



### 3. Berliner Woche der Pathologie

## 94. Jahrestagung der Deutschen Gesellschaft für Pathologie e.V.

in Zusammenarbeit mit dem  
**Bundesverband Deutscher Pathologen e.V.**

in Zusammenarbeit mit der  
**Internationalen Akademie für Pathologie, Deutsche Abteilung e.V.**

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- Mammapathologie
- Hämatopathologie

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94. Jahrestagung der Dt. Ges. f. Pathologie e.V.

Berlin, 27. – 30. Mai 2010

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Institut für Pathologie, Medizinische Hochschule Hannover

## 3. Berliner Woche der Pathologie

### 94. Jahrestagung der Deutschen Gesellschaft für Pathologie e.V.

in Zusammenarbeit mit dem  
**Bundesverband Deutscher Pathologen e.V.**

in Zusammenarbeit mit der  
**Internationalen Akademie für Pathologie,  
Deutsche Abteilung e.V.**

**Berlin, 27.–30. Mai 2010**

*Sehr geehrte Damen und Herren,  
liebe Kolleginnen und Kollegen!*

*Aufbruch in die Genompathologie* – unter diesem Motto stand die Jahrestagung 2003 der Deutschen Gesellschaft für Pathologie (DGP). Seitdem haben methodischer Fortschritt und molekulare Forschung unserem Fach einen atemberaubenden Wissens- und Kompetenzzuwachs beschert. Die kommende Jahrestagung in Berlin vom 27. bis 30. Mai 2010 will eine Zwischenbilanz ziehen und sichtbar machen, was in der *molekularen Tumorphathologie* an der Schwelle zur Anwendung in der klinischen Pathologie steht und ihre Aussagen zur Tumorklassifikation, Graduierung, Prognose und Prädiktion erweitern und präzisieren wird oder bereits kann. Wo stehen wir im Jahr 2010?

Beim *Mammakarzinom* ist die molekulare Tumorphathologie bereits in die diagnostische Sphäre eingedrungen; in den USA werden mittlerweile 60% aller nodal negativen Östrogenrezeptor-positiven Mammakarzinome mit einem Genexpressionstest auf ihre Progressionswahrscheinlichkeit hin untersucht und die Entscheidung für oder gegen eine Chemotherapie daran ausgerichtet. Dieser so genannte „Recurrence Score“ wird angeboten und ausgeführt von Ge-

nomie Health, dessen leitender Pathologe R. Baehner neue Ergebnisse, auch zu anderen Organtumoren, vorstellen wird. Ob ein molekulares Grading schon heute das Potenzial hat, prognostische Aussagen genauer als die traditionelle Pathologie zu treffen, wird von J. Reis-Filho, London, und A. Schneeweiss, Heidelberg, diskutiert werden. Inwieweit die Molekularpathologie bei der schwierigen Einordnung von Vorläuferläsionen hilfreich sein kann, wird von F. Moynfar, Graz, beleuchtet werden. Schließlich sollen wichtige Fragen der Prädiktion und Prognostik zur Diskussion gebracht werden, wie die sicherste Bestimmung des Her2-Targets, durch In-situ-Hybridisierung oder Immunhistochemie, wozu G. Sauter, Hamburg, und J. Rüschoff, Kassel, Stellung beziehen werden. Die biologische Signifikanz von disseminierten Tumorzellen und Mikrometastasen wurde in einer großen Studie in Holland untersucht, die von J. van Diest vorgestellt werden wird.

Abgesehen von der Präzisierung diagnostischer Aussagen sind die molekularen Forschungsergebnisse nicht minder von Bedeutung für ein verbessertes pathogenetisches Verständnis von Neoplasien. Kaum ein Organtumor ist zu nennen, zu dem nicht eine Fülle von molekulargenetischen Forschungsbefunden vorliegt. Ne-

ben einer Auswahl der besten aktuellen Forschungsbeiträge zur molekularen Tumorphathologie wird es daher ein Reihe von Übersichtsreferaten von namhaften Vertretern unseres Faches zu den wichtigsten Organtumoren geben, die nach Art eines „Up-date“ den aktuellen Wissensstand unter besonderer Berücksichtigung der Belange der klinischen Pathologie zusammenfassen und würdigen werden. Thematisch stehen dabei das Kolonkarzinom (T. Kirchner, München), das Pankreaskarzinom (A. Tannapfel, Bochum), das Lungenkarzinom (I. Petersen, Jena), das Prostatakarzinom (A. Hartmann, Erlangen), das Urothelkarzinom (R. Knüchel-Clarke, Aachen), das Nierenzellkarzinom (H. Moch, Zürich), die Karzinome der Schilddrüse (K. Schmid, Essen) und Weichteiltumoren (R. Büttner, Bonn) im Vordergrund.

Besonders erfreulich ist, dass sehr viele hoch qualitative Beiträge zur molekularen Pathologie angemeldet worden sind und die erfolgreiche Forschung der deutschsprachigen Pathologie in diesem Bereich eindrucksvoll dokumentieren. Von besonderem Interesse für den wissenschaftlichen Nachwuchs wird ein Forum mit den Hauptdrittmittelgebern – Deutsche Forschungsgemeinschaft (DFG), Deutsche Krebshilfe und Bundesministerium

für Bildung und Forschung (BMBF) – sein, das von P. Schirmacher, Heidelberg, organisiert werden wird.

Zu den ersten Organtumoren, in denen die Genompathologie ihr Potenzial unter Beweis zu stellen versuchte, gehörten die Leukämien und Lymphome sowie das Mammakarzinom. Zu diesen beiden weiteren Hauptthemen der Jahrestagung werden aktuelle Forschungsbeiträge präsentiert, und es werden Übersichtsreferate gehalten, wobei das großzellige B-Zell-Lymphom, der Morbus Hodgkin, das Plasmozytom und die myeloproliferativen Neoplasien in der *Hämatopathologie* thematisiert werden. Hierzu werden u. a. Beiträge von M. Hansmann, Frankfurt, P. Möller, Ulm, A. Rosenwald, Würzburg, und F. Fend, Tübingen, erwartet. Welchen Stellenwert die „Genompathologie“ in der Hämatopathologie tatsächlich heute hat, soll erörtert und diskutiert werden.

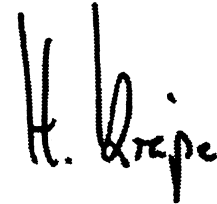
Den Arbeitsgemeinschaften, die um die AG Lungenpathologie und Uropathologie angewachsen sind, wird mehr Raum

gegeben, um auch den spezielleren Themen und Befunden ein Forum zu bieten. Als weitere Arbeitsgemeinschaft soll anlässlich der kommenden Jahrestagung die AG „Molekulare Pathologie“ gegründet werden, die sich insbesondere mit den methodischen Aspekten dieses rasch wachsenden Arbeitsgebietes in der Pathologie auseinandersetzen wird. In zwei gesonderten Sitzungen werden neue Methoden der Gensequenzierung und epigenetischen Diagnostik erörtert. Auch die so genannte „Keynote-Lecture“ fällt in diesen Bereich, in dem P. Lichter, Heidelberg, das „Humane Krebssequenzierungsprojekt“ vorstellen wird.

Die Jahrestagung ist eingebettet in die dritte Berliner Woche der Pathologie, die neben der DGP auch von der *Internationalen Akademie für Pathologie (IAP)* und dem *Bundesverband Deutscher Pathologen* mitbestritten werden wird. Die IAP wird Mikroskopiekurse zur Lymphadenitis, zur Her2-Diagnostik und Mammakarzinomvorstufen anbieten. Der Bundes-

verband tagt am Samstag und Sonntag in denselben Räumen und wird aktuelle Probleme und berufspolitische Fragestellungen thematisieren.

Die Deutsche Gesellschaft für Pathologie freut sich darauf, Sie in Berlin begrüßen zu können.



Prof. Dr. Hans H. Kreipe  
Kongresspräsident

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Hier steht eine Anzeige.

