' end of the HOXA locus (in physical contiguity with HOXA13), called HOTTIP, has been identified. HOTTIP binds the WDR5/MLL complexes driving gene transcription along the HOXA locus. In this study we investigated the roles of HOTTIP and HOX genes in HCC.

Methods. Total RNA extracted from 60-paired biopsies obtained from HCC patients was used to quantify HOTTIP/HOXA13 expression levels via qRT-PCR and subjected to global transcriptome analysis. HOXA13/HOTTIP expression levels have been correlated with patients' clinical-pathological data. Non HCC-conditions have been used as controls.

Results. qRT-PCR data confirmed that HOXA13 is highly deregulated in HCC with no major alteration found in pre-HCC conditions. Furthermore, we outlined that HOTTIP is also deregulated in HCC but not in pre-HCC conditions and that its expression directly correlates with HOXA13 levels. We also found that virus-related (vs alcoholic) HCC patients present the highest expression levels of both HOXA13 and HOTTIP. In addition, we found HOXA13 expression levels to predict patients' overall survival in both treated and untreated HCC patients. However, we did not observe any correlation for either HOXA13 or HOTTIP with other clinical-pathological data. Finally, the global transcriptome analysis revealed that HOXA13 overexpression in HCC identifies a specific subset of genes mostly involved in mRNA processing.

Conclusions. We demonstrated for the first time that HOTTIP expression directly correlates with HOXA13 levels in HCC. Moreover, we confirmed HOXA13 deregulation as a key feature in HCC. Finally, we

outlined HOXA13 and HOTTIP as predictive markers of HCC patients' outcome.

SA-093
SH2D4A is frequently downregulated in hepatocellular carcinoma

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Aims. The aim of this study was to investigate the association between the SH2D4A gene expression, protein level and clinical features in HCC patients.
Methods. Tissue samples from 37 patients with primary HCC (HCCs matched with corresponding non-neoplastic liver parenchyma plus non HCC controls) were profiled using the GeneChip® Human Exon 1.0 ST array (Affymetrix, Santa Clara, CA). To validate the array data SH2D4A mRNA expression on fresh frozen tissues was assessed using qPCR. Immunohistochemistry of the corresponding HCC whole section slides using anti-SH2D4A Ab was performed. Furthermore, SH(2)A protein levels were evaluated on a tissue microarray (TMA) containing 434 samples.
Results. Affymetrix chip analysis revealed that SH2D4A was downregulated in HCC samples compared with their normal counterpart. These results were validated by qPCR in fresh frozen tissues [25 out of 38 samples (65.7%) showed SH2D4A downregulation; p<0.026]. In addition, the combination of qPCR data and immunohistochemistry staining demonstrated a direct correlation between mRNA expression and protein levels in corresponding formalin-fixed paraffin embedded (FFPE) whole tissue slides. Furthermore, the data obtained from TMA analysis also revealed that SH(2)A protein levels were found frequently reduced in HCC and cirrhosis samples compared to normal liver.
Conclusions. In this study we further validate SH2D4A putative tumor suppressor role as we frequently found it downregulated in our cohorts of HCC samples. In addition, we provide new evidence for SH2D4A involvement in HCC pathogenesis demonstrating its deregulation also in cirrhotic pre-neoplastic lesion samples.

SA-094
The perinodular K7/K19 pattern is a valid surrogate for intranodular hepatocellular malignant progression in cirrhotic liver core biopsy samples

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Aims. Diagnoses of a dominant nodule in the setting of cirrhosis can be challenging—especially on core-biopsy samples. From the examination of nodules after resection or explantation it is known that there is a close correlation between perinodular K7/K19-staining presence, pattern and/or absence and intranodular malignant progression. Here, we examined whether this is also valid in liver core biopsy samples.
Methods. An archival search was performed for 'liver biopsy' in combination with ICD-O codes and search terms for cirrhotic nodules (CN), dysplastic nodules (DN) and hepatocellular nodules (HCC). Samples for immunohistological analysis were selected based on histopathological evidence of cirrhosis, presence of lesional tissue, and at least one lesional interface zone. The perinodular K7/K19 pattern was classified as either: complex (slender strings of epithelial cells orientated perpen-

dicular to the perimeter of the nodule with radial extensions between lesional hepatocytes), attenuated (aggregates of thin cores rather than tubular formations without radial extension) or absent. Statistical analysis included intraclass coefficient and test-performance measures.
Results. A total of 23 consecutive cirrhotic liver core biopsy samples fulfilled inclusion criteria and primary diagnoses were 13 non-HCC (composed of 10 CN and 2 DN) and 11 HCC. The K7/K19 patterns around non-HCC were either complex (7 CN and 2 DN) or attenuated (3 CN) but not absent. In contrast, the K7/K19 pattern around HCC was absent (n=10) or attenuated (n=1) but not complex. Intraclass coefficient for primary diagnosis versus K7/K19 pattern was 0.8. Non-HCC (CN plus DN) versus HCC was differentiated with a sensitivity of 92.3%, a specificity of 100%, a positive predictive value of 100% (CI: 75.7–100% for HCC with absent pattern) and a negative predictive value of 90% (CI: 62–98% for non-HCC with complex plus attenuated pattern). An accuracy of 95.6% and a Youden's index of 92.3 indicate that perinodular K7/K19 pattern strongly correlates with intralesional histological findings.
Conclusions. Our findings indicate that the perinodular K7/K19 pattern in liver core biopsy samples is a valid surrogate of the intralesional pathology. Thus, when a difficult hepatocellular nodule is encountered in a cirrhotic liver core biopsy, assessment of the perinodular K7/K19 pattern provides an additional tool for the classification of the lesion.

SA-095
Identification of a novel immunomarker (14-3-3 sigma) for the diagnosis of hepatocellular carcinoma using differential proteomic analysis

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Aims. Hepatocellular carcinoma (HCC) is a major lethal cancer with growing incidence worldwide. Histopathological evaluation of biopsy specimens captures an important aspect of diagnosis, especially in small liver nodules. Immunohistochemical tissue based biomarkers have proven effective as a diagnostic tool, with a panel of glypican 3 (GPC3), glutamine syntethase 6 (GS) and HSP70 having the most favourable sensitivity and specificity. In order to enhance the accuracy of biomarker panels we combined high-throughput methods of systematic proteomic analysis to detect new differentially expressed proteins in HCC and establish them as immunohistochemical markers.
Methods. Initially a set of 7 HCC and 7 normal liver tissue cryo-samples were analyzed by two-dimensional difference in gel electrophoresis (2D-DIGE) and label free quantification via liquid chromatography and mass spectrometry (LC/MS/MS). Candidate proteins were validated (14 HCC and normal liver tissue) and a set of new biomarkers were tested in for consistency in 83 HCC, 25 hepatocellular adenomas and non-tumor liver tissue by immunohistochemistry in formalin-fixed paraffin embedded tissue (FFPE). Immunoreactivity of the biomarker candidates were compared to the combination with GPC3, GS and HSP70-immunostaing.
Results. Initially, 573 differentially expressed proteins were detected and 14 candidate proteins were identified. After validation 6 potential biomarkers (MVP, TRAP-1, pyrophosphatase, MST1/2, CLIC, 14-3-3 sigma) were selected. Combination of GPC3, HSP70 and 14-3-3 sigma vs. the panel of GPC3, HSP70 and GS resulted in a sensitivity of 88.5% vs. 79.5%, a uniform specificity (both 81.8%) and lower ratio of wrong correlations (14.6% vs. 19.4%) in differentiating HCC vs. normal liver tissue. GCP3 had the lowest rate of inter-observer variance (8%), followed by 14-3-3 sigma (9.5%), HSP 70 (19%) and GS (39.3%).

Conclusions. We identified novel biomarkers in HCC by means of high throughput proteomic analyses and evaluated them for use as biomarkers in differential diagnosis of liver nodules. A panel of GPC3 and HSP 70 including 14-3-3 sigma proved best and resulted in an increase of 9% in sensitivity and decrease of 4.8% in wrong correlations. Apart from its role in tumourigenesis and tumour progression, 14-3-3 sigma might therefore be used as a biomarker to enhance the significance of immunohistochemical panels in the differential diagnosis of liver nodules.

Postersession Gastroenteropathologie (unterer GI-Trakt)

SA-096
Dual function of Chk1 in promoting survival and proliferation in a cellular model of hydrogen peroxide-associated colitis

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Aims. Oxidative stress is a major contributor to inflammatory bowel disease (IBD)-associated neoplasia. In our recently developed cellular model of H_2O_2-associated colitis, we found p-JNK-dependent induction of DNA damage checkpoints in human colonic epithelial cells (HCEC) following oxidative stress. Due to the known role of the checkpoint kinase 1 (Chk1) in DNA damage checkpoint control and chromatin remodelling, we focused on this protein to be responsible for cellular survival and enhanced proliferation of altered HCEC.
Methods. Acute inflammation was mimicked by treating HCEC with H_2O_2. Altered HCEC were generated by subjecting HCEC to repeated H_2O_2 cycles (chronic inflammation). Western blot analyses and Chk1 siRNA transfection were performed to analyze DNA damage checkpoint proteins. Cell cycle progression and apoptosis were studied via FACS. The proliferation of HCEC and altered HCEC was investigated. Chromatin remodelling was examined through subcellular fractionation of HCEC, altered HCEC, and ulcerative colitis (UC) samples.
Results. Chk1 knockdown in H_2O_2-treated HCEC revealed the induction of p-JNK. Consequently, p21WAF1 and γ-H2AX were up-regulated, resulting in an activation of the G1/S checkpoint. Thus, Chk1 negatively regulates DNA-damage checkpoint proteins p21WAF1 and γ-H2AX. Indeed, in altered HCEC, we detected increased protein levels of p-Chk1 accompanied by checkpoint override, enhanced proliferation, and undetected DNA damage. Chk1 knockdown in altered HCEC showed decreased proliferation. Moreover, we detected accumulation of Chk1 and acetylated histones on chromatin of altered HCEC and of UC samples.
Conclusions. Chk1 is able to circumvent DNA damage checkpoint control through suppression of p-JNK, and this resulted in undetected DNA damage and enhanced proliferation. Indeed, Chk1 knockdown showed restoration of the normal proliferative phenotype. On the other hand, increased Chk1 binding on chromatin of altered HCEC is accompanied by chromatin remodelling as shown by elevated association of acetylated histones. This could cause enhanced binding of transcription factors, thus promoting survival and proliferation. The in vivo importance of chromatin remodelling has been shown in UC samples. In summary, we found a dual function of Chk1, acting on both signal transduction and epigenetic level, in survival and proliferation in H_2O_2-associated colitis.

SA-097
Increased G2/M cell population and present p-Chk1 are associated with apoptotic resistance of human colon epithelial cells

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Aims. The development of apoptosis resistance in colon mucosa is an important factor in colon carcinogenesis. Several studies focus on unraveling genetic changes, such as p53 mutations, that cause apoptosis resistance. In contrast, much less is known about the question of whether changes in signal transduction, such as phosphorylation, might induce apoptosis resistance. Thus, we aimed at unraveling a function of physiologic reactive oxygen species (ROS) level that is known to alter signal transduction pathways and that occurs in vivo to select apoptotic resistant human colon epithelial cells with altered signal transduction.
Methods. To prove whether low ROS may induce apoptosis resistance, and if so, to gain insights into the underlying molecular pathways, we selected ten human colon epithelial cell lines (altered HCEC) for adaptation to low level of H_2O_2, an important factor of inflammation-associated colon cancer. Apoptosis resistance was verified by FACS using pathophysiological H_2O_2. Western Blot analysis was performed to analyze DNA damage checkpoint proteins.
Results. Cell cycle analysis of altered HCEC compared to HCEC control revealed an increased G2/M cell population. In this context, we detected up-regulation of the G2/M checkpoint protein p-Chk1 in altered HCEC compared to HCEC control. Moreover, stimulation of altered HCEC with pathophysiogical H_2O_2 was associated with resistance to apoptosis. Altered HCEC showed decreased cell cycle arrest in the S phase, but proper cell cycle arrest in the G2/M phase following pathophysiogical H_2O_2 treatment compared to HCEC. This might rescue altered HCEC cells undergoing apoptosis. Instead, cells re-enter the cell cycle in the G1-phase, promoting cellular survival.
Conclusions. In this study, we found an increased cell population in the G2/M phase of the cell cycle in altered HCEC. Moreover, this was accompanied by an altered level of the signal transduction protein Chk1 in its phosphorylated form. We therefore hypothesize that increased p-Chk1, a G2/M checkpoint protein, may cause accumulation of cells in the G2/M phase via G1/S and intra-S-checkpoint override in altered HCEC. This seems to be associated with apoptosis resistance. Furthermore, proper G2/M cell cycle arrest might protect cells against apoptosis.

SA-098
Acute appendicitis with transmural perforation caused by endometriosis: a case report

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Aims. Endometriosis is the presence of ectopic endometrial tissues outside the lining of uterine cavity. It is a well known cause of chronic pelvic pain and infertility in females. Endometriosis of the appendix is a very rare condition and may present with acute or chronic abdominal pain. Preoperative diagnosis is difficult. The pathological examination revealed an acute appendicitis and in addition, small nodules found in the wall of the appendix. The patient's ectopic endometrial glands were surrounded by endometrial stroma.

Methods. We present a case of a 40-year-old Caucasian female with abdominal pain and symptoms of acute appendicitis. For treatment an appendectomy was performed, which resulted in a good outcome. The appendix measured 6.5×0.6 cm at its widest diameter. In the following histological examination after routine H&E-staining an acute appendicitis with transmural perforation was diagnosed and in addition small nodules were found in the wall of the appendix. The patient's ectopic endometrial glands were surrounded by endometrial stroma.

Results. Immunhistochemically, using the Roche benchmark and Roche antibodies (ready for use kit) we identify in the endometriosis a positive reaction for cytokeratin 7, estrogen and progesterone receptor within the epithelial component and a negative reaction for cytokeratin 20. In contrast in the mucosa of the appendix we could detect a positive reaction for cytokeratin 20 and negative reaction for cytokeratin 7, estrogen and progesterone receptor.

Conclusions. The true prevalence of extragenital endometriosis is unknown because of a lack of large, well-defined case series. Case reports throughout the literature describe extragenital endometriosis in almost every organ and tissue in the body. Interestingly, one of the only sites where extragenital endometriosis has not been reported is the spleen. Appendiceal endometriosis is diagnosed pathologically. Glandular tissue, endometrial stroma and hemorrhage are typical examinations conducted in patients with endometriosis. About half of endometriosis of the appendix involves the body and half involves the tip of the appendix. Muscular and seromuscular involvement occurs in two-thirds of patients, while the serosal surface is involved in only one-third of patients. The mucosa is typically not involved, but the submucosa was involved in one-third of patients with endometriosis of the appendix. Our patient is presenting an endometriosis in direct association with acute appendicitis.

SA-099

Diagnostic criteria for mesenteric desmoid-type fibromatosis with special emphasis on differential diagnosis of mesenteric mesenchymal tumors

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Aims. Desmoid-type fibromatosis (desmoid) is a fibroblastic tumour that shows locally aggressive growth. Mesenteric desmoids (intraabdominal fibromatosis) constitute an entity with certain and specific clinical and diagnostic issues. From our experience as a reference centre for soft tissue tumours and GISTs, mesenteric desmoids are frequently subject to misdiagnosis. In this study we present a series of mesenteric desmoids, among them cases previously misdiagnosed as GIST. In addition cases of retroperitoneal fibrosis were investigated. Special emphasis is put on the differential diagnosis of mesenteric mesenchymal tumors.

Methods. Immunohistochemical nuclear β-catenin staining and mutational status of the CTNNB-1 gene were analysed and compared among all cases (n=67). Diagnostic criteria for mesenteric desmoids and important differential diagnosis are reviewed.

Results. Mesenteric desmoids tend to grow macroscopically circumscribed, and reveal nuclear positivity for β-catenin (93%) which was never found in retroperitoneal fibroses (0%; p<0.001) or GIST. CTNNB-1 mutations were found in 92% of desmoid cases and not in retroperitoneal fibroses (0%; p<0.001). Further important differential diagnoses include GIST (immunohistochemically positive for DOG-1 and CD117) and dedifferentiated liposarcomas (overexpression/amplification of MDM2 and/or CDK4). Additional differential diagnosis may include leiomyosarcoma, extragenital stromal tumor, myofibroblastic tumor and synovial sarcoma.

Conclusions. Diagnostic criteria for mesenteric desmoids type fibromatosis include nuclear immunohistochemical positivity for β-Catenin

and the presence of CTNNB-1 mutations. These features are absent in the most important differential diagnosis i.e. retroperitoneal fibrosis, GISTs and dedifferentiated liposarcomas.

SA-100

Morphologic-genetic coherence in serrated lesions of the colorectum based on the European study of interobserver variability in the diagnosis of colorectal polyps

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Aims. In 2012 we presented a European multicentre study on Inter-observer variability in the assessment of colorectal polyps with special consideration of serrated lesions. Now we addressed the question if morphology of the polyps and tumor (epi)genetics are associated.