# 94. Jahrestagung der Deutschen Gesellschaft für Pathologie e. V.

gemeinsam mit dem  
**Bundesverband Deutscher Pathologen e.V.**  
 und der **Internationalen Akademie für Pathologie,  
 Deutsche Abteilung e.V.**

im Rahmen der 3. Berliner Woche der Pathologie

**Berlin, 27.–30. Mai 2010**

## Sitzung: AG Gastrointestinale Pathologie

### Vorträge: Oberer GI-Trakt

#### Do-001

#### **EGFR, HER2 and GRB7 expression are differentially expressed in esophageal squamous cell carcinoma versus Barrett's adenocarcinoma**

I. Kohler, A. Schöpflin, Tang L<sup>1</sup>, J. Neubauer, O. Opitz<sup>2</sup>, H. Geddert<sup>3</sup>, U. Hopt<sup>4</sup>, G. Faller<sup>3</sup>, D. Klimstra<sup>1</sup>, M. Werner, S. Lassmann

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**Aims:** To investigate protein expression of EGFR, HER2 and GRB7 in esophageal squamous cell carcinomas (ESCC) and Barrett's adenocarcinomas (BAC) as well as to examine the effect of GRB7 inhibition in ESCC and BAC cell lines.

**Methods:** Pretreatment biopsies of ESCCs (n=49) and BACs (n=61) were stained for EGFR, HER2 and GRB7 (immuno-histochemistry). ESCC (OE21, Kyse-410) and BAC (OE19, OE33) cell lines were analyzed for EGFR, HER2 and GRB7 at DNA (Q-PCR, FISH), mRNA (Q-RT-PCR) and protein (Western blot, Immunofluorescence) levels. GRB7 was inhibited by siRNA and effects on downstream signalling were examined (Western blot).

**Results:** Strong membranous EGFR and HER2 expression occurred in ESCC (p=0.072) and BAC (p<0.001), respectively. Strong GRB7 expression was linked to BACs (p=0.042) and HER2 expression (p=0.0053). HER2 and GRB7 were co-amplified and co-expressed in BAC cell lines. Full down-regulation of GRB7 by siRNA reduced phosphorylation of AKT in Kyse-410 cells. In contrast, weaker GRB7 down-regulation in BAC cells (OE33, OE19) was linked to Cyclin-D1 and Survivin reduction. **Conclusions:** The distinct expression patterns EGFR, HER2 and GRB7 in ESCCs versus BACs may differently affect ESCCs and BACs cellular responses to EGFR-, HER2 and/or GRB7-targeted inhibition.

#### Do-002

#### **Phenotypic and genotypic characterization of the side population of gastric cancer cell lines**

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**Aims:** The side population (SP) of tumor cell lines share characteristics with tumor stem cells. In this study we phenotypically and genotypically characterized the SP of gastric cancer cell lines.

**Methods:** SP cells were obtained from MKN45- and AGS-gastric cancer cells using Hoechst 33342 staining and fluorescence-activated cell sorting (FACS).

SP cells were subsequently studied morphologically (cytology, immunocytochemistry), on the transcriptional level (gene array) and in cell culture (recultivation assays). Genes differentially expressed in the SP cells were finally searched by immunohistochemistry in neoplastic and non-neoplastic gastric tissue from gastric cancer patients.

**Results:** SP cells were reproducibly obtained from gastric cancer cell lines. The SP cells were smaller and rounder than non-SP cells. SP cells self-renewed in re-cultivation experiments and differentiated into SP- and non-SP cells. Recultivated SP- and non-SP cells showed distinct phenotypes in culture regarding cell shape and colony-formation. SP cells had increased levels of the stem cell markers CD133 and Musashi 1. Transcriptional analyses demonstrated that SP cells express genes that encode for stem cell properties like FZD7, HEY1, SMO and ADAM17. Finally we detected the transcripts of these genes in tissue samples from patients with gastric cancer.

**Conclusions:** Human gastric cancer cell lines enclose a phenotypically and genotypically distinct cell population with tumor stem cell features. Phenotypical characteristics of this distinct cell population are also present in gastric cancer tissue.

#### Do-003

#### **Promotor methylation in gastric cancer and its impact on differentiation, proliferation and tumour antigenicity**

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**Aims:** We aimed to identify and functionally analyse new methylation-regulated genes in gastric carcinogenesis.

**Methods:** Four gastric carcinoma cell lines (AGS, KATOIII, 23132, HGT-1) were screened before and after 5-AZA-treatment in a whole genomic mRNA expression assay (Affymetrix Assay U132 2 Plus). Genes showing more than 3-fold expression changes in at least three cell lines were defined as highly regulated genes. First promoter studies were performed in selected genes e.g. CDX1 via EMSA and CHIP-assays. The methylation profile was verified along the gastritis-metaplasia-carcinoma-sequence on human FFPE-material by Pyrosequencing.

**Results:** The 25 newly identified candidate genes regulated by methylation in gastric cancer have been subdivided into three functional groups (tumour differentiation, proliferation, tumour antigenicity). Single genes showed a complex methylation profile along the gastritis-metaplasia-carcinoma-sequence. Direct regulatory interactions between signalling cascades, e.g. the NFκB pathway and promoter methylation, have been found.

**Conclusions:** Promotor methylation along the gastritis-metaplasia-carcinoma-sequence influences relevant aspects of carcinogenesis like differentiation, proliferation and tumour antigenicity. Complex methylation patterns including hyper- and hypomethylation could be observed as well as functional interactions between promotor methylation and cell signalling in gastric cancer.

**Do-004****HER2 expression in gastric cancer: rare, heterogeneous and of no prognostic value conclusions from 924 cases of two independent series**W. Müller, S. Sivakumar<sup>1</sup>, S. Gray<sup>1</sup>, H.E. Gabbert<sup>2</sup>, H.Grabsch<sup>1</sup>

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**Aims:** Patients with gastric cancer (GC) have a poor survival and biologicals such as Trastuzumab have not been used routinely. Studies in breast cancer indicate that response to Trastuzumab depends on membranous HER2 over-expression or HER2 amplification. Existing data on the clinical relevance of HER2 expression in GC are still limited and controversial.

**Methods:** HER2 expression was investigated by immunohistochemistry using full sections from 418 GC from Germany and tissue microarrays constructed from 506 GC from England. The „DAKO score“ and a continuous percentage scale were used for scoring. Results were compared to clinicopathological parameters and patient survival.

**Results:** HER2 expression showed marked intratumoural heterogeneity. Using a 5% cut off, 6% (n=57) of all GC were classified HER2 positive and 91% of these were intestinal type GC. In both series, no relationship was found between HER2 expression, patient survival or TNM stage.

**Conclusions:** This is the largest study to date demonstrating in two independent series that HER2 expression is not related to GC patient prognosis and that only a very small subgroup of intestinal type GC may potentially respond to HER2 targeting therapy. In contrast to breast cancer, due to prominent heterogeneity of HER2 expression in GC HER2 testing in endoscopic biopsies before treatment will be prone to false negative results.

**Do-005****Histopathological tumor regression after neoadjuvant chemotherapy in adenocarcinomas of the upper gastrointestinal tract: findings from a 20 years-experience**R. Langer, A. Novotny<sup>1</sup>, D. Reim<sup>1</sup>, K. Ott<sup>2</sup>, M. Feith<sup>1</sup>, H. Höfler, K. Becker

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**Aims:** We reviewed a large series of 589 upper gastrointestinal (GI) adenocarcinomas treated by neoadjuvant chemotherapy (CTX) between 1989 and 2009, which all were consistently worked up and evaluated by a standardized tumor regression grading system (TRG) in our institution.

**Methods:** 108 Esophago-gastric Adenocarcinomas (EA), 175 Adenocarcinomas of the esophago-gastric junction (AEJ) and 306 gastric carcinomas (GC) treated by a cisplatin/5FU based CTX were included. TRG was determined using a four tiered system based on the estimation of the percentage of residual tumor in relation to the previous tumor bed.

**Results:** In total, 25 patients (4%) had a complete tumor regression (TRG1a), 111 patients (19%) had a subtotal regression (TRG1b: 1–10% residual tumor). Partial tumor regression (TRG2: 11–50%) was observed in 151 cases (26%), 302 patients (51%) had minimal or no regression (TRG3: >50%). Tumor regression was significantly associated with posttreatment ypT, ypN, ypL category, R-status and survival (p<0.001). Some characteristics relating to the tumor site were, in particular, the higher frequency of total or subtotal tumor regression in EA and AEG (p<0.001) and the association of pretherapeutic tumor grading and Lauren's classification with tumor regression (p=0.001) in GC.

**Conclusions:** We present our 20 years experience of a highly standardized evaluation of upper GI adenocarcinomas after neoadjuvant CTX. Our findings reveal highly objective and prognostic relevant informations from a very large collective of these tumors.

**Do-006****Large scale array-based methylation analysis in GISTs reveals differences according to anatomical localisation and mutation type, and correlates with tumor progression**F. Haller, D. Zhang<sup>1</sup>, Ö. Sahin<sup>1</sup>, B. Korn<sup>1</sup>, A. von Heydebreck<sup>2</sup>, L. Füzesi<sup>3</sup>

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**Aims:** Only few genes have been analysed for methylation status in GIST so far, with methylation of the CDKN2A locus at 9p21 encoding for p16INK4A being a prognostically unfavourable and frequent event.

**Methods:** The methylation status of 800 oncogenes and tumor suppressor genes was analysed using the Illumina Golden Gate platform in 96 GISTs with different mutation types, from different anatomical localisations, and also in metastases.

**Results:** Several genes were found to be methylated significantly differently according to anatomical localisation, mutation status, or in progressive GISTs and metastases.

**Conclusions:** Demethylation of oncogenes, as well as hypermethylation of tumor suppressor genes, are important and frequent genetic events in GISTs, and may contribute to the well known differences associated with anatomical localisation and mutation status. Moreover, these regulatory events may also contribute substantially to tumor progression and metastasis in GISTs.

**Do-007****„Was ist neu?“****Europäische Leitlinie „Kolorektales Karzinom“**

M. Vieth

Bayreuth

**Do-008****„Was ist neu?“****Aktualisierte S3-Leitlinie „Colitis ulcerosa“**

G. Baretton

Dresden

**Do-009****„Was ist neu?“****Biopsische Diagnostik der nicht-alkoholischen und alkoholischen Steatohepatitis (NASH und ASH)**

A. Tannapfel

Bochum

**Do-010****„State of the art“ – Vortrag****Zystische Pankreasläsionen – pathomorphologische Diagnostik und klinische Relevanz**

J. Lüttges

Saarbrücken

## Vorträge: Unterer GI-Trakt

### Do-011

#### Identification of new predisposition genes in patients with adenomatous polyposis by genome-wide high-throughput approaches

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**Background:** In up to 70% of patients with hereditary gastrointestinal polyposis syndromes no germline mutation in the known predisposing genes can be identified, so far. However, the uncovering of the underlying genetic cause is essential for differential diagnosis, risk prediction, predictive testing of asymptomatic relatives, and surveillance recommendations.

**Current research project:** The Institutes of Human Genetics and Pathology of the University Hospital Bonn, both part of the Comprehensive Cancer Center Bonn-Cologne, have a long-standing and successful engagement in the study of hereditary gastrointestinal tumour syndromes in particular HNPCC and adenomatous polyposis. In a current research project, funded by the Deutsche Krebsstiftung, we focus on the identification of new predisposition genes in mutation-negative adenomatous polyposis by applying up-to-date whole-genome approaches such as SNP-array technologies including genome-wide association (GWA) and copy number variation (CNV) analyses, next generation sequencing, and other novel techniques to a large and well-characterised sample of around 300 unrelated patients.

**Tumour tissue analysis:** To successfully perform this challenging enterprise a comprehensive procedure is essential which includes not only the analysis of leukocyte DNA but also tumour tissue regarding histologic subtypes, immunohistochemical features, and molecular genetic profiling. Hence, the collection of paraffin-embedded adenoma tissue is an integrated and important part of our present and subsequent research projects.

**Conclusions and appeal:** We present preliminary results of our genome-wide screening project that will generate a huge amount of genetic data in polyposis patients and hopefully provides new valuable molecular insights into etiology and pathogenesis. However, to enable high-quality molecular research in rare conditions a multidisciplinary cross-linked approach and long-term storage of biomaterial is crucial. We ask the German pathology institutes to support our efforts in building up a tumour tissue collection for rare hereditary polyposis syndromes.

### Do-012

#### Aurora-A inhibition causes down-regulation of Aurora-B in microsatellite-unstable colorectal cancer carcinoma cell lines

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**Aims:** In this study, we examined the effect of Aurora-A inhibition on the chromosomal passenger protein Aurora-B in chromosomal- (CIN) and microsatellite- (MIN) unstable colorectal cancer (CRC) cell lines.

**Methods:** Aneuploid CIN-(HT29, CaCo-2) and diploid MIN-(HCT116, DLD1)-type CRC cell lines were analyzed for Aurora-B DNA copy number (FISH), mRNA (Q-RT-PCR) and protein expression (Western blot, immunofluorescence). Cells were either treated with Aurora-A-specific or unspecific siRNAs or were left untreated (medium only). Untreated and treated cells were investigated for Aurora-B mRNA/protein expression and localization.

**Results:** Aurora-B DNA copy numbers were normal (n=2) in all cell lines. In untreated cell lines, Aurora-B was highly expressed, except in CaCo-2 cells. Aurora-A inhibition induced a marked down-regulation of Aurora-B mRNA (HCT116 cells, p=0.001) and protein (HCT116, DLD-1) expression. Also, in all cell lines, Aurora-A inhibition arrested mitoses in prometaphase, which showed DNA ring structures with central centrosome(s). These central areas were further characterized by Aurora-B accumulation in up to 60% of HCT116, DLD-1 and CaCo-2, but only in 19% of HT29 cells.

**Conclusions:** This study implicates that Aurora-A inhibition influences Aurora-B expression in MIN-type, but not in CIN-type CRC cell lines, with different Aurora-B regulation. Potential future therapeutic inhibition of Aurora-A, respective its associated cellular mechanisms and effects on Aurora-B may cause distinct tumor cell responses in the molecular subgroups of CRC.

### Do-013

#### WTX mutations in high-grade microsatellite instable (MSI-H) colorectal cancers

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**Aims:** Colorectal cancers (CRCs) are mostly characterized by stabilization and activation of  $\beta$ -CATENIN. In high grade microsatellite instable (MSI-H) CRCs several genes in the canonical Wnt-signaling pathway are mutated, like the APC-, AXIN2- and the  $\beta$ -CATENIN gene itself. Recently, WTX (Wilms tumor gene on the X-chromosome) was discovered as a new component of the  $\beta$ -CATENIN destruction complex in Wilms tumors. Here, WTX functions as a tumor suppressor which leads to the stabilization of  $\beta$ -CATENIN when mutated. As the WTX-gene harbors a short T<sub>C</sub>-microsatellite in its N-terminal coding region, we hypothesized that frameshift-mutations might occur in MSI-H CRCs in the WTX gene, thus additionally contributing to the stabilization of  $\beta$ -CATENIN in human CRCs.

**Methods:** Test for MSI-H, fragment length analysis, RT-PCR.

**Results:** Among 632 metastasized CRCs (UICCIV) we identified 41 MSI-H cases (6.5%). Analyzing their WTX-T<sub>C</sub> microsatellite, we found two of the 41 MSI-H cases (4.8%) harbouring a frameshift mutation resulting in a T<sub>4</sub> sequence. To narrow a functional role of the WTX alteration (driver mutation) we investigated if the frameshift was also seen on the mRNA level, and found this in a single case (one male patient).

**Conclusions:** We show that 6.5% of metastasized CRCs belong to the MSI-H type of tumors. Of these, a small proportion (4.8%) displays frameshift mutations in the tumor suppressor gene WTX which might when expressed (2.4%)- contribute to the stabilization of  $\beta$ -CATENIN.

### Do-014

#### Up and down regulation of p16<sup>INK4a</sup> expression in the serrated route to colorectal cancer with BRAF mutation

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**Aims:** In tumorigenesis initial BRAF mutations are known to induce p16<sup>INK4a</sup> associated oncogene-induced senescence (OIS). Loss of OIS can be a decisive step for malignant transformation. In the alternative serrated route to colorectal cancer (CRC) BRAF mutations are frequently observed but the role of OIS in the serrated route is presently unknown. The aim of this study was to evaluate the role of OIS in the serrated route.