Methods. The study comprised 200 lesions with assessment of histomorphological criteria by 10 pathologists and consensus diagnosis and included hyperplastic polyps, classical adenomas, and serrated adenomas such as TSA, SSAs and mixed polyps. From all lesions DNA was isolated after microdissection and mutation analysis was performed with a multiplex SnaPShot PCR covering BRAF, KRAS, NRAS and PIK3AC mutations (Lurkin et al. PLoS One 2010). Bisulfit-Pyrosequencing was performed to assess the MLH1 methylation status. Correlation of mutation/methylation status was performed with diagnostic categories of polyps and with the defined single morphologic criteria published in the German consensus guidelines for diagnosis of colorectal polyps.

Results. As previously reported BRAF mutations were highly associated with hyperplastic polyps (86%, 62/72) and sessile serrated adenomas (80%, 41/51). KRAS mutations were associated with classical adenomas (20%, 10/50) and more frequently with traditional serrated adenomas (78%, 7/9). The number of NRAS and PIK3AC mutation (each 0.5%) was too low to show distinct distribution patterns between the lesions. MLH1 methylation was generally infrequent in the present series of consecutive polyps (mean <5%). Specific histomorphological criteria with high frequency in SSAs like T+L shaped crypt, basal serration and columnar dilatation showed significant correlation with BRAF mutation (all p≤0.001). Specific histomorphological criteria for classic type of adenomas or traditional serrated adenomas like ectopic crypt foci, nuclear stratification, eosinophilic cytoplasm, nuclear atypia correlated significantly with KRAS mutations (all p<0.001). Other proposed histomorphological criteria for diagnosis of sessile serrated adenomas, for instance general serration >20%, could not be correlated to specific molecular changes.

Conclusions. Precisely defined morphologic criteria can predict alterations in colorectal polyps. In our study this is especially possible for BRAF and KRAS mutations. Regarding low frequency mutations like PIK3AC and NRAS and also MLH1 methylation status an increased number of polyps should be analysed with special regards on high grade IENs as a further step towards colorectal cancer.

SA-101

Expression of Cx43 in colorectal carcinoma upregulated by tumor-stroma interactions is associated with fibroblast activation

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Aims. To study the expression alteration of Cx43 and its specific mechanism during the interactions of colorectal cancer cells and fibroblasts.
Methods. We imitated the tumor (human colorectal cancer cell line SW480 and SW620)-stroma (human embryonic lung fibroblast, HELF and human skin fibroblast, HSF) interactions in vitro by construction of directly co-culture model, indirectly double-layer co-culture model and two double layer cell spheroid co-culture model. The expression of Cx43 and the activation of fibroblasts in co-culture models were detected by immunofluorescence. The protein expression of Cx43 in double-layer co-culture model was also detected by Western blotting. The effect of activated fibroblasts on the proliferation of tumor cells was measured by MTT.
Results. The expression of Cx43 in the colorectal cancer cell line was increased after directly or indirectly co-cultured with fibroblasts while the fibroblasts were activated to cancer associated fibroblasts (CAFs). CAFs can promote the proliferation of tumor cells.
Conclusions. Tumor-stroma interactions up-regulate the expression of Cx43 in tumor cells, coinciding with the activation of the stroma. Tumor microenvironment may play an important role in tumor progression and can ultimately determine the tumor's "fate".

SA-102

Aurora-B and phosphorylation of linker histone H1.4 at S27 correlate with specific stages of mitosis in colorectal carcinomas

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Aims. Serine 27 phosphorylation of the linker histone H1.4 (H1.4S27p) is a novel mitotic marker, which is set by the Aurora-B kinase, as shown in experimental model systems. Here we examined the presence of Aurora-B and H1.4S27p in the different stages of mitosis in aneuploid, Microsatellite-stable (MSS) and near-diploid, microsatellite-instable (MSI) colorectal carcinomas (CRC).
Methods. Immunohistochemistry (IHC) was performed for Aurora-B and H1.4S27p on 3 μm thick serial FFPE tissue sections of 36 previously characterized colorectal carcinomas. Stained sections were screened by x40 dry objective and 10 HPFs were evaluated for each case, by counting all mitotic figures, including prometaphase, metaphase and anaphase. Statistical analyses were done by Mann-Whitney test using the SPSS software.
Results. As reported before for experimental model systems, both Aurora-B and H1.4S27p were significantly associated with mitoses when looking at prometa-/metaphases (Aurora-B: p<0.001; H1.4S27p: p<0.01). Comparison of MSS (n=25) and MSI (n=11) CRCs revealed no significance for H1.4S27p or Aurora-B positive mitoses. However, when comparing diploid (n=19) versus aneuploid (n=16) colorectal carcinomas, H1.4S27p (p=0.035), but not Aurora-B (p=0.148) -positive metaphases were significantly higher in aneuploid CRCs. Thereby, the overall mitotic rate was statistically similar in diploid and aneuploid CRCs of the present cohort (p=0.249).
Conclusions. The present study reveals for the first time the link between Aurora-B and H1.4S27p in human colorectal carcinomas. The data suggests a role of Aurora-B for setting H1.4S27p in a physiological pattern in (mainly diploid) colorectal cancer cells. In contrast, in aneuploid colorectal cancer cells, the association of Aurora-B and H1.4S27p-positive metaphases appears to be lost.

SA-103

Functional PI3K/AKT pathway components correlate with shorter disease-free survival in stage II colon cancer

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Aims. Patients with stage II colon cancer have a relatively high five year overall survival rate after surgery. Nevertheless, a significant subgroup of patients (about 20%) develops tumour recurrence and has a dismal prognosis. Currently, there are no clinically established biomarkers available to identify this patient group. Here we used functional pathway activation mapping, based on frequently altered signaling cascades in colon cancer, in formalin-fixed and paraffin-embedded (FFPE) tissue samples to identify patients at high risk for recurrence after surgery for stage II colon cancer.
Methods. Full-length proteins were extracted from FFPE tissue samples from 121 patients with stage II colon cancer. Quantitative reverse phase protein array technology was used to analyse expression and/or phosphorylation levels of PI3K, AKT, pAKT(Ser473), GSK3-beta, pGSK3-beta(Ser9), mTOR, pmTOR(Ser2448), S6RP, pS6RP(Ser235/236), 4E-BP1 and p4E-BP1(Thr37/46).
Results. The strongest prognostic factors for disease-free survival were pAKT (HR=3.52; p=0.032), S6RP (HR=6.3; p=0.044), and p4E-BP1 (HR=4.12; p=0.011). Neither microsatellite instability nor the BRAFV600E mutation status was associated with disease-free survival in a subgroup of 83 patients of our patient cohort. In this subgroup the KRAS codon 12 mutation status was found to be negatively associated with disease-free survival (HR=3.61; p=0.034).
Conclusions. Our data indicate that activated components of the PI3K/AKT pathway might be valuable prognostic markers to stratify patients for their risk of tumour recurrence after surgery. The benefit of adjuvant chemotherapy for high risk patients with stage II colon cancer has not been proven unequivocally. However, since our analysis clearly demonstrates an up-regulation of PI3K/AKT signaling in high-risk patients, specific targeting of components of this pathway may be an attractive strategy for treatment in this patient group. This work has received funding from the Munich Cluster of Excellence M4 (www.m4.de).

SA-104

L1CAM: a prognostic surrogate marker of colorectal cancer?

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Aims. L1 cell adhesion molecule (L1-CAM) is a neuronal cell adhesion molecule in the immunoglobulin family that plays an important role in the development of the central nervous system. It was investigated in a large variety of malignant tumors, among them the highly prevalent colorectal carcinoma. The expression in tissue of primary tumor has been reported as an independent prognostic marker for worse outcome and correlated significantly with a higher prevalence of micrometastases in lymph nodes and bone marrow. The aim of our study was to investigate the correlation between L1CAM expression in the primary tumor and the L1CAM serum concentration.
Methods. From 2009 to 2011 we investigated 61 primary tumor samples of 59 Patients with colorectal cancer as well as 39 serum samples. Immunostaining of L1CAM in primary tumors was performed and classified as negative, weak and strong staining. Serum L1CAM concentrations were measured by ELISA. A spearman rank correlation was performed

to determine the relation between serum L1CAM expression and histology findings.

Results. L1CAM expression was found in 41% of tumors (13% strongly positive staining), among them only tumors that had infiltrated at least tunica muscularis (T2). Correlation of serum L1CAM concentration with immunostaining results, we found a trend to higher serum levels in strongly positive tumors that was not significant due to low patients count. We find a significantly higher L1CAM serum concentration in patients with lymph node status N1 respectively without macroscopic lymph node metastasis.

Conclusions. Our pilot study provide evidence that serum L1CAM is associated with status of lymph node metastasis, while the correlation between L1CAM expression in the primary tumors and micrometastasis in lymph nodes was already known. Further studies are necessary to investigate the correlation between metastasis-related L1CAM expression and L1CAM serum concentrations.

SA-105
The c-MYC target AP4 mediates epithelial-mesenchymal transition and metastasis in colorectal cancer

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Aims. The basic helix-loop-helix transcription factor AP4 is encoded by a c-MYC target gene and displays up-regulation concomitantly with c-MYC in colorectal cancer (CRC) and numerous other tumor types. However, the transcriptome regulated by AP4 in human cells and its specific function during the progression of colon cancer have so far not been characterized. Therefore, we set out to globally determine AP4 target genes and thereby deduce its cancer relevant functions.

Methods. We performed a genome-wide characterization of AP4 DNA binding and mRNA expression after ectopic expression of AP4 in CRC cells using a combination of microarray, genome-wide chromatin-immunoprecipitation, next-generation sequencing and bioinformatic analyses. After exemplary confirmations we subjected a set of direct AP4 targets involved in EMT-related processes to functional analyses in cell culture and xenograft based assays. Furthermore, the expression of AP4 was determined in a case-control study of colon cancer patients with and without distant metastasis (n=110), and in a cohort of CRC patients with long-term follow-up (n=227).

Results. Hundreds of induced and repressed AP4 target genes were identified. Besides many genes involved in the control of proliferation, the AP4 target genes included markers of stemness (LGR5, CD44) and epithelial-mesenchymal transition (EMT), such as SNAIL, E-cadherin/CDH1, OCLN, VIM, FN1, and the Claudins 1, 4 and 7. Accordingly, ectopic expression of AP4 induced EMT and enhanced migration and invasion of CRC cells. Conversely, down-regulation of AP4 resulted in mesenchymal-epithelial transition (MET), and inhibited migration and invasion. In addition, AP4 induction was required for EMT, migration and invasion caused by ectopic expression of c-MYC. Inhibition of AP4 resulted in decreased formation of lung metastasis of CRC xenografts in mice. Detection of elevated AP4 expression in primary colon cancer significantly correlated with liver-metastasis. Furthermore, elevated AP4 expression in primary CRC significantly correlated with poor patient survival.

Conclusions. Our studies identified AP4 as a new regulator of EMT which participates in metastatic processes during progression of colorectal cancer.

SA-106
Endothelial CCR2 signaling induced by colon carcinoma cells enables extravasation via the JAK2-Stat5 and p38MAPK pathway

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Aims. Metastasis is the primary cause of cancer-related mortality. Recent results suggest that tumor cell-derived CCL2 attracts inflammatory monocytes to facilitate tumor metastasis. However, the exact mechanisms how tumor cell-derived CCL2 can enable the independent steps of metastasis upon attraction of monocytes remain elusive. Moreover, the role of the corresponding chemokine receptor CCR2 is only poorly characterized in metastasis.

Methods. By using various experimental mouse models such as wild-type (C57BL/6), knock-out (Ccr2-/-), transgenic (Tie2CCR2/Ccr2-/-) and conditional knock-out mice (LysMCreCcr2fl/fl) as well as by performing bone marrow reconstitution experiments, we are exploring the role of CCL2 and CCR2 in tumor cell extravasation and metastasis. In a simplified approach focusing on the step of tumor cell extravasation using primary pulmonary endothelial cells we are trying to confirm our in vivo data and focus on pathways downstream of CCR2. Furthermore the role of tumor cell-derived CCL2 in this setting is studied using various tumor cell lines.

Results. Here, we demonstrate that CCR2 deficiency prevents colon carcinoma extravasation and metastasis. CCR2 expression on radio-resistant cells or endothelial CCR2 expression restores extravasation and metastasis in Ccr2-/- mice. Reduction of CCR2 expression on myeloid cells decreases but does not prevent metastasis. Tumor cell-derived CCL2 activates the CCR2+ endothelium to increase vascular permeability in vivo. CCL2-induced vascular permeability and metastasis is dependent on JAK2-Stat5 and p38MAPK signaling.

Conclusions. Our results indicate that a tumor cell-derived chemokine can induce vascular permeability and enable efficient tumor cell extravasation, suggesting a so far undescribed role for chemokines in tumor cell extravasation. JAK2/Stat5 and p38MAPK pathways were identified as two targets for the suppression of CCL2-dependent tumor cell extravasation during colon carcinoma metastasis and might therefore represent novel therapeutic targets.

Abstracts

SA-107
FOXC2 is a marker of colorectal cancer progression and induce EMT by targeting E-Cadherin promoter

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Aims. Epithelial-mesenchymal transition (EMT), a key step in tumor invasion and metastasis, has been implicated in the invasion of colorectal cancer (CRC). However, the regulatory mechanisms of EMT triggering in CRC remains unclear. The Forkhead family protein member FOXC2 is deregulated in several human cancers. In the current study, we sought to investigate the potential role and the underlying mechanism of FOXC2 in EMT and CRC progression.

Methods. The relationship between FOXC2 expression and clinical characteristics of CRC was analyzed in 206 paraffin-embedded archived CRC specimens by immunohistochemistry (IHC). The effects of FOXC2 on EMT, migration and invasion, as well as on metastasis, were examined both in vitro and in vivo, using Western blotting, immunofluorescent staining and migration assay and experimental metastasis assay in nude mice. TOP/FOP luciferase assay was using to detect E-Cadherin promoter activity. ChIP assays were performed to confirm the binding of FOXC2 on the E-Cadherin promoter.

Results. FOXC2 protein level was significantly correlated with advanced Dukes stage (p=0.01), T stage (p=0.006), distant metastasis (p=0.011), higher proliferation index (p=0.015) and poor survival of patients (p=0.002). Enforced expression of FOXC2 induced a EMT-like phenotype in CRC cells SW480 and significantly enhanced cell migration in vitro and metastasis in vivo. Conversely, knockdown of FOXC2 in HCT15 and HCT116 cells caused an increase expression of epithelial markers and decrease expression of mesenchymal markers, accompanied by inhibition effects of cell migration in vitro and metastasis in vivo. Moreover, upregulation of FOXC2 led to upregulation of Snail, a central regulator of EMT, partially through activation of modulation of MAPK and AKT/GSK3/Snail signaling cascades. More importantly, Chromatin immunoprecipitation assays revealed that FOXC2 transcriptionally downregulated the expression of the key epithelial marker E-Cadherin through direct association with the E-Boxes located in E-Cadherin promoter. This observation was consistent with the statistically inverse correlation between FOXC2 and E-Cadherin expression in a cohort of human CRC tissues by immunohistochemical analysis.

Conclusions. Our findings suggest that FOXC2 is a valuable marker of CRC prognosis and plays an important role in the progression of human CRC. Our results also provide new functional and mechanistic links between the FOXC2 and E-Cadherin in EMT and the progression of CRC

SA-150
The relevance of Bmi1 in cancer stem cells of human colorectal carcinoma

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Aims. Metastasis and chemoresistance are unsolved and central problems in recent therapies of colorectal cancer and thereby responsible for the most deaths of this disease. Both central mechanisms are discussed to be closely related to cancer stem cells (CSCs). For this reason the hypothesis arose, that CSCs are an interesting therapeutic target for metastasis and chemoresistance. Due to this, it is important to find genes with a functional relevance for CSCs. One candidate is Bmi1 a marker for CSCs in colorectal cancer, which correlates positive with the progression of the disease. Thereof our working-hypothesis was: what is the functional relevance of Bmi1 in the process of colorectal carcinogenesis, metastasis and chemoresistance?

Methods. CSC-cultures were established out of tissue samples from colorectal carcinoma and then analyzed with two gold standards for CSCs: growth of spheroids in 3D cell cultures, as well as subcutaneous growth of tumors in immunodeficient mice. Additionally, mutations in wnt-signaling, MAPK-signaling and microsatellite-, CIMP-state were determined. Further gene expression analyses, TOP-flash assays and FACS analysis were performed.

Results. In previous work we have described that maintaining Bmi1 expression is essential for metastatic traits, chemoresistance and simultaneously reduce the amount of CSCs in colorectal cancer cell lines. In recent work we established primary CSC-cultures and for comparison differentiated spheroid derived adherent cell cultures. Here we correlated an increased Bmi1 expression of the primary CSC-cultures with higher stemness traits. In further work we want to knockdown Bmi1 in primary CSCs to investigate the impact of this molecule on metastasis and chemoresistance.