**Methods:** KRAS and BRAF mutational analyses were done on 91 serrated tumour cases. 60 serrated lesions with BRAF mutation comprising 17 hyperplastic polyps, 8 traditional serrated adenomas (TSA), 24 sessile serrated adenomas (SSA), 2 SSA with low grade intraepithelial neoplasia (LGIEN), 1 TSA with LGIEN, 4 SSA with high grade intraepithelial neoplasia (HGIEN), 1 TSA with HGIEN and 3 invasive carcinomas were found and p16<sup>INK4a</sup> and Ki67 immunohistochemistry as well as analyses of the methylation status of the p16<sup>INK4a</sup> promoter were done.

**Results:** All benign serrated polyps with BRAF mutation showed high p16<sup>INK4a</sup> and low Ki67 expression indicating OIS. Malignant transition shown by HGIEN and early invasive growth was accompanied by loss of p16<sup>INK4a</sup> expression, which significantly correlated with hypermethylation of the p16<sup>INK4a</sup> gene promoter.

**Conclusions:** Our data indicate for the first time that OIS occurs in the serrated route to CRC initiated by BRAF mutation. Loss of p16<sup>INK4a</sup> expression caused by p16<sup>INK4a</sup> gene promoter hypermethylation seems to be an important mechanism during malignant transformation.

**Do-015****KRAS mutation status in primary colorectal cancers and in matched metastases**

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**Aims:** Mutations in KRAS and BRAF are shown to be associated with clinical resistance to treatment with the epidermal growth factor receptor (EGFR) targeted monoclonal antibodies in colorectal cancer. KRAS mutation analysis is often complicated due to lack of previous primary tumors in pathologic archives. Instead of that frequently newly appearing metastases are used for KRAS mutation analysis. However, so far little data are available about the question if the KRAS and BRAF mutation status of primary tumors and metastases are equally informative when considering the multiple steps during metastatic processes.

**Methods:** To address this question we analysed the KRAS mutation status (codons 12, 13, 61) by pyro-sequencing technology in 111 colorectal cancers and matched metastases in lymph nodes, liver, lung, ovary, peritoneum, brain, urinary bladder and bone. BRAF mutation analysis was done in all KRAS mutation-negative tumors. In addition, multiple ( $\geq 2$ ) matched syn- or metachronous metastases and corresponding primary tumors were analysed in 44 cases. Tumor cells were gained either by microscopically controlled manual microdissection or by laser assisted microdissection.

**Results:** Using laser microdissection we finally did not find any discrepancies in the KRAS and BRAF mutation status in primary tumors versus matched syn- or metachronous metastases.

**Conclusion:** KRAS and BRAF mutation analysis in metastases appears to be as valid as in primary tumors and should be a reliable alternative in cases where primary tumors are not available to predict response to therapies with anti-EGFR targeted monoclonal antibodies.

**Do-016****KRAS-/BRAF-status in stage III colorectal cancer correlation with morphological parameters and prognostic impact**

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**Aims:** KRAS and possibly BRAF mutations are negative predictors for anti-EGFR therapy in colorectal cancer (CRC). The prognostic impact of these mutations in CRC is less clear. This study aimed to assess the correlation of KRAS-/BRAF-status with morphological characteristics and its prognostic impact for long term outcome in UICC-stage III CRC.

**Methods:** FFPE material was collected from 493 CRC treated with adjuvant 5-FU monotherapy in the PETACC2 trial. KRAS mutations were detected by direct sequencing (ABI Prism 310), BRAF mutations by pyrosequencing (Pyromark Q24). Statistical analysis was done using Fisher exact test and Kaplan Meier survival analyses at a level of significance of 0.05.

**Results:** While KRAS mutations did not correlate with any pathological parameters, BRAF mutations were significantly associated with mucinous histology, higher pT-stage, poor differentiation and location in the right-sided colon. Neither KRAS- nor BRAF-status showed any impact on disease-free and overall survival in univariate analyses.

**Conclusions:** KRAS-/BRAF-status does not have any prognostic impact in CRC treated with 5FU-monotherapy. While BRAF mutations are associated with certain morphological characteristics, KRAS mutations are not.

**Do-017****Linking epidemiological and molecular data in colorectal cancer**

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**Aims:** To investigate the correlation of risk factors with the molecular features of colorectal cancer by analysing colorectal cancers from the patients of the DACHS-Study.

**Methods:** The DACHS-Study (Darmkrebs: Chancen der Verhütung durch Screening) is a population-based case-control study prospectively collecting data on risk and preventive factors of colorectal cancer. 1300 colorectal cancer tissues from patients of DACHS-Study were available for analysis. Tumor tissues were assembled by Tissue-Micro-Array (TMA) technique and immunohistochemical stainings were performed. In a preliminary study the immunohistochemical data were compared to the tumor location, grading and gender.

**Results:** Immunohistochemical analysis on TMA slides provided reliable results for expression loss of MLH1 and MGMT. The loss of MGMT expression was found to be significantly more common among female compared to male patients ( $p=0.003$ ).

**Conclusions:** Immunohistochemical analysis on TMA slides of colorectal cancers collected from a population based study (DACHS) provides a rational approach to grouping tumorigenic pathways and to link the molecular findings with prospectively generated data on patient and tumor characteristics with well documented follow-ups.

**Vorträge: Leber/Pankreas****Do-018****Signal transduction in hepatocellular carcinoma. What is of significance for the pathology**

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Most cellular processes are regulated by reversible phosphorylation of proteins, which play a critical role in the regulation of cell signaling. Signals from outside the cell are transferred to the nucleus by a process of signal transduction in which kinases phosphorylate other proteins resulting in a signal cascade. Dysregulation of these kinases and their pathway components has been connected with tumor cell proliferation, anti-apoptosis angiogenesis and invasive behavior.

In recent years a new class of enzyme inhibitors which blocks protein kinases was introduced. These drugs have names ending with -nib (e.g. Imatinib, Sorfenib).

We have recently shown that phosphorylation of p70S6 kinase predicts the overall survival of patients with hepatocellular carcinoma (HCC). p70S6 kinase is the key downstream target of the mammalian target of rapamycin (mTOR) which can be blocked by rapamycin. The phosphorylation of p70S6 kinase indicated aggressive tumor behavior in patients and this marker may identify high-risk patients. The extracellular regulated kinase (ERK1/2) is involved in the regulation of cell proliferation and apoptosis in HCC. Phosphorylation and therefore activation of ERK in the tumor was associated with poor prognosis. Thus, the immunohistochemical determination of pERK1/2 status is a promising factor for the identification of patients who may benefit from new kinase inhibitors which target the ERK pathway.

In order to find new differential activated pathways we present a new high-throughput technique to generate relevant kinase activity profiles in HCC.

**Do-019****Liver spots – clonal spots in HCC-bearing non-cirrhotic liver tissue indicate hepatocarcinogenesis?**

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**Aims:** About 80% of HCC develop in cirrhosis of known etiology (e.g. hepatitis B, alcoholic liver cirrhosis, hemochromatosis, tyrosinemia). Most unclear is hepatocarcinogenesis in non-cirrhotic liver tissue. We compare in the present study for the first time normal and HCC liver tissue areas with regard to clonal proliferation.

**Methods:** As a marker for this purpose and as an indicator of mtDNA alterations, we use specific mutations of mtDNA, which are detectable within these fields. By laserdissection, sequencing, immunohistochemical and histochemical staining, we examine fields which cell population is demarcated by the reduction / deficiency or overactivity in the mitochondrial cytochrome c oxidase compared to the remaining liver tissue.

**Results:** In non-neoplastic altered liver parenchyma from HCC both the density as well as the size of these clonal fields were significantly higher than in normal liver tissue.

**Conclusions:** These observations permit the following conclusions: 1. For the regeneration of liver parenchyma in HCC-diseased livers just far fewer proliferating progenitor cells are available than in healthy liver tissue of the same age. 2. The remaining precursor cells have to form larger fields than in normal parenchyma. 3. The increased density of the demarcated clonal fields carrying mtDNA mutations indicates a significantly increased mutation rate in the diseased liver. We describe a new method for the determination of the “mutational load” as an expression of the genetic age as an indicator lesion in the liver.

**Do-020****Cell density-independent activity of YAP protects from TRAIL-induced apoptosis and supports viability and invasion in liver cancer cells**

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**Aims:** The hippo signalling pathway is evolutionary highly conserved and plays a pivotal role in organ size determination. Since cell/cell contact inhibition and density-independent growth are frequently observed in cancer, we aimed to analyze the role of the hippo-signalling-dependent transcriptional regulator yes-associated protein (YAP) in human hepatocellular carcinoma (HCC).

**Methods:** Expression of YAP in human normal livers, premalignant lesions (dysplastic nodules), and HCCs was analyzed using real-time PCR, western blotting, and tissue-micro-arrays. The expression of different mammalian hippo pathway kinases (Mst1/2, Lats1/2) and YAP was inhibited using gene-specific siRNAs. Functional assays (cell viability, migration, and invasion) were performed using independent HCC cell lines (HuH-7, PLC/PRF/5, Hep3B).

**Results:** Increased expression of YAP was detected in 66% of human HCCs compared to normal livers at the mRNA and protein levels. Nuclear and cytoplasmic expression of YAP significantly correlated with tumor dedifferentiation ( $r=0.7$ ,  $p<0.001$ ) and proliferation ( $r=0.55$ ,  $p<0.001$ ). The knock-down of nuclear YAP resulted in decreased tumor cell viability, migration, and matrix invasion. TRAIL treatment after YAP inhibition was associated with increased PARP cleavage in HCC cells. Surprisingly, depletion of different hippo pathway kinases did not change the subcellular localization of YAP.

**Conclusions:** Overexpression and nuclear enrichment of YAP is frequently observed in human hepatocarcinogenesis. Nuclear YAP supports tumor growth and tumor cell dissemination independent of hippo pathway activity, suggesting uncoupling of YAP functions from cell density. Inactivation of YAP bioactivity represents a potential mechanism for tumor cell sensitization to apoptotic stimuli.

**Do-021****HSF1 supports the development and progression of human hepatocellular carcinoma by sustaining MAPK and AKT/mTOR cascades**

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**Aims:** In the present study, we assessed the functional relevance of HSF1 activation in hepatocarcinogenesis by using HCC cell lines and human hepatocellular carcinoma (HCC) samples.

**Methods:** Levels of HSF1 and its effectors were assessed via real-time RT-PCR, western blotting, and immunohistochemistry in human HCC and corresponding surrounding non-tumorous tissues. Effects on cell growth of either reactivation by transfection or suppression via siRNA of HSF1 were assessed in human HCC cell lines.

**Results:** HSF1 expression progressively increased from surrounding non-neoplastic livers to HCC, reaching the highest expression in tumors characterized by shorter patients' survival length following liver partial resection. Forced overexpression of HSF1 in human HCC cell lines via transient transfection resulted in increased activity of MAPK and AKT/mTOR pathways and suppression of JNK cascade, leading to a rise in proliferation and a decline of apoptosis in vitro. Conversely, suppression of HSF1 in HCC cell lines decreased MAPK and Akt/mTOR pathways and induced apoptosis, with a consequent decrease in cell growth. A striking inhibitory effect on tumor growth was obtained when silencing of HSF1 was associated with MAPK and mTOR inhibitors.

**Conclusions:** HSF1 sustains tumor growth and the activity of MAPK and AKT/mTOR cascades in human hepatocarcinogenesis. Treatments aimed at suppressing HSF1 might be highly beneficial for the treatment of human HCC.

**Do-022****Expression and methylation status of miR-148a in pancreatic ductal adenocarcinoma and its impact on proliferation**

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**Aims:** Upon microRNA array analyses in ductal pancreatic adenocarcinoma (PDAC) tissues we identified miR-148a to be frequently down-regulated. Therefore, the aim of this study was to validate the miR-148a expression status in microdissected pancreatic tissue and to further characterize its mode of regulation and its functional impact on PDAC growth behaviour.

**Methods:** MiR-148a expression was analyzed via TaqMan assays in microdissected ductal, acinar and PDAC cells. MiR-148a expression was reconstituted in the PDAC-cell line IMIM-PC2 via retroviral gene transfer. Its impact on tumor growth was analyzed by MTT and wound healing assays. The role of methylation for miR-148a expression was assessed in primary PDAC tissue by MSP analyses and in-vitro via 5-aza-dC treatment of PDAC cell lines followed by miR-148a TaqMan assays.

**Results:** Downregulation of miR-148a in PDAC could be demonstrated. MiR-148a over-expressing cells showed in-vitro a decrease in proliferation. Migration behavior was not altered by miR-148a expression. Finally we found that approximately 50% of the analyzed PDAC are hypermethylated at miR-148a promoter site.

**Conclusions:** Down regulation of miR-148a expression in PDAC results frequently upon its promoter methylation. Reconstitution miR-148a expression is able to reduce PDAC growth rate, indicating that miR-148a down regulation is likely contributing to PDAC development and progression and may thus be a therapeutic target for miRNA based approaches.

**Do-023****Epithelial-stromal interaction in pancreatic cancer: the role of Collagen type V and Tenascin-C**Igor Paron<sup>1</sup>, Sonja Berchtold<sup>1</sup>, Madhavi Shamarla<sup>1</sup>, Irene Esposito<sup>1</sup>  
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**Aims:** The aim of our project is the characterization of the functional role of Tenascin-C (TNC) and collagen type V (Col V) - two proteins of the extracellular matrix that activate the integrin signaling pathway - in pancreatic cancer progression, in order to gain insight into the stromal-epithelial cross-talk in pancreatic ductal adenocarcinoma (PDAC).

**Methods:** We performed immunohistochemistry on human pancreatic tissues to determine the expression of Col V in precursor lesions and invasive PDAC. Further, to study the effect of Col V and TNC on pancreatic cancer cell lines, in vitro assays (wound healing, invasion and adhesion assays, MTT viability test) were performed. The integrin signaling pathway was studied by immunoblotting and immunofluorescence.

**Results:** We demonstrate that Col V is mainly expressed by pancreatic stellate cells and that its stromal expression levels increase in the progression from low-grade pancreatic precursor lesions (PanIN 1) to PDAC. Moreover, we show that Col V significantly promotes pancreatic cancer cell adhesion, migration and invasion, without affecting cell viability. Interestingly, PDAC cell lines grown on Col V coated plates display an increased resistance to gemcitabine and 5-fluorouracil. TNC, whose levels of expression are already known to increase in cancer progression through PanIN lesions, is able to decrease pancreatic cancer cell adhesion to fibronectin and, in addition, has an enhancing effect on cell migration, invasion and adhesion to the uncoated growth surface.

**Conclusions:** Col V is up-regulated in pancreatic cancer progression and affects important properties of pancreatic cancer cells, such as motility and chemoresistance. TNC may play a role in spreading and metastasis in vivo by affecting cell motility and invasiveness.

**Do-024****Präsentation ausgewählter Poster aus der GI-Pathologie**C. Langner  
Graz**Sitzung: AG Hämatopathologie****Do-025****Detection of isocitrate dehydrogenase 1 mutation R132H in myelodysplastic syndrome by mutation specific antibody and direct sequencing**Mindaugas Andrusis<sup>1</sup>, David Capper<sup>2</sup>, Thomas Luft<sup>3,4</sup>, Christian Hartmann<sup>2,5</sup>, Hanswalter Zentgraf<sup>6</sup>, Andreas von Deimling<sup>2,5</sup><sup>1</sup>Department of General Pathology and<sup>2</sup>Department of Neuropathology, Institute of Pathology, Heidelberg<sup>3</sup>Department of Medicine V, Universitätsklinikum Heidelberg<sup>4</sup>Adaptive Immune Regulation Unit, DKFZ, Heidelberg<sup>5</sup>Clinical Cooperation Unit Neuropathology, DKFZ, Heidelberg<sup>6</sup>Monoclonal Antibody Unit, DKFZ, Heidelberg

**Aims:** Sequencing of the acute myeloid leukemia (AML) genome revealed somatic mutations in isocitrate dehydrogenase-1 (IDH1). The aim of the study was to test whether myelodysplastic syndrome (MDS) also carries IDH1 mutations.