Conclusions. Our experiments showed that Bmi1 plays an important role for the maintenance of CSC in colorectal cancer. In further work it shall be investigated if Bmi1 has as well an essential role for metastasis and chemoresistance.

SA-108
Microsatellite instability, KRAS and BRAF gene mutations in patients with locally advanced rectal adenocarcinoma before and after neoadjuvant 5-FU radiochemotherapy

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Aims. Multiple activating mutations of the signal- and repair pathway, such as BRAF-, KRAS-mutations and microsatellite instabilities are involved in colorectal cancer pathogenesis. Molecular characterization of specifically locally advanced rectal cancers is scarce. Therefore the retrospective study addresses the intratumoral status of KRAS, BRAF and microsatellites loci with respect to tumor response and patients' antecedent including nicotine abusus, familial history, and health care to further molecularly identify rectal cancer patients.

Methods. The study assesses the molecular status of 50 rectal cancer samples (25 before and 25 after neoadjuvant 5-FU radiochemotherapy). KRAS and BRAF mutations were examined through two independent analytical methods (sequencing and SNaPshot) to ensure efficient mutation detection. The microsatellite analysis was conducted using a fluorescent multiplex PCR-based method.

Results. KRAS mutations were found in 9 of 25 (36%) rectal cancer patients and were not significantly associated with the response to therapy (p=0.577), age (p=0.249) or sex of the patient (p=0.566). No link exists between KRAS mutation status and nodal (p=0.371) or metastatic stage (p=0.216). For two patients KRAS mutation status changed after application of neoadjuvant 5-FU radiochemotherapy. All tumour samples were diagnosed BRAF-negative. Two rectal cancer patients exhibited a MSI-H phenotype and showed no tumor response.

Conclusions. 1) KRAS mutations status may change after neoadjuvant 5-FU radiochemotherapy relevant for further therapeutic decisions 2) MSI-H patients do not respond to neoadjuvant 5-FU radiochemotherapy. Further prospective studies are needed to validate these results.

SA-109

BRAF V600E-specific immunohistochemistry for the exclusion of Lynch syndrome in MSI-H colorectal cancer

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Aims. The differentiation between hereditary and sporadic microsatellite-unstable (MSI-H) colorectal cancer is a crucial step in Lynch syndrome diagnostics. Within MSI-H colorectal cancers, the BRAF V600E mutation is strongly associated with sporadic origin. We here asked whether BRAF V600E-specific immunohistochemistry (clone VE1) is helpful in separating sporadic from Lynch syndrome-associated MSI-H colorectal cancers.

Methods. To that end, we performed VE1 immunohistochemistry and BRAF sequencing in a series of 91 MSI-H colorectal cancer specimens from patients tested for Lynch syndrome.

Results. Concordance of VE1 immunohistochemistry and molecular BRAF mutation status was observed in 90 out of 91 (98.9%) MSI-H samples. All eleven tumors classified as BRAF V600E mutation-positive by Sanger sequencing were immuno-positive, and 79 (98.8%) out of 80 tumors classified as BRAF wild type showed negative staining. All VE1-positive tumors were MLH1 and PMS2-negative by immunohistochemistry. None of the tumors from MMR gene germline mutation carriers (n=28) displayed positive VE1 staining, indicating that BRAF V600E mutation-specific immunostaining has a low risk of excluding Lynch syndrome patients from germline mutation analysis.

Conclusions. In conclusion, implementation of VE1 immunohistochemistry was able to detect BRAF mutated MSI-H colorectal cancers with a sensitivity of 100% and a specificity of 98.7%. Among MLH1-negative colorectal cancers, the rate of VE1-positive lesions was 21%, offering the exclusion of these patients from MMR germ line testing. We therefore suggest the integration of VE1 immunohistochemistry into the diagnostic panel of Lynch syndrome.

SA-110

Low to moderate expression without gene amplification of Her-2/neu predominates in colorectal cancer localized in colon sigmoideum and rectum

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Aims. HER-2/neu is an important biomarker in various cancers but HER-2/neu expression is only reported in a few colorectal carcinomas (CRC) and nothing is known about the localization of HER-2/neu-positive CRCs. In this study we analyzed the frequency of expression and amplification of HER-2/neu in CRC and the localization of HER-2/neu-positive CRCs.

Methods. Immunohistochemical analysis of HER-2/neu expression in 52 CRCs and 38 matched non-tumorigenic mucosa samples was performed. HER-2/neu expression pattern was documented for circularity and intensity of membranous staining according to ASCO guidelines. Statistical analyses are based on interval scaled H-scoring data. Samples with elevated HER-2/neu expression were subjected to gene amplification analysis by fluorescence in situ hybridization (n=15).

Results. 79% of normal mucosa samples showed a low to moderate degree of HER-2/neu expression. This was seen in all regions of CRC. 42% of CRCs showed a low to moderate HER-2/neu expression whereof 14 of 21 CRCs with HER-2/neu expression were localized in colon sigmoideum and rectum. CRCs localized in caecum and colon ascen-

dens exhibit less HER-2/neu expression than CRCs localized in colon sigmoideum or rectum (p=0.072). FISH analysis did not show any gene amplification within the investigated samples.

Conclusions. CRCs show low to moderate HER-2/neu expression. There is no gene amplification in CRCs samples with elevated HER-2/neu expression. CRCs with low to moderate HER-2/neu expression predominate in colorectal cancer localized in colon sigmoideum and rectum.

SA-111

Expression analysis of Her2 in colon carcinoma

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Aims. Alterations of Her2 (ERbB2) occur in various cancers representing an attractive therapeutic target for cancer therapy. Since this is introduced in the therapy of gastric cancers it has also gained more interest for other gastrointestinal malignancies. The aim of the present study was to investigate and characterize the role of Her2 in colon carcinoma.

Methods. Her2 was determined by immunohistochemistry (IHC) on a tissue microarray containing 353 primary resected colon carcinomas. Her2 IHC 2+ cases were additionally analysed by fluorescence in situ hybridisation (FISH). Results were correlated with pathologic features (UICC pTNM category, tumor localisation, tumor differentiation), additional moleculargenetic characteristics (BRAF, KRAS, HSP70 and HSP90, MSI) and survival.

Results. 51 cases (14%) showed a positive expression for Her2 (score 2+ and 3+). 16/43 cases (37%) with Her2 2+ showed an amplification of Her2 using FISH, resulting in a total number of 24 "Her2 positive" cases (7%) according to the criteria of the FDA/EMEA for gastric cancer. Her2 expression or amplification was not associated with pT category, presence of lymph node and distant metastases, tumor localization (right sided vs. left sided carcinomas) tumor differentiation and MSI status. KRAS mutations of codon 12/13 were detected in 51% (26 cases) and BRAF mutation in 10% (5 cases) of the Her2 IHC positive cases, which is within the range of the usual frequency of these alterations in colorectal cancer. Her2 status had no impact on patient's survival. Interestingly, there was an association between Her2 expression and the expression of Her2-linked molecular chaperones HSP70 and HSP90 (p=0.042 and p=0.013).

Conclusions. Her2 overexpression and amplification can be observed in a small subset of colon carcinomas offering potentially treatment options for a selected group of patients. In this context, the association with Her2 linked cellular chaperones may offer additional therapeutic targets with recently introduced HSP targeting molecules.

SA-112

ypN0 nodal status after neoadjuvant chemoradiotherapy for rectal carcinoma is not associated with adverse prognosis as compared to pN0 after primary surgery

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Aims. Neoadjuvant chemoradiotherapy (nCRT) has been established as standard treatment for locally advanced rectal cancer (cT3/4 or cN+ M0) to minimize the rate of locoregional recurrence and enhance local disease control. As a consequence of downstaging, a significant proportion of patients show non-viable tumor deposits within regional lymph

nodes that qualify as ypN0 according to the current TNM classification irrespective of the actual pre-treatment status. Accordingly, the prognostic relevance of ypN0 status as compared to true pN0 without preceding nRCT is not known.

Methods. We retrospectively analyzed 132 patients who underwent standard TME surgery after nCRT for rectal carcinoma and were classified as ypN0 and compared the prognosis with 341 patients with pN0 without nCRT.

Results. Re-evaluation of regional lymph nodes after nCRT showed no evidence of previous metastasis in 91 cases [ypN0(-)], sure non-viable metastasis in 14 patients [ypN0(+)] and unsure status in 27 patients [ypN0(uk)]. The observed, disease-free and cancer-related survival was similar in both groups (p=0.339, 0.055 and 0.670).

Conclusions. Our results showed similar outcome in patients with ypN0 and in the control group with pN0. Validation of these results are necessary to clarify the questions regarding postoperative adjuvant chemotherapy for patients with ypN0(+).

SA-113
High chance to identify metastases under the first 12 lymph detected lymph nodes in colorectal cancer

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Aims. Many authors recommend evaluating as many as possible lymph nodes (LN) in colorectal cancer specimens to exclude understaging by missing LN metastases. The latter is thought to be the reason for the worse outcome in cases with poor LN harvest. In order to simulate the effect of LN harvest on the probability to establish the diagnosis of LN positivity we performed sequential LN dissection.

Methods. In order to simulate the effect of LN harvest on the probability to establish the diagnosis of LN positivity we performed sequential LN dissection.

Results. 35 cases (37%) showed LN metastases. The rates of positive LNs in the 4 portions were 63%, 23%, 3% and 9%, respectively. In 31 of 35 nodal positive cases (89%) the first positive LNs were found among the first 12 detected LNS. In 3 of the remaining 4 cases (1 right hemicolon, 2 sigmoid colon and 1 rectal specimen) the first LN metastases were detected in portion IV. Only in one case was a LN metastasis found exclusively outside of the defined tumor region. 96% (90 out of 94) of cases could have been correctly classified by evaluating 12 LNs from the tumor region.

Conclusions. The recommendation to investigate at least 12 LNs in colorectal cancer seems reasonable. LNs out of the area close to the tumor bear the highest chance of metastatic involvement.

SA-114
Tumor deposits in colorectal cancer and their role in TNM classification

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Aims. Extranodal tumor deposits (TDs) in the pericolic fatty tissue are a frequent finding in colorectal cancer. According to their contour they have been interpret as either complete lymphnode occupation (round contour, pN+) or as a consequence of vascular invasion (irregular contour, pV1). In the new, 7th edition of the TNM classification tumor deposits are summarized in the pN-category and classified as pN1c in otherwise lymph node negative cancer. Thus, pN1c results in an upstaging from UICC stage II to stage III, the critical stage for adjuvant chemotherapy.

Methods. We were interested in the prognostic significance of TDs including pN1c and in the frequency of UICC upstaging. Therefore, 350 colon cancers TNM classified according to the older 6th TNM version were reviewed for tumor deposits (TDs). The results were compared to the 5-year disease free survival rates.

Results. TDs were identified in approximately 25% of colorectal cancers, rarely resulting in an UICC upstaging. TDs were a significant prognostic factor in nodal positive and negative cancer.

Conclusions. In conclusion, TDs are an important prognostic marker and likely follow a different mechanism than classical lymph node metastasis. Tumor deposits should therefore be mentioned separately. Our data further suggest that UICC upstaging in lymph node negative cancers and consequently the application of adjuvant chemotherapy is justified.

SA-115
Assessing the infiltration type of colorectal cancer: presentation of a highly prognostic score

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Aims. To date, the TNM classification is the main prognostic marker in colorectal cancer. Tumor grading is less important for two reasons. Firstly, poor differentiation, particularly of MSI-H carcinomas may not be associated with poor prognosis and secondly, the current grading system highly favours G2 tumors. Thus, G2 tumors dominate the grading spectrum while the frequency of for example G1 carcinomas is almost negligible.

Methods. In analogy to the situation in the stomach (diffuse versus intestinal infiltration type) we developed a simple three-tier score for the assessment of the infiltration type and applied this scoring system to 350 colorectal cancers for which a 5-year follow up was available.

Results. By this approach, we did not only identify a strong association of score 3 cancers (highly infiltrative, radiating) with poor prognosis but also achieved to reach a more balanced distribution of the 3 different scores as compared to the conventional grading.

Conclusions. Our data show a significant association of growth pattern and prognosis in colorectal cancer and suggest to include an infiltration score in routine diagnostics of colorectal cancer.

Postersession Bioinformatik, digitale Bildanalytik, Biobanking

SA-116
DZIF harmonized biobanking infrastructure

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Aims. Access to a comprehensive biobanking infrastructure is mandatory for multi-site infectious disease research and its translation into application. Within the German Center for Infectious Diseases (DZIF), a biobank coordination and technology platform will be established and during the starting phase (until 2012), the existing and complementary main biomaterial banks in Munich, Heidelberg, and Braunschweig

will provide specific infection-related biomaterial collections for DZIF members.

Methods. Nowadays, a harmonized biobanking structure for infectious diseases and special expertise are missing in Germany particularly at some DZIF partner sites. Biomaterial banking as a common DZIF resource will be based on existing banks, collectives, and their respective expertise, content, and documentation as initiated during the starting phase (until 2012). The DZIF biobank structure will be governed by a board consisting of the three applicants and the biobanking representatives of all other partner sites. The three applicants will be responsible for the three columns microbial pathogens and producers (Overmann), liquids (Wichmann), and tissues (Schirmacher). Central coordination is essential for further development, harmonization, and coordinative embedding of DZIF biobanking. Wherever applicable, ELSI-, IT-, QM-, IP- and structural solutions will be harmonized between the three columns and with existing national (TMF, AG Tissue Banking) and international structures (BBMRI). Strenghts of the project are to build up an early-on working platform by integrating pre-existing leading expertise (e.g. ELSI, QM, databases, project management) and technologies. Using these established and proved structures adjusted to the DZIF allows a rapid generation of annotated collections and derivatives by integrating existing resources when possible.

Results. This platform will provide a structured ELSI-, QM-, and IT-frame for DZIF biobanking and respective solutions for other DZIF partner biobanks (roll-out). Another aim is to establish and characterise microbial pathogen and producer, as well as human biomaterial collectives and respective derivatives for DZIF and other partners.

Conclusions. The harmonized biobanking infrastructure of the DZIF will rapidly provide researchers and outside collaborators access to high quality biomaterials in a sustainable manner, in order to support the development of DZIF and new research in the field of infectious diseases.

SA-117
The BMBF Initiative to build up centralized biomaterial banks in Germany: The popgen 2.0-Network

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Aims. Funded by the Federal Ministry of Education as one of the five facilities supported within the National Biobank Initiative, the popgen 2.0-Network (P2N) will merge seven data and probe collections currently existing at the Medical Faculty of the Christian-Albrechts-University (CAU) Kiel. This network will aim towards a uniform and centralised research infrastructure which will enhance scientific usability by means of fast and easy access to vast quantities of quality-controlled data and probes that have been collected in compliance with legal and ethical regulations.

Methods. Under coordination of popgen, a well established population-based biobank, P2N will comprise the data and probes of seven collections, namely of popgen itself, the Cancer Centre North, the Department of Neurosurgery, the Institutes of Pathology and Pharmacology, the University Lung Centre North and the Centre of Family Medicine. In order to harmonise, standardise and ease access to these manifold probe types together with the both comprehensive and sensible data attached to them, P2N has performed a survey of the participating biobanks by evaluating infrastructure, workflows, probe and data types, recruitment and consent policies. As far as applicable, this information is presently being mapped to the newly acquired biobank information management system (BIMS), and first steps aiming towards a common broad consent policy have been undertaken.

Results. A shared network of peripheral biobanks with centralised access is merely as useful as the consistent and uniform quality of the probes and data collected and provided by the network partners. Due to the broad heterogeneity of the participating probe and data collections the usage of one common efficient albeit flexible BIMS is a crucial ingredient for a properly functioning biobank network. Equally important is a standardised handling with the probes itself and the accompanying data, in particular the informed consent documents. Ideally, these routines must adequately be supported by the BIMS and a uniform set of SOPs.

Conclusions. Within the next few years P2N will have set up one of Germany's largest probe and data collections comprising approximately probes of more than 800,000 individuals. Standardised IT and quality control infrastructures will facilitate an efficient and data safety conform access.

SA-118
The Interdisciplinary Bank of Biomaterials and Data Würzburg (ibdw)—high quality liquid and solid biomaterials for research

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Aims. The Interdisciplinary Bank of Biomaterials and Data Würzburg (ibdw) aims to simultaneously collect liquid and solid biomaterials (BM) from consenting patients and trial subjects of the university hospital and its associated research centers. The implementation of a consistent labeling, registration, storage, tracking, and retrieval system enabling parallel analysis of matching blood and tissue samples along with clinical data poses a major challenge. Integration of sample and data collection into clinical routine requires smooth and stable processes.