**Methods:** A series of bone marrow biopsies from patients with MDS were stained using a newly developed antibody specific for the IDH1 R132H amino acid exchange. The presence of mutation was confirmed by direct sequencing.

**Results:** Three out of 71 patients exhibited antibody binding to myeloid precursor cells. The presence of the R132H mutation was confirmed by DNA sequencing in one case. Two patients with IDH1 R132H mutation progressed to AML.

**Conclusions:** Taken together, our data confirm the presence of IDH1 mutation in MDS and prove that R132H mutations are already detectable in precursor lesion of AML. Furthermore, we demonstrate that standard immunohistochemistry with mutation specific antibody is a sensitive and reliable method to detect IDH1 mutation in MDS.

**Do-026****Expression of CD30 in neoplastic mast cells in aggressive forms of systemic mastocytosis**K. Sotlar, K. Petat-Dutter, S. Berezowska, P. Valent<sup>1</sup>, H-P Horny<sup>2</sup>  
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**Aims:** CD30 (TNFRSF8) is a cell membrane protein of the tumor necrosis factor family. It represents a well established marker for certain lymphatic neoplasms like Hodgkin's lymphoma and anaplastic large cell lymphoma but was found not to be expressed on reactive mast cells. Thus, the potential diagnostic utility of CD30 in mastocytosis was largely unknown.

**Methods:** A series of cases of systemic mastocytosis (SM; n=100) were studied by anti-CD30 immunohistochemistry in order to assess or exclude CD30 expression by neoplastic mast cells.

**Results:** The following preliminary results were obtained: 1) CD30 is expressed in only a small subgroup of SM; 2) in positive cases, CD30 is expressed in a largely varying number of mast cells, ranging from about 5% to almost 100%, 3) there is a marked tendency for a strong expression of CD30 in high-grade variants of SM like aggressive SM or mast cell leukemia.

**Conclusion:** Our findings indicate that CD30 might be a powerful marker for the identification of the aggressive subvariants of SM. Thus, CD30 should be added to the panel of markers used for the diagnosis and, especially, the subtyping of SM in routine work-up. The confusion of positive results with malignant lymphoma can be avoided by the additional use of a combination of appropriate mast cell markers (i.e., tryptase and CD117).

**Do-027****Sensitive detection of KIT<sup>D816V</sup> in systemic mastocytosis by determination of mutant allele burden on the DNA and cDNA level**

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**Aims:** Reactive and neoplastic mast cells strongly express CD117/KIT. The quantity of compact mast cell infiltrates in a bone marrow biopsy might be small. Thus, whether KIT<sup>D816V</sup> can be detected on the DNA level depends on the number of mast cells and the background signal of non-mast cell haematopoiesis harbouring the wild-type KIT. Purification of mast cells by laser microdissection (LMD) and allele-specific competitive blocker PCR are two established methods for detection of KIT<sup>D816V</sup>. However, the former depends on accessibility to a LMD device and is time-consuming. The latter allows no quantification of mutant alleles (e.g. for comparative follow-up analysis). It has been suggested that transcription from the mutant DNA allele in samples carrying the KIT<sup>D816V</sup> is considerably high leading to mutant mRNA abundance and thus enabling a more sensitive approach by cDNA analysis even in unsorted cells.

**Methods:** DNA/RNA extraction from formalin-fixed and paraffin-embedded bone marrow biopsies (n = 27) from SM cases. Pyrosequencing<sup>®</sup> for mutant allele quantification and HinfI restriction analysis.

**Results:** Mutant allele burden was significantly higher on the transcript versus genomic level (cDNA median 48%, DNA median 5%-DNA, p<0.001). KIT<sup>D816V</sup>-cDNA was positive in all cases and restriction enzyme analysis confirmed mutant KIT<sup>D816V</sup>. KIT<sup>D816V</sup>-DNA showed <5% alleles/not detectable in 9 cases.

**Conclusions:** Routine diagnostics for KIT<sup>D816V</sup> should be performed combined on the DNA and mRNA level.

#### Do-028

##### **Allele specific PCR for the JAK2-V617F mutation is a valuable approach to detect residual disease in patients after hematopoietic stem cell transplantation**

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**Aims:** Reduced intensity conditioning allogeneic stem cell transplantation (RIC-alloSCT) is a potentially curative procedure in the therapy of myeloproliferative neoplasms. However, 30–50% of all patients relapse depending on the subentity, the stage of the disease, the genetically based risk profile and the remission status at the time of the RIC-alloSCT. Therefore, optimization of the diagnostic schedules in the post transplant period aiming at the earliest possible therapeutic intervention has gained increasing attention.

**Methods:** Peripheral blood, bone marrow aspirates and bone marrow biopsies were gained at defined times. DNA was extracted and allele specific oligonucleotide specific PCR as well as allele specific wild type blocking PCR were performed for the JAK2-V617F mutation.

**Results:** Both procedures are similar sensitive techniques to detect residual disease after SCT for JAK2-V617F positive disease. Furthermore no significant differences were observed when performing blinded paired analysis on gDNA from PB or BM compared to bone marrow biopsies.

**Conclusions:** In comparison to other parameters in most BCR-ABL negative, JAK2 positive MPN, the value of quantitative PCR (QPCR) turns out to be the most important tool for the determination of minimal residual disease (MRD) load.

#### Do-029

##### **The effects of Polo-like kinase 1 (Plk1) inhibition on leukaemic blasts in bone marrow biopsies of patients with acute myeloid leukaemia**

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**Aims:** The mitotic serine/threonine kinase Polo-like kinase 1 (Plk1) is an important regulator of the cell cycle especially at the G<sub>2</sub>/M transition and during mitosis. Plk1 is overexpressed in several solid tumors as well as in acute myeloid (AML) and lymphoblastic leukaemia, where it is a therapeutic target. In this study we show the effect of therapeutic Plk1 inhibition on AML blasts in pre- and post-treatment bone marrow biopsies (BMBs) of AML patients.

**Methods:** We analyzed BMBs of six AML patients who were treated with the selective Plk1 inhibitor BI2536, before and one day after the first therapy cycle. We performed routine diagnostic stains (Gomori, iron stain, CAE, Giemsa) and immunohistochemistry for Plk1 and Cleaved Caspase 3.

**Results:** Compared to the BMBs before therapy, all post-treatment BMBs displayed an increase of atypical mitoses (range 3- to 189-fold) and also apoptotic cells (up to 14-fold). Immunohistochemistry demonstrated that the correct centrosomal and/or spindle pole associated localisation of Plk1 in AML blasts before therapy, was lost in the AML blasts in the post-treatment BMBs (weak diffuse cytoplasmic staining). Only few atypical mitoses stained positive for Cleaved Caspase 3 (mitotic catastrophe) one day after the first therapy cycle.

**Conclusions:** Our data confirm the sensitivity of AML blasts to Plk1 inhibition with small molecule inhibitors. We show that the atypical mitoses lack correctly localized Plk1. Moreover, only few of the atypical mitoses were in transition to apoptosis, thus substantiating data from previous cell culture experiments.

#### Do-030

##### **Acute panmyelosis with myelofibrosis clinicopathological and molecular features of an aggressive myeloid neoplasm**

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**Aims:** Since specific genetic alterations have not yet been defined, acute panmyelosis with myelofibrosis (APMF) still remains a clinicopathologically assigned AML category. We aimed to further characterize this rare variant of a myeloid neoplasm by clinicopathological and molecular studies.

**Methods:** Our files were searched for patients fulfilling the 2008 WHO criteria of APMF and for age- and gender-matched cases with refractory anemia with blast excess and fibrosis (RAEB-F). Our approach included the analysis of the JAK2-V617F, the MPL W515L and the GATA1 mutational status, the imaging of nucleophosphamin1 (NPM1), the review of laboratory, cytogenetic and clinicopathological data including patient's survival.

**Results:** APMF was associated with a significant shorter median survival (p=0.000), a lower MCV (p<0.001), a higher LDH (p<0.001), a higher grade of fibrosis (p<0.001) and higher blasts counts (p<0.001) than RAEB-F. A JAK2 V617F mutation was detected in 36% of APMF and in 21% of RAEB-F patients (p>0.05). An aberrant NPM1 was visualized in 12.9% of the APMF vs. 7% of the RAEB-F specimens (p>0.05). No MPL W515L and GATA1 mutations were found. In the APMF group, age ≤60 years was associated with a more favourable outcome (p=0.013). Hematopoietic cell transplantation (HCT) resulted in a significantly longed OS than conventional chemotherapy even in elderly patients (p=0.005).

Hier steht eine Anzeige.

**Conclusions:** Our results point to significant morphological and molecular differences between APMF and RAEB-F. Survival was significantly shorter in APMF than in RAEB-F. We detected a higher frequency of mutated JAK2 V616F in the APMF samples than reported for other categories of AML or MDS which was, however, similar to the NPM1 localization, not predictive of outcome. In contrast to data published for acute megakaryoblastic leukaemia, no MPL W515L mutation was found. Our data show that a rapid diagnosis and a proper management including the option of HCT even in elderly patients are mandatory for the management of APMF.

#### Do-031

##### **Thrombospondin-1 (THBS1) overexpression in different disease stages of primary myelofibrosis is exclusively megakaryocyte-derived – no evidence for stroma-cell involvement**

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**Aims:** Primary myelofibrosis (PMF) is a chronic myeloproliferative neoplasm with reactive remodeling of the bone marrow architecture including increased angiogenesis, progressive matrix accumulation and fibrosis development. Thrombospondin-1 (THBS1), a factor with pro-fibrotic but also anti-angiogenic properties, has not been investigated in PMF before.

**Methods:** Because of the adverse effects mediated by THBS1 we investigated the expression in PMF related to the stage of myelofibrosis (n=51) and in individual follow-ups by real-time PCR, immunohistochemistry and confocal laser scanning microscopy.

**Results:** THBS1 was significantly overexpressed ( $p < 0.05$ ) in all stages of PMF. Individual follow-ups showed involvement of THBS1 during progressive myelofibrosis. Megakaryocytes and interstitial proplatelet formations were shown to be the only source for THBS1 overexpression. Stroma cells like endothelial cells were unlabelled for THBS1.

**Conclusions:** We conclude that THBS1, overexpressed by megakaryocytes and their progenies, apparently can not prevent exaggerated angiogenesis in PMF but is rather involved in the development of myelofibrosis.

#### Do-032

##### **Expression analysis of microRNA in atypical chronic myeloid leukaemia (aCML) and chronic myelomonocytic leukaemia (CMML)**

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**Aims:** BCR-ABL-negative atypical chronic myeloid leukaemia (aCML) and chronic myelomonocytic leukaemia (CMML) show dysplasia in all three myeloid differentiations with a prominent proliferation of the granulopoiesis in aCML and monopoiesis in CMML. The molecular basis of this differentiation prevalence is not known but a subset of microRNAs is associated to granulopoietic or monopoietic differentiations.

**Methods:** Real-time PCR expression analysis of myelopoiesis-associated microRNAs (miR-10a, miR-17-5p, miR-155, miR-223, miR-424) in bone marrow cells of aCML (n=3), CMML (n=5), BCR-ABL-positive chronic myelogenous leukaemia (CML; n=5) and non-neoplastic control cases (n=3). Molecular-pathological characterisation includes analyses of CBL exon 8/exon 9 and JAK2 exon 14 gain-of-function mutations, BCR-ABL-RT-PCR and cytogenetic analysis.

**Results:** Analysis of bone marrow cells revealed that median relative microRNA expression levels were increased in specific entity:

- miR-424 in aCML (median 5.8-fold)
- miR-223 in CMML (median 4.9-fold)
- miR-155 in CML (median 5.2-fold)

Down-regulation of miR-10a was observed in aCML, CMML and CML (median 0.28, 0.40 and 0.24, respectively). MiR-17-5p was not deregulated in neoplastic hematopoiesis.

**Conclusions:** The expression of miR-424 and miR-223 is increased in aCML and CMML.

#### Do-033

##### **Thymomas reveal different levels of epithelial cell maturation and a majority shows cortico-medullary differentiation**

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**Aims:** To clarify the differentiation and the level of developmental maturity of neoplastic thymic epithelial cells (TECs)

**Method:** Comparison of gene expression profiles of thymomas with those published for purified cortical and medullary TECs

**Results:** Gene expression profiling of WHO-defined thymomas and hierarchical cluster analysis separate WHO-defined thymomas from each other and from thymic carcinomas. Compared to purified normal bulk cortical and medullary TECs (Gotter et al, 2004), type A thymomas showed but a slight medullary and ~no cortical signature, while type AB, B2 and B1 thymomas revealed progressively more cortical differentiation. While the almost mature medullary signature of B1 thymomas (including expression of the medullary master gene AIRE and its targets) was expected, the stronger medullary footprint in B2 compared to AB thymomas was a surprise. Profiles of B3 thymomas were largely unambiguous; however, the key cortical chemokine CCL25 that recruits thymocyte precursors to the thymus was among the top genes expressed in B3 but not in type A thymomas.

**Conclusions:** The WHO thymoma classification is supported by our gene expression data. Most thymomas show cortical and medullary features that both increase from AB through B2 to B1 thymomas. These findings argue for founder mutations in bi-potent TEC progenitor cells in AB, B1 and B2 thymomas.

#### Do-034

##### **T- and B-cell clonality in acute infectious mononucleosis and atypical EBV-associated lymphoproliferative disorders**

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**Aims:** 40 cases of atypical Epstein Barr Virus (EBV)-associated lymphoproliferation (AEAL) with an uncertain malignant potential were compared with 65 cases of acute infectious mononucleosis (AIM), with special regard to clonal rearrangement of the heavy chain (IgH) and the T-cell-receptor (TCR) genes and clinical outcome.

**Methods:** Histology, immunohistochemistry, EBER in situ hybridization, DNA extraction, polymerase chain reaction and fragment analysis of all cases. The clinical data were assessed.

**Results:** The median age of the cases with AEAL (61,1 y) was significantly higher than the median age of the cases with AIM (18,9 y,  $P < 0.005$ ). Only 3 cases of AIM showed a monoclonal rearrangement of IgH; none a monoclonal rearrangement of TCR. In the group with AEAL monoclonal rearrangement of IgH (30%) and/or TCR (25%) were found however, a copious number of cases was polyclonal. The clinical outcome was variable and did not significantly correlate with the results of clonal rearrangement. Some patients developed malignant lymphoma in the course of disease, others were treated by chemotherapy with regression and some showed spontaneous regression.

**Conclusions:** Clonal rearrangement of IgH is a rare event in AIM and not associated with poor clinical outcome. In cases with AEAL, clonal rearrangement of IgH or TCR is more frequent, but prognosis is still unpredictable. Further standardized prospective studies are necessary for a better understanding and to plan therapeutic strategies.

### Do-035

#### Functional interactions of novel RUNX1 isoforms in normal and malignant T lymphocytes

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**Aims:** RUNX1 (AML1) is an essential transcription factor involved in intrathymic T-cell maturation and development of malignant T-cell lymphomas. However the expression and functional relevance of recently by us described novel RUNX1-isoforms in T cells is so far unknown.

**Methods:** RNase Protection Assay, radioactive qRT-PCR, Western Blot, immunofluorescence, electro mobility shift assay, co-immunoprecipitation, cell transfection and generation of stable T cell transfectants, siRNA knock down, cDNA microarrays.

**Results:** We could show that in normal and malignant T cells the activating/inhibiting RUNX1 isoforms p48/p30 interact with FoxP3 and NFAT transcription factors and regulate the expression of target genes such as IL-2. Moreover we could determine by double immunofluorescence stainings the expression pattern of RUNX1 isoforms in normal lymph nodes and malignant T cell lymphomas.

**Conclusions:** We conclude that the interaction of novel RUNX1 isoforms with other transcription factors such as FoxP3/NFAT is crucial for the differentiation and proliferation of normal and malignant T cells. Identification of further RUNX1 dependent signalling pathways will elucidate tumor specific signalling T cell lymphomas and will be the basis for gene targeted immunotherapies.