Methods. The concept for broad patient consent has been approved by the local ethics committee and the consenting process has been integrated in the patient admission procedure. Each patient is assigned a non-speaking, random identifier not associated with any patient identifying data. Unique identifiers are assigned to all samples and data and are linked to the patient's identifier. Thus, all data within the ibdw is stored pseudonymized. Depseudonymization can only be done on request and through an independent gatekeeper. Public access to data offered for search requests is fully anonymized. Workflows ensuring high quality and implementation of standard preanalytical criteria (SPREC) according to ISBER recommendations are implemented. All liquid BM is stored as 350 µl aliquots at −80°C in two fully automated freezer modules which can hold about 500,000 samples each. Solid BM is stored in manual freezers at −80°C. Selected liquid or solid BM can be stored in the gas phase of liquid nitrogen. The tubes that are used for storage carry their unique identifier in a 2D bar code on the bottom.

Results. Soft integration of the BM-collection process into clinical routine together with informing potential BM-donors about the scope and aims of the ibdw provides high acceptance among both, clinical staff and patients. This requires a high degree of automation where e.g. mouse-clicks being replaced by touch-screens. This process transparency is a prerequisite for an unbiased quality assessment.

Conclusions. The ibdw offers a unique professional biobanking service for both clinicians and researchers as collection of BM and corresponding data is done in a standardized and highly automated way. Simultaneous collection of liquid and solid BM samples offers new possibilities for future biomarker research. Networking of modern biobanks will provide access to sufficient sample sizes and serve as a basis for high quality national and international research.

SA-119

Next generation biobanking—the centralized biomaterial bank of the Charité (ZeBanC)

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Aims. Biobanks have already been established in many institutions and are regarded as essential prerequisites for biomedical research. However, previous biobank efforts by many different scientists have led to an enormous diversity in quality, legal and ethical aspects and IT infrastructure. This heterogeneity raises many costs and largely prevents cooperation between different biobanks. Moreover, since most of these previous biobank activities are project-driven, there is no sustainability in most of the cases. The Federal Ministry of Education and Research (BMBF) recognized this unsatisfactory situation and launched a call for funding of a national biomaterial bank initiative aiming at the establishment of central biobank infrastructures which condensate all biobank activities of an institution under one roof.
Methods. The establishment of a huge central biobank infrastructure is a complex multi-step procedure which includes the installation of technical equipment for storage and laboratories. Workflows for the acquisition and processing of biospecimens (including liquid samples and tissues) have to be designed, tested and implemented. Legal and ethical aspects including patient information and consent have to be established. Last but not least, an IT infrastructure needs to be raised including interfaces to several other (clinical) databases. Finally, rules for the storage (including fees) and distribution of samples and data have to be introduced.
Results. The ZeBanC is up and running. The equipment is almost complete and the laboratory and storage space is identified and prepared. The workflows are defined and the technicians are trained to handle the biospecimens and the data. The IT system is installed and currently being configured. Most importantly, the ZeBanC is already productive. First ZeBanC partners are actively recruiting their samples for storage and processing in the ZeBanC. Additional ZeBanC partners are currently being integrated. A number of additional services are in place such as production of tissue microarrays, acquisition of whole slide images (WSI), extraction of DNA and RNA including quality control and next generation sequencing.
Conclusions. In conclusion, the concept of a centralized biobank bears first fruits at the Charité. More and more partners are willing to share their samples and data in the ZeBanC and are actively using the offered services. Thus, the ZeBanC provides an excellent basis for the future challenges in personalized medicine research.

SA-120

Biospecimen quality management at the RWTH centralized biomaterial bank (RWTH cBMB)

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Aims. High impact research on biospecimens largely depends on the quality of the sample, which must be obtained according to rigorous procedural standards. To achieve this, biomaterial banks, such as the RWTH centralized biomaterial bank (RWTH cBMB), are challenged to develop and apply standards warranting that research data represent unique biological features of the specimen rather than pre-analytical process variation.
Methods. Best practice protocols were defined as standard operating procedures (SOP) before acquiring any type of human biomaterial. For solid tissues, a quality management (QM) development program has

been set up. This includes active research on biospecimens, which assesses the stability of molecular analytes in relation to specimen handling time, rate and method of freezing, or aliquot size. Next, this knowledge is integrated with protocols in order to generate evidence-based SOPs. Molecular tests are established that assess a biospecimen's quality. Lastly, pathological sample characterization is performed before samples are provided to researchers.
Results. The biobank management system StarLIMS has been established to keep track of a sample's pre-analytical history from acquisition to storage by a chain-of-custody principle, including the SPREC code. Currently, the most frequently collected tissue is liver, followed by gynaecological, breast, and colorectal samples. Molecular tests to assess sample quality will be adopted from guidelines of two organizations specialized in this field, i.e. ISBER and OBBR. Further, digital photography/macroscopy of larger tissues can be associated with a biospecimen in StarLIMS in order to document the exact sample localization within the surgically resected tissue. Ultimately, any solid tissue is reviewed on H&E sections by board-certified pathologists. Diagnosis must be reconfirmed and cellular content be appropriate before samples are finally provided to researchers.
Conclusions. Biospecimen QM enables the collection and provision of samples with highest quality, improving the accuracy of molecular research data. Biospecimen QM is a multidisciplinary challenge, involving clinicians, biologists and computer scientists.

SA-121

Improving medical research by centralizing fragmented biorepositories: the RWTH centralized biomaterial bank (RWTH cBMB) as an example

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Aims. The RWTH Aachen University Hospital is one of Europe's largest hospitals providing medical care for more than 250,000 in- and outpatients by 33 clinical departments every year. Previously, human tissues and bodily fluids obtained in the course of diagnosis and treatment have been collected with ethical committee approval for research purposes by several clinical departments. Although considered valuable, these tissue collections have a significantly greater potential for medical research after centralization and standardization by applying common protocols for tissue acquisition and handling, as well as management of medical data or patient and sample identities.
Methods. Within participating clinical departments, standard operating procedures are being developed and applied with regard to obtaining informed consent, tissue acquisition, processing and storage, pseudonymization of sample, patient and medical data, as well as process-controlled provision of sample and data sets to requesting researchers.
Results. Within the first 15 months, more than 12,000 solid and liquid biospecimen samples have already been systematically collected, processed and stored within RWTH cBMB. As a disease-based biorepository, tumor samples and matched non-malignant tissues comprise the majority of solid tissue biospecimens, whereas liquid samples (whole blood, plasma, serum, buffy coats, urine, and liquor) were predominantly collected from oncological, cardiovascular and neurological diseases. The laboratory information system StarLIMS has been adapted for the management of a sample's pre-analytical history and storage. An identity management tool has been self-developed and successfully operates the pseudonymization process. A Wiki application was established in order to provide a central communication platform for participating clinicians and to register clinical parameters to be associated with a biospecimen. Association of medical data not contained in clinical information systems will be managed by OpenClinica software.

Ultimately, all information on biospecimens and its associated data will be integrated by a data warehouse.

Conclusions. Centralizing and harmonizing the large biorepository of RWTH cBMB will provide researchers with constantly high quality biospecimens and comprehensive associated data sets following a high standard of patient and data security, altogether improving medical research.

SA-122
Munich Biobank Alliance: implementation of improved standards for collecting biospecimens in the era of personalized medicine

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Aims. Biobanks and biospecimens are critical components for clinical and basic research and are fundamental for the development of personalized medicine and improvement in the quality of human health care. Fresh frozen tissue is the most valuable material for molecular analysis. However, quality of biospecimens and associated data is critical and must be collected according to standardized procedures, in order to prevent spurious analytic results.

Methods. A major aim of the Munich Biobank Alliance (www.m4.de/) is to establish a sustainable infrastructure for state of the art biobanking in the era of personalized medicine. Moreover, a comprehensive ethical and legal concept in line with current national and international guidelines has been developed and implemented in cooperating institutions in the Munich area.

Results. As a member of the Munich Biobank Alliance the "Gewebebank of the Klinikum Rechts der Isar der medizinischen Fakultät TU München" aimed at improving standard operating procedures for collecting, processing and storing of tissue samples, as well at expanding and substantiate annotation, in consideration of personalized medicine. For data management, an in-house biobanking information system has been developed interfacing the clinical (PKIS) and the pathological information systems and automatically incorporating clinicopathological data. Parameters recorded for each samples include:

– clinical data: clinical diagnosis (primary and secondary), prior permanent medication, medication before surgery, serological findings, chemotherapy or radiation,

– quality and processing-related data: time of vessel ligation, time of hand-over to pathologist, time of preservation (liquid nitrogen),

– biospecimen related data: type of sample (primary tumor/metastases/non-neoplastic), origin of the tumor sample (central/periphery), type of non-neoplastic tissue,

– pathological data: histopathological diagnosis, TNM staging system, the parameters grade (G), resection status of the operation (R) and an in-house pathological annotation.

Conclusions. We believe that a high quality biospecimen collection together with a highly standardised clinicopathological annotation will advance the field of translational research and is critical for discovery, validation and implementation of biomarkers in clinical practice as well as the identification of new targets for personalized cancer therapy.

SA-123
Invitation of a tumor bank in Malawi

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Aims. Cancer in Africa has high mortality rates and has become a serious health problem. Knowledge about epidemiology, pathogenesis and genetic of many kind of cancer in the African population is scarce. Pathology plays an important role not only in diagnostics, but also in cancer research. However, especially in sub-Saharan Africa there are only few laboratories which can provide constant service for surgical pathology. The aim of the project is to initiate a tumor biobank as a basis of structured, significant tissue-based research targeting cancer in sub-Saharan Africa.

Methods. Formalin fixed paraffin embedded (FFPE) probes, routinely used for diagnostics, may be a valuable source for tissue based research. One of the most crucial issues, though, is the quality of the specimens. Currently existing infrastructure allows diagnostic standard staining. Application of further molecular analysis, however, is hampered by the lack of standardization of pre-analytic tissue handling (variable fixation times, unclear fixation agents etc.).

Results. A basic approach for building up a biobanking structure in the low resource settings in Malawi will encompass: a) structuring and standardization of tissue processing, allowing FFPE tissue to serve as a firm base for scientific projects, b) evaluation of recently developed alternative fixation agents, which show certain advantages over FFPE in terms of usage for molecular analysis and may offer alternatives for robust tissue preservation, c) complementary to tissue collection, a clinical data bank will be established in order to document corresponding relevant clinical information, d) consideration of ethical issues for the usage of tissue (informed consent, respect of cultural aspects, etc.) is of high importance. Tight collaboration with ethics commissions is mandatory.

Conclusions. The initiation of tissue biobanks in sub-Saharan Africa is a valuable step towards tissue-based research in order to gain insight into cancer in the African population. It is a prerequisite for subsequent focusing on distinct cancer entities using targeted molecular analyses (e.g. HPV-analyses and other immunohistochemistry or DNA based research).

SA-124
Development of a biobank linked database

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Aims. The translational research approach of modern Comprehensive Cancer Centers is embedded in structured and strictly interdisciplinary cancer care, which includes well-documented patient populations and correlating tumor- and biobanking systems. Fundamental for functioning is a standardized implementation of pathological information of tumor tissue and the linkage to clinical data together with strict rules of admission concerning patients' data privacy. In times of scientific and technological changes, a flexible database is needed to guarantee sustainability including that new generated research data has to be easily linked. Here we describe the development and the features of our biobank-linked database.

Methods. The database was developed in MySQL 5.1, a programming language designed for managing data in relational database manage-

ment systems, with the development environment Eclipse Hellios and the database development assistant Toad for MySQL. It runs on a Suse Linux Enterprise server, a virtual server at the central IT facility of the University of Freiburg. The interface for data import was developed with Java, the web-interface was developed using JavaServer Pages 2.0 and Servlet-Container ***Tomcat 7.0. The Servlet-Container Tomcat 7.0 was connected upstream for security and performance reasons to the Apache HTTP Server 2.2 to act as a Proxy. It inquires to the Tomcat-Servlet-Container and transfers the encryption of the dynamic generated HTML-pages. Consolidating of data of the facilities into structured text data is achieved by the mark-up language XML. Furthermore programs for data import from our intranet application for comprehensive tumor documentation called CARAT established for the entry of all cancer data elements, as well as from excel sheets were programmed. Data integrity is evaluated by random checks of patients by the IT officer and a physician.

Results. We developed a flexible biobank-linked database. Patients consent, as well as clinical data can be updated either automatically or manually from our facility and extern clinical partners. Patients' personal data are only accessible for the administrator. All clinic-pathological and research data are electronically protected. Access for clinic-pathological and research data is only admitted for accredited persons. Access can be guaranteed after written request.

Conclusions. A well planned and flexible clinicopathological biobank-linked database is the fundament of sustainable biobanking as well as future research.

SA-125
Effective handling of bioprobes using a new STARLIMS application for 2D barcode rack scanners

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Aims. Due to a national funding initiative of BMBF for the setup of centralized biobanks in the clinical environment (cBMB), currently many large-scale biobanks are evolving with the urgent need of modern storage techniques and adequate IT infrastructure for managing the sample related information. With raising sample numbers, aliquoting, tracking and picking of samples gets more time-consuming and error-prone. Compared to conservative methods, 2D bar-code rack scanners enable the user to register samples more rapidly and reliably, as whole racks can be registered. Combined with a modern LIMS, the management and picking of samples can be done more efficiently as well. Here, we present a new STARLIMS application for the registration and management of 2D bar-coded samples that works in combination with any rack scanner.

Methods. Our solution uses filling templates that help to standardize the process of aliquoting and subsequent registration of mainly liquid patient samples. Different templates can be predefined and deposited in STARLIMS, for different biological materials (tissue or liquid) and different sampling procedures (universal or individual) in any common rack format (96 or 24 tubes). Additionally, it is also possible to manually register samples, as it may occasionally be necessary to adapt individual sample characteristics (i.e. material type, filling volume).

Results. The user is guided through the registration process to supply all the information needed for the scanned samples. To speed-up the registration process, already known information as well as metadata are automatically assigned to all samples from the same registration subset. When scanning registered racks, the application detects moved samples automatically and synchronizes the new positions with the database. Missing samples require user interaction to decide whether these samples have been used, moved or disposed.

Conclusions. By using this new application, the whole process of aliquoting and registration of biosamples can be optimized, thus minimizing

the "time to freeze" of the biological material and improving sample quality.

SA-126
Automated nucleic acid extraction from FFPE material—a comparison of five different systems in the view of biobanking

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Aims. Biobanking activities in translational studies using nucleic acid extracts are often performed close to pathological archives and thus close to daily clinical routine. We wanted to test whether roboter-based automated nucleic extractions from FFPE materials are comparable in results and applicability for different techniques independent from the used platform.

Methods. FFPE materials of colorectal specimen for DNA and RNA extractions were transferred to five different roboter platforms of four companies. The extractions were tested for photometric RNA or DNA yield, Agilent assays, qPCRs for DNA, mRNA and miRNA and pyrosequencing.

Results. Due to degradation of nucleic acids because of formalin fixation no RINs could be calculated in the Agilent assays. Photometry yielded concentrations of RNA between 2.4 to 171.0 ng/µl and of DNA between 4.3 to 81.8 µg/µl. As an indicator for the variability qPCR results the SEM of delta CTs from three different experiments were taken. In the q-RT-PCRs of RNA SEMs between 0.46 and 4.18, in qPCRs of DNA SEMs between 0.54 and 2.11 could be calculated. Test-dependent interclass correlations coefficients revealed values from very high (0.851 DNA or 0.938 RNA) to only moderate or poor (0.573 DNA or 0.146 RNA) concordance. In pyrosequencing only one platform achieved complete analysis of all specimens. For miRNA analysis one platform failed.

Conclusions. Different extraction platforms for FFPE material in one study should be used with care. qPCRs experiments either from RNA or DNA showed reliable results. Other techniques in nucleic acid research like pyrosequencing or miRNA analysis might be more sensitive to the used platforms and underlying chemistry. Especially in setting up different strategies for multicentric studies, e.g. either using material collections in one central study laboratory or distributing the methods to the centres and wiring data collections, should enclose these considerations.

SA-127
100 years Institute of Pathology Mannheim—a longitudinal study on clinical obductions and causes of death over time in a single center

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Aims. Die Medizin befindet sich in stetigem Wandel. Die Bedeutung des Obduktionswesens hat sich im letzten Jahrhundert stark verändert, hierin spiegeln sich auch einschneidende allgemeine und medizinhistorische Ereignisse des vergangenen Jahrhunderts wider. 100 Jahre Pathologisches Institut in Mannheim gaben Anlass zur Analyse der Obduk-

tionszahlen sowie der Todesursachen Anfang des 20. Jahrhunderts im Vergleich zu denen des 21. Jahrhunderts.

Methods. Für diese Untersuchung wurden die Obduktionsbücher des Pathologischen Instituts Mannheim für die Jahre 1902 bis 1952 und 2000 bis 2011 herangezogen. Die Daten der insgesamt 34.884 Obduktionen wurden mithilfe der Programme Microsoft-Access sowie Microsoft-Excel ausgewertet. Als Vergleichsgrößen dienten die Statistischen Berichte der Stadt Mannheim und des Statistischen Landesamtes sowie ausgewählte Kennzahlen der Klinikumsverwaltung. Die rein statistischen Untersuchungen wurden durch ein umfangreiches medizinhistorisches Quellenstudium ergänzt.

Results. Von 1902 bis 1952 stieg die Anzahl durchgeführter Obduktionen von 240 auf über 1000 Obduktionen pro Jahr an. Im 21. Jahrhundert nahmen die Obduktionszahlen von 250 im Jahr 2000 auf knapp 100 im Jahr 2011 ab. Die Obduktionsrate der am Klinikum Mannheim Verstorbener lag 1902 bis 1952 bei 60–90%, ab dem Jahr 2000 bei unter 20% und seit 2009 unter 10%. Bezogen auf die Gesamttodesfälle der Stadt Mannheim lag die Obduktionsrate im ersten Beobachtungszeitraum durchschnittlich bei 25%, in den letzten 12 Jahren hingegen bei knapp 5%. Haupttodesursache bei Kindern und Erwachsenen stellten 1902 bis 1952 Infektionskrankheiten wie Tuberkulose und Pneumonie dar. Im 21. Jahrhundert standen die Herz-Kreislauf-Erkrankungen bei Erwachsenen an führender Position, während Kinderobduktionen meist zur Klärung der Todesursache von Fehlgeburten durchgeführt wurden.

Conclusions. Das Obduktionswesen am Pathologischen Institut Mannheim hat sich in den letzten hundert Jahren stark gewandelt und spiegelt die technologischen Fortschritte der klinischen Medizin beispielhaft wider. Die Abnahme der Obduktionszahlen folgt dem allgemeinen Trend in Deutschland, die Bedeutung der klinisch-pathologischen Obduktion nimmt weiter ab. Die Todesursachen der beiden Beobachtungszeiträume lassen sich aufgrund der unterschiedlichen Obduktionsraten und Änderungen im Obduktionsgut nicht vergleichen. Sie müssen mit großer Vorsicht und in Bezug auf den jeweiligen historischen Hintergrund interpretiert werden.

SA-128

Terminologic analysis as an efficient way to improve learning and teaching of pathology

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Aims. Improving education in pathology necessitates cutback of passive explanations to gain time that can be devoted to help students become active learners and problem solvers. Given that pathology consists in large parts of definitions, very detailed terms and concepts, its education includes complicated terminology. Here we examined manual categorization of disease descriptors into a pre-defined classification system as a way to identify terminologic difficulties that can help both educators and students.

Methods. Assignment of pre-defined categories onto a source text was performed manually (category-based terminology analysis). The categories were defined as four basic disease types consisting of "inflammatory-/immune-related" (-itis), "degenerative" (-osis), "neoplastic-/proliferative-/tumorous" (-oma) and "not classifiable" (other). As a source text, we chose a dermatopathology chapter of a standard pathology textbook (Pathologic Basis of Disease, Robbins 8th ed). To capture a student's perspective, one of the authors (SD) was asked to manually assign at least one of the four classifiers to each descriptive term during an ongoing pathology course. Analysis was performed globally for the chapter, its subchapters and per disease entry.

Results. The selected chapter (dermatopathology) consists of 19 pages organized into 5 subchapters with a total of 19 disease entities. A total of 250 assignments [n=81 -itis (32%); n=88 -oma (35%); n=15 -osis (6%); n=66 other (26%)] were performed in ~2 hours and resulted in an average of 50 assignments (range 27–104) per subchapter and 13±1.2 assi-

gnments per disease entity (range: 7–31). Vectorial representation allows assessment of the perceived complexity of individual terms (e.g. 26% not classifiable), diseases or subchapters, which is reflective of the appropriateness of the provided explanations for the current understanding by the student. Disadvantages of the method include the time-commitment (10 min per page), restrictions imposed by the source text (related to author, edition, etc.), and difficulties imposed by the classification categories (here only four). Key advantage is sensitization to difficult terms on the students and educators side.

Conclusions. The delineated approach is a straightforward method to identify terminologic strengths and weaknesses. Such classifiers may represent an efficient way to re-focus and improve pathology education.

SA-129

A web-based virtual data archive as virtual research environment

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Aims. The aim of the project is to develop a virtual research environment on an academic level. We concentrated on the establishment of a standardised metadata scheme for archiving digitalized histological sections, clinical data and findings like radiological pictures in a virtual library. This systematic assignment of tags to diseases and findings will be useful to improve communication and data exchange between different medical disciplines, with pathology being the principal reference and link between participants.

Methods. Standardized metadata about digitalized histological sections were identified and applied. A pilot data demonstrator based on MS SharePoint was implemented. SharePoint was extended by an import-export interface for Web-Ontology-Language (OWL), enabling the construction of ontologies in ontology editors (Protegé).

Results. A metadata scheme consisting of 26 fields was worked out. 16 fields are regarding the medical description level, 10 fields specify the administrative, technical or proprietary context. Controlled vocabulary, terminology systems, thesauri and classifications (e.g. ICD-O, ICD-10, MeSH, B-Classification) were implemented. Users can adapt fields according to their needs. Clinical data and digitalized material from different disciplines can be easily found by using the tags in the virtual library.

Conclusions. Standardized descriptions of histological images are crucial to prevent misunderstandings or misinterpretations in a multidisciplinary setting. The multidisciplinary platform could be used during interdisciplinary conference meetings, but also as a platform for virtual interdisciplinary conferences when specialists are separated spatiotemporally. Last but not least, a multidisciplinary platform can be used for scientific, intern and student teaching purposes.

Abstracts

SA-130
3D reconstruction of lung adenocarcinomas—one module for the development of mathematical multiscale models of lung cancer

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Aims. The BMBF-funded LungSysII consortium integrates information derived from molecular biology, cell biology, and histology using systems biology approaches to generate integrative multiscale-models of non-small cell lung cancer (NSCLC). In this context, we aim to define the three dimensional spatial relationship of the vascular system and the tumor mass in human pulmonary adenocarcinomas (ADC) as well as adjacent non-tumorous tissues based on histological data. We here report our most recent progress to generate a comprehensive 3-dimensional (3D) picture of ADC.

Methods. Material was collected from freshly resected ADC patients and systematically cut into pieces of up to 1 cm in diameter. Samples were processed for optical projection tomography (OPT) scanning, utilizing tissue autofluorescence or specific epitope staining using directly labelled smooth muscle actin (SMA)-specific antibodies. In addition, alternate staining of serial sections derived from tumor samples were performed including H&E-, factor VIII (FVIII)-, and pan-cytokeratin (KL1) staining. Automated whole slide imaging was performed using the Hamamatsu NanoZoomer Digital Pathology system. The resulting 2D information was used to generate a 3D representation of the data by means of a non-linear elastic image registration.

Results. Whole tissue OPT-scanning revealed the spatial distribution of bronchial and vasculature structures in the tumor and adjacent non-tumorous lung tissue. The image quality of the 3D vessel structure was improved solving a non-negatively constrained, L2-based reconstruction problem iteratively (MRNSD) on the raw-data produced by the OPT system. To reconstruct the serial sectioning data to a 3D volume, a special non-linear image registration algorithm was developed and applied. Specialization of the algorithm was needed due to cutting artefacts such as shape distortion and staining variation. The optimized non-linear algorithm was successfully applied on the H&E-, FVIII-, and KL1-staining.

Conclusions. We here present approaches for 3D reconstruction of the vascular system and tumor mass in ADC as well as bordering healthy tissue. This quantitative information covers the range μm to cm and can be used for computational tissue modelling and for integration in mathematical multiscale models, which are currently under development.

SA-131
Matrix-Assisted Laser desorption/ionization imaging mass spectrometry (MALDI IMS) for the analysis of FFPE biopsies

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Aims. Fresh-frozen biopsies represent attractive samples for proteomic biomarker investigation, however, in large numbers logistics and archiving is difficult and expensive. Alternatively, FFPE samples are routinely prepared for pathological characterization and are abundantly available in tissue archives. The ability to conduct MS-based proteomic analyses of FFPE tissue opens new opportunities for clinical studies and biomarker discovery.

Methods. We have used matrix-assisted laser desorption/ionization (MALDI) imaging mass spectrometry (IMS) to directly analyze peptides from FFPE sections by adapting the heat-induced antigen retrieval method. Trypsin and matrix deposition onto FFPE and fresh frozen biopsy specimens was carried out using the ImagePrep device (Bruker, Bremen) followed by subsequent MALDI MS analysis using a Bruker Autoflex Speed in profiling and imaging mode. Statistical analysis was done with ClinProTools 3.0. Each analysis was performed as an independent experiment to ensure statistical independence. We first investigated the reproducibility of the method by comparing the peak distribution of 5 different biopsies taken from the same patient. We then performed an on-tissue digestion experiment on a fresh-frozen tissue and on FFPE tissue taken from the same patient, to determine the comparability of these two types of samples.

Results. Using ImagePrep, high-quality MALDI mass spectra and high-spatial-resolution ion images were obtained from fresh frozen and FFPE biopsies. The resulting MS spectra showed remarkably similar peptide profiles between fresh-frozen and FFPE tissues.

Conclusions. Our results highlight the use of MALDI IMS technology for the rapid detection of peptides in FFPE samples with high accuracy and reproducibility. This is of crucial importance and provides the basis for the introduction of MALDI IMS into routine histopathological practice.

SA-132
MALDI imaging of sinonasal adenocarcinoma of intestinal type (SNAIT) and colorectal carcinoma (CRAIT)

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Aims. Despite their different sites of origin SNAIT bear a strong histological resemblance to colorectal carcinomas (CRAIT). Even in immunhistology no major differences were detected so far. We used MALDI imaging to compare both types of carcinomas.

Methods. Paraffin sections of 20 SNAIT and 20 CRAIT were mounted on ITO-coated conductive glass slides for MALDI analysis. After Paraffin removal we performed a tryptic digestion of tissue section. Trypsin solution was applied directly onto the section by an automated spraying device. Tissue incubation with the trypsin solution was performed for two hours at 37°C. The tissue section was coated with alpha-cyano-4-hydroxycinnamic acid. The MALDI Imaging data sets were acquired in the mass ranges m/z 400 Da to 4.000 Da and evaluated by principal component analysis (PCA).

Results. We obtained good peptide profiles, showing differences between SNAIT, colorectal carcinomas (CRAIT) and normal colorectal mucosa, but we got also differences within the carcinoma groups. Preliminary results however seam to allow a distinction between SNAIT and CRAIT on the basis of MALDI imaging.

Conclusions. In situ MALDI imaging is suitable for detecting characteristic differences even in tumors with high morphological similarities. Further research is needed to classify the peptide profile differences in detail.

SA-133

imaging mass spectrometry analysis to distinguish follicular lymphoma (FL) and diffuse large B-cell lymphoma (DLBCL)

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Aims. Diagnosis of lymphoma includes besides H&E diagnostics a number of histochemical and immunohistochemical methods because of the variety of different lymphoma subtypes. Compared to other tumor entities, single lymphoma subtypes are characterized by a relative homogeneity. For this reason, lymphomas are good models for matrix-assisted laser desorption/ionization (MALDI) mass spectrometry (MS) imaging and profiling experiments. Since MALDI MS represents a fast and reliable method, the aim of this study is to investigate the ability of MALDI imaging mass spectrometry (IMS) to generate protein profiles that can distinguish clinical samples of FL from DLBCL, thereby replacing (immune-) histochemistry.

Methods. MALDI IMS was used to directly analyze proteins from in total 14 fresh-frozen biopsy sections from patients with either FL or DLBCL. H&E stained sections were evaluated by a pathologist and serial sections were prepared for analysis by applying matrix solution with a spraying device, followed by MALDI IMS analysis using an Autoflex Speed in linear mode. MS images were compared and a subset of the statistically significant discriminator proteins in the regions of interest were then selected and evaluated on the basis of P values from the Wilcoxon/Kruskal-Wallis test.

Results. Our preliminary analysis has shown promising results with the detection of twelve protein signatures that could differentiate between follicular lymphoma and DLBCL (p>0.01, 1,7-fold change). Among the detected peaks were m/z values consistent with annotated values for histones.

Conclusions. This pilot study was done to assess the efficacy of MALDI MS to discriminate between follicular lymphoma and DLBCL tissue samples. A more detailed and rigorous study involving a larger sample set is currently being developed, in order to build a model to molecularly classify DLBCL and lymphoma subtypes. In addition, simultaneous investigation of FFPE-biopsies and peptide profiling is necessary in order build a model and apply this technique for a more individualized therapeutic approach in histopathological practice.

SA-134

Challenges and possibilities of translational core projects— an example

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Aims. Translation of basic scientific findings into applied clinical setting is of utmost importance in cancer research. Pathology integrates morphological, histological and molecular information of (human) tissue specimens and links this to alterations of basic (patho)biochemical processes and to (patho)physiological/clinical characteristics. As such, pathology is central to the understanding of complex biological systems in disease. This report discusses the challenges and possibilities of a translational core project exemplarily in view of a request of "validating SNAIL expression in colon cancer".

Methods. The project included 1) establishment of 5 antibodies to "Snail" by immunohistochemistry, 2) validation of antibody specificity in protein extracts from microdissected cell populations by Western Blotting and 3) the set up and application of RNA in situ hybridization using RNAScope technology.

Results. IHC stainings were successfully established for 4/5 SNAIL antibodies from a mere technical viewpoint (e.g. no background stai-

ning). However, further critical evaluation revealed (suspicious) nuclear SNAIL expression in almost all cell types, including normal epithelial cells. Validation of SNAIL antibody specificity in protein extracts of microdissected normal epithelial and invasive tumor cells showed unspecific bands for all commercially available SNAIL antibodies. To allow for the requested SNAIL expression analysis in a morphological context, next RNAScope technology was established. Specificity of SNAIL signals were proven in FFPE cell pellets of negative and positive control cell lines. A PolR2A (positive) and DapB (bacterial, negative) probes served as controls. Further analysis of FFPE tissue specimens of colorectal carcinomas (n=6) yielded generally low numbers of SNAIL signals in all cases, except few single tumor cells with increased SNAIL signals. These were distributed throughout the tumor, without close context to epithelial-mesenchymal transition as expected.

Conclusions. This report shows the extensive work-flows that may challenge small pathology teams of translational core projects upon critical evaluation and discussion of the analyses requested by other scientists. Still, it also provides an insight into the possibilities that may arise of such projects for further development and contribution of pathology to translation of basic research "ideas" into a realistic clinic-pathological setting.

SA-135

Single cell mass cytometry: quantitative multi parametric imaging of primary breast cancer

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Aims. According to molecular alterations, breast cancer has been classified into different subtypes with distinct features and clinical behavior, including normal like, luminal A (estrogen receptor (ER)+, low grade), luminal B (ER+, high grade), human epidermal growth factor receptor 2 (HER2) enriched (ER-, progesterone receptor (PR)-, HER2+), basal like (ER-, PR-, HER2-), and Claudine low (often ER-, PR-, HER2-, low cell-to-cell contacts). Each stage of tumor development is influenced by cells of the tumor microenvironment, i.e. normal cells, soluble factors, signaling molecules and extracellular matrix (ECM) in which the tumor cells are embedded. The underlying cellular stimuli and activated signaling pathways determine the effects of tumor microenvironment and cancer cell interactions. As a result, the state of the microenvironment has a strong impact on tumor cells and can support proliferation, invasiveness, and confer drug resistance during cancer therapy.

Methods. A comprehensive analysis of the underlying cellular stimuli and activated signaling pathways of tumor cells and their microenvironment in a spatially resolved manner has been difficult to achieve due to a lack of suitable technologies. To address this need, laser ablation (LA) [1] as one of the suitable imaging techniques was applied in combination with "mass cytometry", a novel mass spectrometer-flow cytometer hybrid, which determines stable metal isotopes tagged to antibodies by metal-chelating reagents [2]. Mass cytometry is based on an inductively coupled plasma time-of-flight mass spectrometer (ICP-TOF-MS), which enables a simultaneous analysis of up to 100 biomarkers on a quantitative, sub-cellular level with high sensitivity (9 orders of magnitude dynamic range).

Results. Here, we will present first results, analyzing formalin-fixed paraffin-embedded primary breast cancer samples by LA-mass cytometry at sub-cellular resolution to (i) investigate the intricate interplay of tumorigenic cells with their microenvironment, and (ii) to recapitulate molecular portraits of breast cancer.

Conclusions. Application of quantitative single cell LA-mass cytometry can provide a detailed picture of phenotypic diversity and cellular interplay and may mark an important step towards personalized precision medicine in the future.

Literatur:
1. Hattendorf et al (2003) Analytical Chemistry 75(15):341A–347A
2. Bodenmiller et al (2012) Nature Biotechnology 30(9):858–867

SA-136

Quantitation of Cancer heterogeneity using a novel tissue microarray platform

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Aims. Cancer is often heterogeneous both on a morphological and on a genetic level. Resected tumors are often large. Even if they are not completely embedded in paraffin, multiple blocks are usually taken. Despite of this, molecular tumor analysis is usually restricted to one tissue block. The aim of this study was to systematically analyze cancers on a molecular level across many tumor tissue blocks.
Methods. Heterogeneity tissue microarrays (TMAs) were manufactured containing 6–9 different tissue spots per tumor. Spots were taken as distant from each other as possible and they were collected from at least 3 different tumor containing paraffin blocks. Heterogeneity TMAs contained 8 samples each from 350 colon cancers, 9 samples each from 113 gastric, and 6 samples each from 224 pancreatic cancers. In addition, the TMAs contained a different number of corresponding lymph node metastases. Heterogeneity TMAs were analyzed by immunohistochemistry for expression of p53, HER2 and by fluorescence in situ hybridization (FISH) for alterations of HER2, EGFR, MYC and CCND1.
Results. Variable but often significant heterogeneity was observed for the analyzed molecular features. Among amplified cases, FISH findings were for example homogeneous in gastric cancer for HER2. Genomic heterogeneity was more prevalent for HER2 amplification in pancreatic cancer (5 of 9 amplified cases were heterogeneous) and HER2 expression in colon cancer (17 of 19 positive cases were heterogeneous). Heterogeneity was also seen for MYC amplification in gastric cancer whereas p53 mutations as detected by IHC showed a very low degree of heterogeneity in colorectal cancers.
Conclusions. The data demonstrate that molecular heterogeneity is highly variable between molecular parameters and tumor types. High homogeneity argues for an early role in tumor development and high suitability as a therapeutic target. TMAs are a valid tool for the analysis and quantitation of molecular heterogeneity.

Postersession Oralpathologie und Dermatopathologie

SA-137

Multiple simultaneous KIT-mutations in a case of acral lentiginous melanoma and its metastases

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Aims. Approximately 10–20% of melanomas, particularly the acral lentiginous (11%), mucosal (21%), and chronic sun-damaged cutaneous (17%) subtype, harbor mutations of the KIT tyrosine kinase gene, predominantly in exon 11, followed by exons 13, 17, and 18. On the other hand, up to 50% of melanomas in intermittently sun-exposed skin display BRAF mutations, offering the possibility of a targeted therapy with small molecular kinase inhibitors. To date, little is known about the occurrence of multiple KIT mutations in the same tumor and the consistency of the mutational status between primary and metastases.
Methods. We here describe a case of a 64-year-old patient with an acral lentiginous melanoma of the left thumb (tumor thickness 3.5 mm, Clark level III) who subsequently developed a gluteal skin metastasis after 8 years and pulmonary metastases after 9 years, respectively, without receiving tyrosine kinase inhibitors. KIT (exon 9, 11, 13, and 17) was performed in all samples by using direct sequencing of PCR products as well as BRAF mutation analysis (codon 600 and 464–469) by using pyrosequencing. Testing was performed independently in two institutions (Göttingen/Cologne) to exclude technical errors.
Results. The primary tumor harbored a KIT exon 13 (p.N655K) and an exon 17 mutation (p.N822I). Interestingly, the skin metastasis displayed the same exon 17 mutation (p.N822I) and an additional exon 11 mutation (p.Y553S). The pulmonary metastases showed the same mutations as the skin metastasis (p.Y553S; p.N822I), but displayed an additional exon 11 mutation (p.K558E). All examined tumors showed a BRAF wildtype status.
Conclusions. The simultaneous occurrence of multiple KIT mutations in melanomas is not well documented, yet. The present case suggests that mutations observed in the primary tumor may persist in the metastases, but additional mutations in the same gene may occur during tumor progression, even in the absence of tyrosine kinase inhibitors. The prognostic relevance of additional KIT mutations, such as exon 17 mutations, and their sensitivity towards tyrosine kinase inhibitor treatment are, however, still unclear and require further investigation.

SA-138

Copy number alterations and massive genomic remodeling determine poor outcome in malignant melanoma

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Aims. The clinical course of malignant melanoma (MM), a tumor with increasing incidence, is difficult to predict. The diagnosis of MM is based on histology, and disease prognosis depends mainly on mitotic rate, Breslow tumor thickness and ulceration. Initial findings of our group, however, revealed considerably more chromosomal aberrations in MM with metastases than in MM without metastases. Recently a phenomenon termed chromothripsis was reported and describes a single catastrophic cellular event, in which one or a few chro-

mosomes, chromosome arms or chromosomal subregions are shattered into tens to hundreds of pieces and reassembled incorrectly. However, the significance of chromothripsis for prognosis in patients with MM is entirely unclear.

Methods. We applied array comparative genomic hybridization (aCGH) and for confirmation paired-end sequencing in 10 patients who died of MM 3.7 years (median, range 0.9 to 7.6 years) after diagnosis and in 10 patients who had a median disease-free survival of 14.8 years (range 12.5 to 16.7 years; p=0.00001).

Results. We were able to analyze 18 of the collected 20 MM samples by aCGH (8 MM with poor prognosis and 10 MM with good prognosis). Of the eight analyzed MM cases with poor prognosis, all eight (100%) were found to have copy number changes by aCGH, a proportion differing significantly from the MM cases with good prognosis (p=0.004), where only three of 10 (30%) samples showed aberrations. MM associated with good prognosis showed predominantly whole chromosome or chromosome arm gains and losses while MM with poor prognosis demonstrated focal events, culminating in two cases in a pattern consistent with the phenomenon of chromothripsis, which was confirmed by paired-end sequencing.

Conclusions. In conclusion, for the first time we could demonstrate that histopathologically indistinguishable MM associated with either good or poor prognosis differ significantly not only in number but also in structure of chromosomal aberrations. MM with poor prognosis showed a significantly higher incidence of genomic imbalances and harbored significantly more copy number changes than MM associated with good prognosis. In addition, while genomic imbalances in MM with good prognosis, when present, virtually always affected whole chromosomes or chromosome arms, focal copy number alterations and chromothripsis patterns were restricted to MM with poor prognosis.

SA-139
Deciphering the genomic heterogeneity in malignant melanoma by genomic profiling of clonal tumor populations

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Aims. Melanoma is a heterogeneous disease and even though consistent genetic patterns among different subtypes have been revealed, clinical trials have failed to significantly improve survival. However, most of the genomic studies do not take into consideration the underlying genomic heterogeneity. In this study, we used genomic profiling of sorted clonal tumor populations in order to investigate the genomic heterogeneity and the clonal evolution in malignant melanoma.

Methods. Multiple biopsies from melanoma patients were subjected to flow-sorting based on DNA content. The tumor specific marker SOX10 was used as an additional parameter to exclude contribution of normal tissue. Resulting clonal tumor populations were genomically characterized by usage of high resolution aCGH. A TMA was constructed for validation of the findings.

Results. DNA content based flow sorting of small human melanoma biopsies was successfully implemented and revealed the co-existence of distinct clonal populations (diploid as well as aneuploid) within these samples. The use of the additional marker SOX10 allowed us to uncover and sort pure diploid tumor populations from diploid fractions admixed with normal cells. These findings were confirmed by aCGH.

Conclusions. Human malignant melanoma is composed of different clonal populations with population-specific genomic aberrations. The use of SOX10 allowed unraveling of pure tumor populations within the diploid peaks, which would have been obscured by the use of DNA content alone. Further genomic analyses of these sorted clonal tumor

populations are fundamental for the understanding of the genomic heterogeneity and its potential impact on metastasis and therapy response in malignant melanoma.

SA-140
The correlation between blood vessels and bone formation after maxillary sinus augmentation

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Aims. Maxillary sinus augmentation (MSA) represents a predictable technique to successfully graft severe atrophic posterior maxilla prior to implant placement. For this, autologous bone, bone substitute materials or mixtures of both can be used. Independent of the used material, it is the goal to obtain a maximum amount of bone, while bone formation is related to a complex cascade of biological factors. Angiogenesis is thought to be directly linked to new bone formation. However, there are no studies which have evaluated the correlation between the blood vessel count and the amount of bone after MSA. In this study, the correlation between the count of blood vessels and the percentual distribution of bone as well as soft tissue was investigated after MSA with bone and bone substitute materials in ex vivo samples.

Methods. A total of 35 trephine samples were retrieved 5 months after grafting the maxillary sinus either with autologous bone (control, n=8), composites of autologous bone and beta-tricalciumphosphate (n=15, group A) or autologous bone and beta-tricalciumphosphate/hydroxyapatite (n=11, group B). The samples were processed according to standardized methods for histological (HE, Goldner) and immunohistochemical (anti-CD31 stain) examination. The count of vessels per sample was determined as well as the distribution of bone, substitute material and soft tissue. The data were plotted against the percentual distribution of bone and soft tissue and correlations were evaluated by means of a Spearman test.

Results. The mean distribution of soft tissue was the highest in group A (42.3%) and lowest in group B (32.2%). The mean distribution of bone was the highest in the control (27.8%), followed by group A (mean=24.2%). The lowest amount of bone was seen in group B (mean=23.5%). Most blood vessels (mean=53.8 vessels per sample) were counted in the control. Group A revealed 47.1 vessels per sample, the lowest amount (26.9 vessels per sample) was detected in group B. Altogether, the count of blood vessels significantly correlated with the amount of soft tissue in this study. No correlation could be found between the number of blood vessels and the percentual distribution of bone.

Conclusions. In this study angiogenesis mainly occurred in soft tissue independent from the grafted material used after MSA. Further studies are necessary to investigate the mechanisms of bone formation and the role of the blood supply in MSA.

SA-141
Head and neck manifestations of IgG4-related disease: a review on histopathological features and potential mimics

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Aims. IgG4-related systemic disease (synonyms: hyper-IgG4 disease, IgG4-related fibrosclerosis, etc.) is a recently defined emerging condition with a highly heterogeneous clinicopathological features and variable disease manifestations. This disorder is characterized by unifocal or multifocal (multi-organ) involvement by plasma cell-rich inflamma-

tory infiltrates associated with prominent fibrosclerosis that not uncommonly interferes with organ function resulting in diverse clinical symptoms and signs. Autoimmune pancreatitis represents the prototype of this disease. However, to date, diverse organs/organ systems have been reported to be involved by IgG4-disease including hepatobiliary system, lung, lymph nodes, salivary and lacrimal glands, mediastinum, retroperitoneum, kidney, pituitary and others.

Methods. We analyzed 72 histopathological specimens from the head and neck containing prominent lymphoplasmacytic infiltrates (15 obstructive sialadenitis, 27 inflammatory lesions of the oral cavity, 10 sclerosing sialadenitis/Küttner tumor, 7 dacryocystitis, 5 sinusitis/polyposis, 5 cervical lymph nodes and 3 inflammatory middle ear polyps) for numbers of IgG-positive plasma cells.

Results. High counts of IgG4 plasma cells were found in sclerosing sialadenitis (mean: 40 cells/hpf) contrasting sharply with the cases of sialadenitis caused by sialolithiasis (mean: 3 cells/hpf). Chronic dacryocystitis also showed high numbers of positively staining plasma cells for IgG4. Greatly varied but generally high counts of IgG4 positive plasma cells in excess of 70 cells/hpf were seen in inflammatory oral cavity lesions followed by plasma cell-rich inflammatory polyps of the sinonasal tract and middle ear and in plasma cell-rich lymphadenitis.

Conclusions. Our results demonstrate the common occurrence of high numbers of IgG4 positive plasma cells in diverse non-specific inflammatory lesions, indicating that high IgG4 positive plasma cell counts per se does not reliably distinguish IgG4-associated systemic disease from non-specific conditions. Thus IgG4 counts must be cautiously interpreted in the context of appropriate clinical and histopathological features of systemic IgG4-related diseases. The IgG4/IgG ratios may be more powerful in recognizing hyper-IgG4-disorders and their distinction for non-specific localized inflammatory lesions.

SA-142
Necrotizing granulomatous submandibular sialadenitis—uncommon initial manifestation of Wegener's granulomatosis

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Aims. Wegener's granulomatosis is a vasculitis of small vessels with development of zones of bionecrosis in regions of the respiratory tract (nose, paranasal sinus, ear, lung) and kidney. Particularly in paranasal sinus the diagnosis can be difficult because the small vessel vasculitis is difficult to identify and granulomas are likewise difficult to demonstrate.

Methods. Inhere we present the case of a 43-year-old male patient. The patient was admitted to the hospital for a recurrent painful swelling of the submandibular gland. Radiological imaging revealed a homogenous enlargement of the both submandibular glands with more pronounced changes in the right gland. There was no evidence of sialolithiasis or an abscess. Because antibiotic therapy had no influence on swelling and pain submandibulectomy was performed.

Results. Histologically the submandibular gland showed an intensive inflammation with total parenchymal destruction. The inflammatory infiltrate was mixed with areas of neutrophil and eosinophil abscesses and ill-defined granulomas. A conspicuous feature was the presence of multinucleated giant cells. Some of the blood vessels showed evidence of vasculitis. Further clinical investigations confirmed the diagnosis of Wegener's granulomatosis.

Conclusions. The initial, isolated manifestation of Wegener's granulomatosis in salivary glands is extremely rare, being limited in the literature to few case reports. The diagnosis may be challenging. The histologic key features are necrotizing mixed inflammation with occurrence of multi-nucleated giant cells in addition to vasculitis and/or granuloma formation.

SA-143
Hypophosphatemic rickets associated with an odontogenic tumor: a case report

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Aims. Oncogenic osteomalacia caused by hypophosphataemia has been linked with several distinct tumour types, particularly with the phosphaturic mesenchymal tumour, mixed connective tissue variant. Herein, we report a single case of a patient with multiple pathological fractures caused by hypophosphataemic oncogenic osteomalacia associated with an odontogenic tumour.

Methods. Our patient was a 48-year-old man who presented with multiple pathological fractures caused by vitamin D-resistant hypophosphataemic osteomalacia during the last three years. The thorough clinical work up involved laboratory tests and radiographic investigations, including PET-CT using radiolabelled somatostatin analogues. A small jaw tumour was found and resected and subsequently sent to our laboratory together with a trans-iliac bone biopsy.

Results. Microscopically, the tumour showed odontogenic epithelial proliferations embedded in cellular fibroblastic stroma without atypia and areas of cemento-osseous matrix consistent with a central odontogenic fibroma (previously the WHO type, currently the epithelium-rich type). Undecalcified bone biopsy showed moderate osteomalacia. Laboratory values of calcium/phosphate metabolism recovered within the first days after the surgery.

Conclusions. We report a case of systemic tumour-induced hypophosphataemic rickets associated with a small jaw tumour. Our case demonstrated that it is crucial to perform a meticulous clinical and radiographic examination to detect the causative neoplasm.

SA-144
71 cases of carcinoma ex pleomorphic adenoma: prognostic criteria of multistep tumour progression

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Aims. Previously we identified prognostic steps in the progression of pleomorphic adenoma (PA) to carcinoma ex PA (CEPA) including a threshold of 6 mm extracapsular invasion, distinguishing minimally invasive (favourable) and extensively invasive (unfavourable) CEPA. This proved to be prognostically more relevant than classical pT-stages. At this stage, little information is available on the relative proportion of cases in the different progression steps.

Methods. 71 CEPA cases (41 male, 30 female; 53 from primary and 18 from recurrent PA) were recruited from 5 centres and were analyzed according to diagnostic and prognostic criteria.

Results. 24 cases show pure intraductal (ID) CEPA, 11 minimally invasive CEPA, 36 extensively invasive CEPA. Altogether, 47 cases show an ID component, while all myoepithelial CEPA are devoid of an ID component. Invasive carcinomas are: undifferentiated (14), adeno NOS (8), salivary duct (7), myoepithelial (12), other types (7). Nine% of all CEPA,

but 67% of myoepithelial CEPA are low-grade. Myoepithelial and very large CEPA are often misdiagnosed.

Conclusions. CEPA is generally regarded to have a dismal prognosis. However, this is likely to be limited to advanced CEPA with more than 6 mm invasion. In our large series 49% belong to groups with favourable prognosis (pure ID and/or minimally invasive CEPA). Therefore, it is of paramount importance for prognosis and therapy to report these steps of progression. The frequent myoepithelial type of CEPA is a special type as it is often low-grade, has a relatively good prognosis, does not pathogenetically develop from an intraductal precursor and, in addition, is often misdiagnosed.

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FGFR1 expression rather than amplification is the crucial predictive marker for in-vitro sensitivity of HNSCC cells to FGFR small molecule inhibitor treatment

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Aims. Small molecule FGFR inhibitors are currently used in clinical trials in patients with FGFR1 amplified squamous cell cancers. In a recent study, we could detect the FGFR1 amplification in 15% of squamous cell head and neck carcinomas (HNSCC). Aim of our current study was to elucidate whether FGFR1 expression or amplification status is the crucial predictive marker for in-vitro sensitivity of HNSCC cells to FGFR small molecule inhibitor treatment.

Methods. We screened HNSCC cell lines for the genomic FGFR1 status using flourescence in-situ hybridization (FISH). Furthermore, we examined FGFR1 mRNA and protein expression using qRT PCR and Western blot, respectively. Subsequently, cells were treated with a small molecule FGFR inhibitor. We performed cytotoxity assays and assessed phosphorylation status of FGFR1 and downstream signalling targets before and after treatment. Moreover, cell proliferation upon inhibitor treatment was examined by Ki 67 staining.

Results. Two of the HNSCC cell lines (HN and SCC-25) harbored a FGFR1 low level amplification, one (584A2) harbored a polyploidy combined with a deletion of the FGFR1 gene locus and one (HSC-3) had a diploid FGFR1 status. Interestingly, 584A2 cells had the highest amount of FGFR1 mRNA, which correlated with high FGFR1 protein expression. Subsequently, cytotoxity assays pointed out that 584A2 was more sensitive to a small molecule FGFR-inhibitor treatment (IC50=5.8 μM) as compared to HN and SCC-25 cells, which both had an IC50 >10 μM. After treatment 584A2 cells showed a decrease in pFGFR1 and pErk and a reduced proliferative activity.

Conclusions. Our data suggest that high FGFR1 mRNA levels resulting in high protein expression rather than genomic FGFR1 amplification is the crucial predictive marker for response to targeted FGFR treatment in HNSCCs.

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Characterization of MAPK signalling activity in correlation to EGF receptor expression, KRAS/BRAF mutation status and tumour stage in adenoid cystic carcinoma of the salivary glands

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Aims. Salivary gland tumours are a rare and heterogeneous group of malignancies and account for about 1–3% of all neoplasms of the head and neck. The biology of this diverse tumour entity has been subject to research but has not yet been well understood. Although epithelial growth factor receptor (EGFR) pathway has been intensely studied, sufficient meaningful experimental data is still lacking regarding the role of the EGFR-dependent kinases. The aim of this study was to assess the role of EGFR/MAP kinase in signalling pathways of salivary gland tumours with a main focus on adenoid cystic carcinomas (ACC). Furthermore our goal was to identify abnormalities that could be linked to tumour characteristics and malignant behaviour.

Methods. The study cohort consisted of 68 paraffin-embedded samples of salivary gland tumours that had been submitted to the Institute of Pathology Military Hospital Ulm between 2005 and 2012: 35 ACCs, 8 mucoepidermoid carcinomas, 8 acinic cell carcinomas, 3 squamous cell carcinomas, 14 in a group of other histologic types. Expression of EGFR and C-Kit (CD117) was evaluated applying immunohistochemistry (IHC) while mutation status of KRAS and BRAF was tested by DNA-Sequencing. Finally, activity of MAPK signalling was assessed by IHC for phosphorylated ERK1/2. The study was approved by the ethical review committee of the Landesärztekammer Baden-Württemberg.

Results. 85% demonstrated positive EGFR IHC (1+ to 3+) while C-Kit was expressed in 81% regardless of entity. No correlation of T-stage and EGFR or C-Kit expression was found. Neither KRAS nor BRAF showed any activating mutations in our sample set. In 58% of the cases EGFR overexpression (2+/3+) was followed by enhanced MAPK signalling (1+ to 3+) as indicated by pERK1/2 IHC. In EGFR-negative ACC (20%), however, there was also strong immunopositivity for pERK1/2.

Conclusions. Overall, EGFR expression of the various tumour entities correlated with findings previously described in literature. In ACCs, however, there was no significant correlation between T-stage and EGFR expression (p=0.53) in comparison to literature findings. In 20% of ACCs activity of MAPK signalling was neither linked to EGFR overexpression nor KRAS/BRAF-mutation. These findings might explain the lack of value for EGFR IHC as a prognostic marker in ACC and point towards a different mechanism of MAPK pathway activation in these tumours, perhaps via the previously published neurotrophin-3 receptor (NTRK3) signalling.

SA-147

Are papillary microcarcinomas of the thyroid always a simple incidental finding? Preliminary results

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Aims. Papillary microcarcinomas (PMC) are very common, representing about 30–40% of all papillary thyroid carcinomas, often found incidentally. However, some show an aggressive behaviour with local recurrence and lymph node metastases in about 30% of cases. Our aim is to study both histological and molecular characteristics of PMCs with and without lymph node metastases.

Methods. A total of 28 cases of PMCs (9 with lymph node metastases), were retrieved from the pathology's archive of Klinikum Augsburg from 2004 to present. For each case BRAF V600E mutation status was

evaluated by PCR analysis. All cases were evaluated for the following histological parameters: histological subtype, presence of intratumoral fibrosis, superficial tumor location, intraglandular tumor spread/multifocality, presence of capsule and psammomabodies.

Results. The cases with lymph node metastases had a higher incidence of classical variant (89% vs. 42%), fibrosis (56% vs. 42%), superficial location (67% vs. 32%) intraglandular tumor spread/multifocality (78% vs. 19%) and presence of psammomabodies (33% vs. 10%) as those without metastases. None of the cases with metastases showed a capsule, whereas this was present in 21% of the other cases. The incidence of BRAF V600E mutation was almost identical (33% vs. 32%) for both groups. Interestingly, focusing on the presence BRAF mutation, intraglandular tumor spread/multifocality, fibrosis and superficial tumor location, we found that at least 3 of these features are present in 56% of cases with metastases as compared to 15% of cases without.

Conclusions. In this study we observed differences between PMCs with and without lymph node metastases. Some histological features such fibrosis, intraglandular tumor spread/multifocality, classical variant tumor type and superficial tumor location are frequently found in PMCs with lymph node metastases. On the other hand, the incidence of BRAF V600E mutations, usually associated with tumor recurrence and aggressive clinical behaviour, is very similar in the two cohorts. This finding may indicate that BRAF V600E mutation alone could not be a prognostic factor for lymph node metastases. A combination of histological and molecular features may differentiate between cases with and without risk for lymph node metastases.

SA-149
Differential diagnostic value of miRNAs in thyroid nodules

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Aims. MicroRNA (miR) expressions play important roles in multiple biological processes and are consistently found to be altered in thyroid tumors. For pathologists it is often a challenge to differentiate between overlapping subtypes, especially between follicular variants of papillary and follicular carcinoma of the thyroid. The current study explores whether miRNA expressions can be routinely used as an additional differential diagnostic tool in thyroid pathology.

Methods. Based on literature, three different TaqMan microRNA assays (miR-146b, miR-221 and miR-222) were used to determine the miRNA expression level by means of quantitative real-time PCR (Q-PCR). The miRNA strand was first reverse transcribed into its single stranded cDNA (complementary DNA) using the TaqMan MicroRNA Reverse Transcription Kit (ABI). miR-16 and U47 were as well as an Universal miRNA reference used as controls. The miRNA expression analysis was expanded to a series of 10 subtypes of benign and neoplastic thyroid lesions. The ΔCT value was calculated for each specimen.

Results. MiR-146b (cut-off ≤−0.285) shows highly increased expression levels in papillary thyroid variants and is predictable. miR-221 and miR-221 were not differently expressed in normal tissue versus adenomas, papillary and follicular carcinomas.

Conclusions. As compared to the literature miR-146b as well as miR-221 and miR-222 seem to play important roles in tumorgenesis of thyroid carcinomas. A close association is implicated between the elevated miR-146b in papillary thyroid carcinomas which could potentially be exploited and utilized as practical additional routine biomarker for differential thyroid cancer diagnosis, especially for follicular variants of papillary carcinoma.

Postersession Orthopädische und Hämatologische Pathologie

SA-151
Raise the positive rate of molecular diagnosis in B-NHL by FISH combined PCR-Genesca

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Aims. To analyse the immunoglobulin rearrangement types and characteristic of abnormal chromosomes of B-cell non-Hodgkin lymphoma (B-NHL) in Chinese population and provide evidence for the molecular diagnosis platform.

Methods. PCR-Genescan technique to investigate the clonal rearrangement of IGH gene, and Fluorescence in situ hybridization (FISH) to detect the structural alterations of IGH, BCL-2 and BCL-6 were performed in 45 cases of B-NHL.

Results. 1) Combined FR3A and FR2A primers, the clonal rearrangement rate was 61.6% in the 39 B-NHL cases by Genescan (17 monoclonal rearrangement and 7 biclonal rearrangement). 2) In the 35 DLBCL cases, chromosome break rates detected by IGH, BCL6 and BCL2 dual color apart rearrangement probes were 62.9%, 25.7% and 2.9% separately. The proliferation rate of BCL2 was 43%. 25% MALT1 break occurred in 8 cases of MALT lymphoma and IgH/BCL2 was detected in 1/2 follicular lymphoma. (3) The positive rate of molecular diagnosis for lymphoma raised from 61.5% to 81.8% by FISH combined PCR-GeneScan.

Conclusions. Conclusion FISH combined PCR-GeneScan improve the positive rate of molecular diagnosis in lymphoma.

SA-152
Tyrosine phosphatase SHP2 promotes a germinal center-like reaction in GC-derived B-NHL

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Aims. Some key molecular reactions in the germinal center GC have been concerned recently as a critical function in human B-cell lymphomagenesis. A fundamental role for Src-homology 2 domain-containing phosphatase 2 (SHP2) in GC reaction was investigated in human GC-derived B-NHL cell lines in order to elaborate molecular mechanism of SHP2 activity on the redifferentiation of GC-derived B-NHL

Methods. 4 stable Shp2-deletion cell lines (Daudi, OCI-LY6, Ramos, Su-DHL-5, which are available as target cell of GC-derived B-NHL with different conditions) were established. Rescue experiments were performed with pMSCV-neo-SHP2-WT.

Results. SHP2 knockdown in established GC-derived B-NHL cell lines blocks their growth and arrests G1 phase progression. However, the Shp2 knockdown-induced growth inhibition has been rescued with exogenous SHP2. SHP2 knockdown leads to a decrease in Erk1/2 phosphorylation concomitant with a reduction in phosphorylation of Src, whereas Stat3 and Akt phosphorylations are not affected. Similar to SHP2 knockdown in GC-derived B-NHL cell lines, R/E SHP2 protein also inhibits ERK1/2 and Src phosphorylation as well as cell proliferation. SHP2 inhibition leads to decrease in CD20 and CD77 expression in established GC-derived B-NHL, whereas CD38 and CD138 expression level are increased. A preliminary morphological observation under electron microscopy implies that rough endoplasmic reticulum increase in established SHP2 depletion GC-derived B-NHL. Concomitantly, protein levels of Bcl6, E2A, AICDA and Pax5 are reduced in these cells. But expression level of Blimp1 and XBP1 are increased. SHP2 knock-

down results in down-regulation of c-myc in assayed cells. We find that Src and Erk1/2 activities are essential for keeping constitutive GC phenotype in B cells.

Conclusions. SHP2 could regulate GC-derived B-NHL cells proliferation in vitro. Reduction of SHP2 expression leads to decrease in constitutive GC phenotype in GC-derived B-NHL. SHP2 also acts as key mediator that contributes to redifferentiation of GC-derived B-NHL cells trending to plasma cells. These results provide new insights into the signaling cascades influencing GC B cells redifferentiation direction as well as a rationale for targeting SHP2 in GC-derived B-NHL cells. Further researches could be conducted with both in vivo animal model of B-cell lymphomagenesis and lymphoma cases in order to narrow and confirm molecular targets associated with redifferentiation GC-derived B-NHL

SA-153

Bcl-2 expression combined with Chan's algorithm is the best tool to predict the outcome of Chinese diffuse large B-cell lymphoma

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Aims. To investigate the clinical significance of bcl-2 protein expression and two classification algorithms including Hans model and Chan model in patients with diffuse large B-cell lymphoma (DLBCL).

Methods. Two-hundred and thirty-seven cases were collected. Standard two-step EnVision method of immunohistochemical staining was used to assess the expression of Ki-67, CD3, CD45RO, CD20, CD79a, bcl-2, bcl-6, CD10, MUM-1, GCET1, and FOXP1. The phenotypic classifications were assessed according to the standard of the two models. The clinical data and follow-up data were collected for survival analysis.

Results. The male (131 cases) to female (106 cases) ratio was about 1.24:1, the average age was 52.6 years. Seventy-five cases (31.6%, 75/237) showed primarily lymph node involvement. Gastrointestinal tract (71 cases) was the most commonly involved extra-nodal organ. All cases expressed one or more pan B cell markers such as CD20 (99.1%, 231/233) and CD79a (81.7%, 85/104). The high expression of Bcl-2 protein was detected (61.5%, 139/226). All patients with complete clinical follow-up data survived from 1–120 months, the medial survival is 103.9 months. The expression of bcl-2 protein indicated an adverse prognosis, the medial survival of the positive group is 120 months, 62.2 months for negative group, p value is 0.019. Two-hundred and thirty cases were classified according to Hans model, with ninety five GCB cases and one-hundred and thirty five non-GCB cases, the ratio is 1:1.42. Survival analysis showed no difference between GCB and non-GCB subtypes (p=0.102) as listed in sheet 1. According to the Chan's algorithm, sixty eight case of one-hundred and eighty one were belong to GCB group, with one-hundred and thirteen non-GCB cases, the ratio is 1:1.66. GCB subtype showed much better prognosis than non-GCB subtype according to survival analysis (p=0.031). Additionally, the expression of bcl-2 protein showed higher in non-GCB group (67.0%, 71/106) than GCB group (50.0%, 32/64), and Life Table survival analysis showed the expression in non-GCB subtype showed the worst survival.

Conclusions. Non-GCB group is the more common type of DLBCL in China. High expression of bcl-2 protein is detected in the non-GCB group. Not all subgroups classified with different classification models indicate different prognosis. Bcl-2 expression combined with Chan's algorithm may be the best tool to predict outcome.

SA-154

Glucosylceramide synthase (GCS)-derived gangliosides in the membrane lipid microenvironment regulate leptin signaling in hypothalamic neurons

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Aims. Neurons of the hypothalamic arcuate nucleus (Arc) are main regulators of energy homeostasis. Neuronal function depends essentially on plasma membrane-located gangliosides. Our studies highlight a novel mechanism for central nervous system control of body weight.

Methods. We demonstrate that leptin signaling, a major regulatory circuit in hypothalamic control of body weight, depends on GCS-derived gangliosides in a combined in vivo approach using a mouse model featuring an inducible neuron-specific deletion of GCS (Ugcg f/f//CamK-CreERT2 mice) as well as in vitro in a mouse hypothalamic cell line.

Results. Upon leptin stimulation, gangliosides GD1a and GM1 form dynamic complexes with leptin receptors on the plasma membrane of hypothalamic neurons and initiate formation of intracellular signaling metabolites in vitro. These results are in line with well-known findings that gangliosides form complexes with neuronal Trk receptors upon receptor stimulation. Ugcgf/f//CamKCreERT2 mice display obesity, lower sympathetic outflow to peripheral tissues and lower lipid mobilization from peripheral fat stores. We have identified the Arc as an important mediator of body weight deregulation in these mice as Ugcg gene delivery to this hypothalamic nucleus significantly ameliorates obesity. In line with the in vitro data, Ugcgf/f//CamKCreERT2 mice show impaired hypothalamic leptin receptor signaling.

Conclusions. In summary, our studies highlight GCS-derived gangliosides as essential components in the lipid membrane microenvironment that activate leptin receptor signaling in hypothalamic neurons. These findings highlight the loss of GCS activity as a new pathological mechanism for the development of obesity.

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Establishment and characterisation of new Chordoma cell lines

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Aims. Chordoma is a tumour derived from chorda remnants which expresses early embryonic stem cell factors such as members of the sonic hedgehog pathway. We aimed at establishing new chordoma cell lines to define characteristic molecular patterns by gene expression profiling, thereby focusing on the sonic hedgehog pathway.

Methods. Gene expression profiling was done by Agilent Human GE 4x44K Microarray including 19,596 Entrez Gene RNAs. Selected targets were evaluated by PCR, Western blotting, immunocytology, and histology. We also examined effects of treatment with Vismodegib, the potent and specific SMO (smoothened, frizzled family receptor) antagonist and hedgehog inhibitor.

Results. We established three new chordoma cell-lines (U-CH3, U-CH6, U-CH7) in addition to the published chordoma cell-lines U-CH1, U-CH2, and MUG-Chor1. The cell lines highly expressed brachyury, EMA, S100, and vimentin at protein and RNA levels. The six cell lines clustered together when compared to 60 NCI cell lines (derived from carcinomas of breast, ovary, lung, kidney, prostate, and colon; melanoma, central nervous tumors, leukaemias). In all chordoma cell lines, so-

nic hedgehog (SHH) and smoothened (SMO) were highly expressed on RNA and protein levels. In addition, 30 chordomas showed a consistent expression of SHH and SMO in situ. Treatment of the cell lines with Vismodegib induced a senescent phenotype by morphology.

Conclusions. The new chordoma cell lines serve as models for further analysis of this tumour entity and should foster research for successful treatment strategies.

SA-156
BRAF mutation in Erdheim-Chester disease: a case report

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Aims. Histiocytoses are group of rare diseases with manifestations in the musculoskeletal system. Recently, BRAF mutations were reported in histiocytoses, including a group of Erdheim-Chester disease patients. Herein, we report a single case of Erdheim-Chester disease harbouring a BRAF mutation.

Methods. Our patient was a 35-year-old woman suffering from amenorrhoea, diabetes insipidus and knee pain for 5 months. We investigated using radiographs and laboratory test. Furthermore, a bone biopsy was performed and processed both undecalcified and following EDTA decalcification. Immunohistochemical analysis (CD1a, S100, CD163, langerin) was performed. PCR analysis was performed three years after the primary diagnosis.

Results. Radiographically, both femora showed periostitis with wavy contour of the outer margin, cortical thickening, and heterogenous osteosclerosis next to uninvolved epiphyses. Microscopically, trabecular sclerosis with focal hyperosteoidosis and bone marrow fibrosis with infiltration of foamy macrophages were apparent. Our diagnosis was Erdheim-Chester disease. Molecular analysis revealed a BRAF mutation.

Conclusions. Further studies of the pathogenic role of BRAF mutations in histiocytoses are necessary. Therapy for Erdheim-Chester disease with vemurafenib might possibly influence the outcome of the systemic form of this non-Langerhans cell histiocytosis.

SA-157
MYC gene amplification in soft tissue and bone tumours: an analysis of a single center with patients' survival

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Aims. MYC contributes to the pathogenesis of a majority of human cancers and yet strategies to modulate the function of the MYC oncoprotein are very popular. Recently it was shown that high level gene amplification is associated with postradiation or chronic lymphedema in angiosarcomas. In this study we analysed a well characterized cohort of soft tissue and bone tumours for MYC amplification and correlate the results with clinicopathological parameters including survival.

Methods. 160 soft tissue and bone tumours (40 undifferentiated pleomorphic sarcomas, 30 leiomyosarcomas, 16 synovialsarcomas, 20 liposarcomas, 22 angiosarcomas, 9 MPNST, 4 carcinosarcoma, 2 malignant solitary tumors, 3 chondrosarcomas and 14 other soft tissue sarcomas)

were analysed with fluorescence in situ hybridisation for MYC amplification with a centromere probe as reference.

Results. We found low level and high level amplifications in angiosarcomas (40.9%), MPNST (11.1%), leiomyosarcomas (6.6%), liposarcomas (5%), undifferentiated pleomorphic sarcomas (2.5%), and carcinosarcoma (25%). 80% of patients with MYC amplification had a history of radiation therapy. Angiosarcomas showed a significantly shorter survival compared to undifferentiated pleomorphic sarcomas (p=0.034), leiomyosarcomas (p=0.002), synovialsarcomas (p=0.005), and liposarcomas (p=0.003).

Conclusions. Angiosarcomas showed a significantly higher MYC amplification status than other sarcomas and these genomic alterations might lead to a shorter patients' survival. Not all sarcomas with MYC amplification are radiation induced. Given the emerging perspective that MYC has finally become a drugable cellular target, it might be important for angiosarcomas. Amplifications in chondrosarcomas or other sarcomas are a rare event.

SA-158
Neoadjuvant treatment improves capsular integrity and the width of the fibrous capsule of high-grade soft-tissue sarcomas

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Aims. Neoadjuvant treatment is thought to improve resection with margin-negative surgery in locally advanced soft-tissue sarcomas (STS). Treatment-induced alterations of the tumor periphery have not yet been microscopically evaluated. This histopathological study compared limb STS with primary resection and those that had undergone neoadjuvant treatment, emphasizing microscopic changes of the fibrous capsule (FC) and reactive zone (RZ) after neoadjuvant treatment.

Methods. Patients with primary high-grade limb sarcomas (N=76) which have not previously been treated were included. Of those, 37 were primarily resected and 39 were treated with one of the following neoadjuvant treatment modalities: 7× chemotherapy (CTX), 3× radiotherapy (RT), 15× isolated limb perfusion (ILP), 8× CTX+RT, and 6× CTX+ILP. Sizes of the FC and RZ were microscopically measured, and FC-integrity was documented. Histopathologic regression was expressed as a percent.

Results. Only 35.1% of untreated sarcomas showed an intact FC. We observed significantly higher capsular integrity after treatment (76.9%). Additionally, the average width of the FC (0.21 mm vs. 0.61 mm) and RZ (0.67 mm vs. 1.48 mm) increased significantly. The extent of histopathologic regression showed a correlation with capsular integrity and width. The combination of two treatment modalities (CTX+ RT or ILP) showed strongest effects at the tumor periphery.

Conclusions. Neoadjuvant treatment stabilizes the tumor periphery in STS (e.g., the capsule). Concerning local treatment strategies, these novel histopathologic insights might significantly influence the decision as to whether primary resection is advisable in advanced local soft-tissue sarcoma.

SA-159

Pathologic response of synovial sarcomas to hyperthermic isolated limb perfusion with melphalan and TNF-alpha

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Aims. Hyperthermic isolated limb perfusion with TNF-alpha and melphalan (TM-HILP) is a promising local treatment for primarily non-resectable soft tissue sarcomas (STS) of the limbs, which results in remarkable tumor responses and limb-salvage rates. The aim of the study was to investigate the STS subgroup "synovial sarcoma (SyS)" in terms of histopathological response to TM-HILP and to compare the results with clinico-pathological parameters of our complete collective of TM-HILP treated STS.

Methods. Resection specimens of a total 125 patients, including 14 SyS, in whom a TM-HILP was carried out between 2002 and 2012 were investigated. TM-HILP was performed under mild hyperthermia (39°C) and leakage monitoring. The dose of TNF-alpha was adjusted to 0.25 mg/L perfused tissue volume and melphalan to 11–13 mg/L. Regression was assessed by light microscopy as a percent of viable tumor after TM-HILP and additionally divided in responders/non-responders (cutoff 10% viable tumor) using the regression grading scale of Salzer-Kuntschik.

Results. The comparison of the SyS subgroup (n=14) with the complete STS cohort (n=125) revealed a significant higher number of distally located limb tumors (SyS 12/14, 85.7% vs. 48/125, 38.4%; p=0,001). Furthermore, we found a significant smaller tumor size of the SyS compared to all STS (mean size, SyS 6.2 cm vs. 10,1 cm; p=0,018). Percentage of regression (mean percentage, SyS 74.0% vs. 76.0%; p=0.972) or Salzer-Kuntschik responder/non-responder ratio (SyS 7/7 vs. 73/52; p=0.546) showed no divergent response to TM-HILP. By investigating the complete collective of 125 STS we were able to exclude a general discrepancy between proximal and distal tumor localization in regard to pathological response (mean, proximal 78.0% vs. distal 72.0%).

Conclusions. Treatment of STS is more and more dictated by the identification of histological subtype, in a family of uncommon cancers comprising more than fifty subtypes. In this regard published data concerning effectiveness and outcome of TM-HILP treated STS are based on mixed STS collections of common and rare STS entities. Because histology is now felt to be relevant not only for target therapies, but also for chemotherapy or preoperative induction therapies like the TM-HILP, we demonstrate that the STS subtype SyS shows comparable good results in terms of tumor regression after TM-HILP than previously reported for mixed STS cohorts.

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Histopathologic classification of neosynovialitis of endoprothesis with short-endurance-pathologies

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Aims. The so called consenus classification of SLIM can differentiate between infectious, particle-induced, functional und fibrotic (endoprothesis-associated Arthrofibrosis) pathologies. This classification was used with pathologies of endoprothesis with conspicuous short endurance. In this connection a new subtype could be differentiated.

Methods. The histologic preparations have been collected arthroscopically in the routine diagnostics of endoprotheses dysfunction. According to the criteria of the advanced consensus classification the HE-preparations were classified in above-named groups. 7 preparations of type I, 7 preparations of type IV and 16 preparations of a new type, which could not be assigned to another type, were immunhistologically stained with tenascin.

Results. Histopathologic there were 16 cases of bacterial infection, 11 particle-induced cases, 15 cases of endoprothesis-associated arthrofibrosis and 10 cases of the indifferent type. 28 cases could not be defined according to the Classification: histopathologic there were similarities to type IV neosynovialitis. Necrosis and Fibrininsudations were added. Immunhistological a distinct Tenascin-Expression was seen.

Conclusions. As tissue Tenascin expression was seen as a marker of tissue remodeling, the high Tenascin expression in SLIM could be interpreted as an expression of tissue remodeling around the prothesis. Possibly micro-fractures with reparative changes around the prothesis (necrosis) are accountable for the new subtype. By using laser scanning (LSM) or more markers of tissue remodeling the meaning of tissue remodeling processes in the pathogenesis of endoprothesis dysfunction in particular in patients with early dysfunction should be clarified. Maybe the new subtype is an early form of arthrofibrosis.

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Histopathologic wear algorithm

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Aims. Particle-induced inflammation reactions around the endoprothesis are an essential limiting factor for the endurance of endoprothesis. Besides the cost-intensive and device-related elaborative EDX- and FTIR methods the histopathology of conventional HE-stained preparations gives a contemporary and cheap possibility for wear-particle characterisation in the tissue around the endoprothesis (SLIM).

Methods. An algorithm (particle algorithm) is submitted, which facilitates a particle-characterisation by using polarisation-optic analysis, Berliner-Blau-Reaction and Ölrot-Staining in the HE-preparations.

Results. According to the algorithm chemically and mechanically unfixed wear-particle of POL+ and POL− can be identified. Metallic no-iron particle (e.g. Titan, Cobalt, Nickel etc.), metallic no-iron particle of metall-metall-tribological pairing, PMMA- and PE-Particle, ceramic, aluminiumoxide- and zirconium oxide particle can be differentiated. Haemorrhage residua and endogenous crystal depositions (e.g. CPPA) come into consideration as no-wear particle.

Conclusions. Using the above mentioned light microscopic and enzyme histochemical criteria a wear/particle characterisation corresponding to the suggested algorithm is possible. In particle corrosion and particularly in metall/metall-tribological pairing necrosis and lymphozytic infiltration can appear, which can be evaluated as a particle-associated inflammation pattern (toxic particle overload, allergic type IV reaction?). By using this particle algorithm a simple mode for wear particle/particle identification in dysfunction of endoprothesis is given.

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Different expression of miRNAs in synovial tissue of patients with rheumatoid arthritis and osteoarthritis

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Aims. Rheumatoid arthritis (RA) is a common systemic autoimmune disease with unknown etiology, heterogeneous clinical presentation and disease course. Currently, diagnosis is done following the ACR criteria and a limited number of laboratory tests (anti-citrullinated protein antibodies, rheumatoid factor) that are not accurate enough, leading often to delayed diagnosis and irreversible joint damage. In tissue, only a few cases can be clearly diagnosed as RA (rheumatoid nodules). Recent works have demonstrated the emerging role of microRNA expression in autoimmune diseases and rheumatoid arthritis. In this study, the quantitative expression of selected miRNAs is examined with qPCR to discern RA from osteoarthritis (OA) in tissue biopsies.
Methods. Five potential miRNAs candidates and two endogenous reference genes were used. The training set, containing cases of RA (n=30) and OA (n=30), was determined by two pathologists. Total RNA was isolated from FFPE tissue and cDNA was synthesized by reverse transcription followed by quantitative real time polymerase chain reaction (two-step RT-qPCR). Input was normalized with the delta-delta CT-method against the endogenous reference gene and the relative expression was determined by fold-change against a reference pool of OA.
Results. Three miRNAs, miR-146a, miR-155, miR-223, and one endogenous reference gene, snRNA-U6, were chosen for analysis. A minimum of two-fold expression of the selected miRNAs among the RA cases compared to the OA pool could support the diagnosis of RA.
Conclusions. Early diagnosis and treatment of RA is essential to prevent irreversible joint damage. Histopathological diagnosis of RA has several limitations. It was clearly shown that RA could be distinguished from OA by determination of miRNA gene expression in FFPE tissue biopsies. This molecular approach offers a quantitative and objective test to support diagnosis of RA. Differential miRNA expression has impact on gene expression and disease course and these miRNAs represent potential biomarkers for patients with RA and OA.

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MALDI imaging mass spectrometry to investigate compartments of synovial tissue of patients with rheumatoid arthritis

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Aims. Rheumatoid arthritis (RA) is an autoimmune disease with proliferation of lymphocytes, fibroblasts and synovial lining causing cartilage and bone destruction. The etiology and pathogenesis of this disease are still unknown. Early and precise diagnosis is mandatory to choose the appropriate therapy. Diagnosis of RA is made according to the (American Congress of Rheumatology) ACR-criteria. Personalized medicine requires exact description of synovial pathology to target the appropriate cell population in various RA subtypes or stages. The aim of this study is to identify differences in peptide patterns in two compartments of synovial tissue by MALDI TOF Mass Spectrometry (MS) profiling and imaging.
Methods. Patients were classified according to the American College of Rheumatology (ACR) criteria for RA. Adjacent serial FFPE sections were stained with H&E and were reviewed by a pathologist. Areas of interest (synovial lining and sublining layer, 10 discrete areas per compartment) were marked digitally and the histology annotated images were merged with a photomicrograph of the section taken before the MALDI MS measurement. For MALDI MS analysis, trypsin and matrix solution were applied to discrete regions of interest using a robotic spotter. Pixel coordinates of these areas were transferred to a mass spectrometer for spectral acquisition in reflector mode. Generated data were then subjected to biocomputational analysis to reveal biomarker candidates. In total, 20 tissue sections were analyzed. Some tissue sections were chosen for MALDI MS imaging of the whole tissue sections to show the distribution of the m/z values of interest.
Results. Various m/z values representing different peptide profiles were demonstrated in the two tissue compartments. Several peptides were found to be expressed at higher levels in the synovial lining compared to the sublining layer, with a 99.5% confidence level and a 1.7-fold change. Other peptides were more pronounced in the sublining areas.
Conclusions. We clearly show different peptide patterns in synovial lining layer and sublining areas by MALDI MS profiling and imaging. Further studies are required to provide greater statistical power and to identify subtypes or different disease stages that are directly applicable to personalized medicine.

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Imaging mass spectrometry (IMS) approach for the assessment of degeneration of meniscus

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Aims. Exact assessment of meniscus structure degeneration is not possible because of interobserver variability even if histochemical methods are included in the diagnostic procedure. For this we have applied imaging mass spectrometry to support objectivity of meniscus diagnostics.
Methods. 20 FFPE-tissues of meniscus with areas with low degeneration and strong degeneration were included in this study. Additionally, frozen tissue biopsies were collected to identify proteins involved in meni-

scus degeneration. All patients gave informed consent; the study design was approved by the local ethics committee. The grade of degeneration was assessed by two pathologists. Consent could be obtained only when a two grade system with low grade and high grade degeneration was applied. Trypsin and matrix deposition onto FFPE and matrix deposition onto fresh frozen biopsy specimens was carried out using the Image-Prep device (Bruker, Bremen) followed by subsequent matrix-assisted laser desorption/ionization (MALDI) analysis using a Bruker Autoflex Speed in profiling and imaging mode. Statistical analysis was done using ClinProTools 3.0. Each analysis was performed as an independent experiment to ensure statistical independence.

Results. Statistical analysis generated significantly different signals between the two areas of interested in the FFPE-meniscus tissues. 6 peptide ion signals were sufficient to discern low grade (or normal) from high grade degeneration areas. 3 signals were present in normal meniscus and low degeneration areas and 3 signals strongly expressed in areas with high grade degeneration.

Conclusions. MALDI MS technology can discriminate between high grade and low grade meniscus degeneration, either in profiling or in imaging mode. Further studies are required to discriminate old and fresh ruptures as an objective measure to answer questions of clinicians and insurances.

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Sinonasal carcinomas without intestinal differentiation: proof of concept of wood dust unrelated development

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Aims. After occupational exposure to wood dust, sinonasal carcinomas are typically of intestinal type. However, other types of carcinoma are observed and therefore the question whether there is a relation to the employment remains to be discussed.

Methods. From I/1999–10/2012 carcinomas of the inner nose of 397 patients with exposure to wood dust of more than two years were investigated histologically and immunhistochemically. Regularly, antibodies were used to CK7, CK20, CDX2 and Synaptophysin. Optional CK5, p63, Chromogranin, CD117, BRST2, Mammoglobin, MUC2, MUC5, HMB45 a. o. were applicated.

Results. 325 of the 397 carcinomas (82%) exhibited an adenocarcinoma of intestinal type mostly as tubular-papillary subtype of G2. Grade 1 carcinomas were rare. Grade 3 cases revealed some tumor cells with CDX2 or CK20 positivity at least. 72 carcinomas (18%) showed no differentiation of intestinal type. Undifferentiated large cell carcinomas (<3%) and lymphoepithelial carcinomas (<1%) were rare.

Conclusions. The definition of the occupational disease BK4203 demands only the diagnosis adenocarcinoma without the limitation to the intestinal type. However, the rarity of different types of non-intestinal adenocarcinomas in our very large collective of wood dust exposure cases indicates no close relationship to occupational work. On the other hand, it is not to be excluded that undifferentiated carcinomas are an anaplastic phase of primarily intestinal differentiated adenocarcinomas. Therefore, in every unusual case an extensive immunhistochemical investigation is necessary to detect CK20 or CDX2 positive relicts.

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Zusammen mit einem **Lebenslauf und einer Publikationsliste** reichen Bewerber ihre Arbeit ein. (Bitte alle Unterlagen in doppelter Ausfertigung sowie elektronisch einreichen!)

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