### Do-036

#### DNA demethylation and histone acetylation of T cells results in an anaplastic large cell lymphoma-like phenotype

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**Aims:** A characteristic feature of anaplastic large cell lymphoma (ALCL) is their partial or complete loss of the T-cell expression program despite their T-cell origin. Since the loss of the T-cell phenotype in many ALCL reminds of the loss of the B-cell identity of classical Hodgkin lymphoma and the underlying epigenetic mechanisms we investigated whether similar epigenetic events are involved in silencing the T-cell genes in ALCL.

**Methods:** ALCL- and T-cell lymphoma- cell lines (n=4, each) were treated with a combination of 5-aza-2'-deoxycytidine (DNA demethylation) and Trichostatin A (histone acetylation). The RNA of treated and untreated cell lines was used for gene expression profiling (Affymetrix).

**Results:** We show that combined DNA demethylation and histone acetylation induce in T-cell lines an up-regulation of ALCL-characteristic genes and an almost complete extinction of the T-cell phenotype. In contrast, the same treatment of the ALCL cell lines did not reconstitute the T-cell phenotype.

**Conclusions:** Epigenetic mechanisms play a major role in the extinction of the T-cell phenotype in ALCL analogous to the situation in classical Hodgkin lymphoma.

### Do-037

#### Results of a multicentre trial of FISH analysis for non-Hodgkin's lymphoma

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**Aims:** To investigate reproducibility of FISH results as a tool for lymphoma diagnostics.

**Method:** FISH analysis of five non-Hodgkin's lymphomas (one follicular lymphoma with additional BCL6 rearrangement, two B-cell lymphomas intermediate between diffuse large B-cell (DLBCL) and Burkitt lymphoma with C-MYC and BCL2 rearrangement, and two DLBCL without BCL2, BCL6, c-MYC or IgH rearrangement) was performed in eight institutions with commercially available fusion probes for the IgH/BCL2- and the C-MYC/IgH-translocation as well as with break-apart probes (BAP) for the C-MYC and BCL6 loci.

**Results:** In the lymphomas all nine aberrations were readily detected by all centres. In the positive lymphomas the number of positive cells ranged from 30%–87% and was highest for the BCL6 BAP, while fusions probes had positive results between 60%–90%. No false positive or false negative results were obtained. Pearson's correlation coefficient between the centres was always >0.8, mostly >0.95 (very high). T-tests and variance analyses indicated homogeneity of the counts of positive and negative cases within all groups.

**Conclusions:** FISH is a highly reproducible technique which yields substantial additive help for standard diagnostics.

### Do-038

#### Immunophenotype and mutation status of CLL/SLL with plasmacytic differentiation

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**Aims:** To evaluate the expression of various plasma cell markers in relation to the IgH gene mutation status and prognosis in chronic lymphocytic leukaemia/small lymphocytic lymphoma (CLL/SLL).

**Methods:** Immunohistochemistry (CD5, CD20, CD23, CD43, MUM1/IRF4, VS38c, CD138, BLIMP-1, XBP1, kappa/lambda, ZAP-70), molecular analysis using IgH PCR and sequencing.

**Results:** A total of 65 biopsies of nodal CLL/SLL were investigated. All cases showed a characteristic immunophenotype (CD20+, CD5+, CD23+, CD43+). By morphology and monotypic light chain expression, 21/65 CLL/SLL cases were diagnosed as CLL with plasmacytic differentiation and in parallel expressed various plasma cell markers (MUM1/IRF4, BLIMP-1, XBP1) more frequently. IgH PCR and sequencing revealed 38% mutated and 62% unmutated CLL/SLL. More than half of the CLL/SLL cases with plasmacytic differentiation displayed an unmutated IgH status, a finding which correlated well with the expression of the surrogate marker ZAP-70. Currently we are completing the collection of the clinical data.

**Conclusions:** Surprisingly, the majority of CLL/SLL cases with plasmacytic differentiation is derived from unmutated B-cells. According to the preliminary analysis of the clinical data plasmacytic differentiation appears to be an adverse prognostic factor.

**Do-039****Cyclin D1 positive diffuse large B-cell lymphomas are of post-germinal center-immunophenotype and lack alterations in the CCND1 gene locus**

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**Aims:** The association of cyclin D1 expression with specific histological subtypes of DLBCLs and/or in the setting of Richter's transformation has not been studied systematically. The aims of this study were to analyse the incidence of cyclin D1 overexpression in DLBCLs and Richters transformation (RT) cases and to elucidate the possible molecular mechanism.

**Methods:** Seventy-six cases of DLBCLs, including 9 RT cases were included in this study. Immunohistochemical stainings for CD20, CD5, CD30, BCL-2, BCL-6, CD10, MUM1, cyclin D1 and Ki67 were performed and complemented by immunofluorescence double stainings. CCND1 and c-MYC gene loci were analyzed by FISH

**Results:** Of the 67 de novo DLBCLs, 13 cases (19.4%) were cyclin D1+. These cases displayed heterogeneous morphological patterns. All cases were negative for CD10. MUM1 was positive in 11/14 cases. No CCND1 gene locus alterations were identified by FISH analysis, except for one case. No c-MYC translocations were identified.

**Conclusions:** 1) Cyclin D1 expressing DLBCL show a post-germinal B-cell phenotype 2) The abnormal expression of cyclin D1 is not associated with a t(11;14), suggesting an alternative mechanism of cyclin D1 deregulation.

**Do-040****Identification of a chemokine receptor profile characteristic for mediastinal large B-cell lymphoma**

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**Aims:** Mediastinal large B cell lymphomas (MLBCLs) are considered as a subtype of diffuse large B cell lymphoma (DLBCL), however they exhibit completely different patterns of dissemination. To address the preferential positioning of MLBCL, we focused on homeostatic chemokines involved in B cell compartmental homing in secondary lymphoid organs.

**Methods:** We applied immunohistochemistry to assess chemokine receptor and ligand expression in situ. Flow cytometry was used to identify the chemokine receptor profile on a MLBCL-derived cell line, Karpas1106 and on thymic B cells. Migration assays were performed to examine functionality of chemokine receptors. Electrophoretic mobility shift assay was applied to score for NF-κB activity.

**Results:** We obtained an unexpectedly low expression frequency for the chemokine receptors CXCR5 and CCR7 in MLBCL. While the mature B cell marker CCR6 was absent in most cases, the lineage aberrant marker CCR9 emerged in the majority of MLBCL cases. Given the role of NF-κB in the transcriptional activation of CCR7, we identified the involvement of the non-canonical activation pathway in MLBCLs.

**Conclusions:** MLBCLs exhibit a chemokine receptor profile which allows the discrimination from DLBCL not otherwise specified and classical Hodgkin lymphoma. Furthermore, we suggest that low abundance expression of CCR7 and CXCR5 may hinder lymphoma cells from nodal dissemination.

**Do-041****Immunohistochemical characterisation of 145 paediatric lymphoblastic lymphomas treated within the Euro-LB 02 study: results of the EICNHL pathology panel**

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on behalf of the European EICNHL Pathology Panel

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**Aims:** International Panel review and immunohistochemical characterisation of 192 pediatric lymphoblastic lymphomas (LB) treated within the European study Euro-LB 02.

**Methods:** Paraffin material of 145 paediatric LB was available for review by the international pathology panel. A minimal staining panel for diagnosis according to the WHO-08 criteria included CD3, CD79a, MPO and TdT which was expanded according to the lineage of the LB. The EGIL criteria for subtyping of T-LB were evaluated for their use on paraffin embedded tissue. Further markers of specific interest (Pax5, CD1a, CD56, CD34, CD10) were investigated.

**Results:** We will present the completed data of the international review including an extensive immunohistochemical characterisation of 145 pediatric LBs. An interim analysis reveals 85 T-LBs (59%), 45 B-LBs (31%), 3 mixed lineage lymphomas (2%), 3 undifferentiated lymphomas (2%) and 9 unclassified cases (6%). Subtyping into the EGIL-defined subgroups TI-IV was impossible mainly due to the failure of reliable distinction between cytoplasmic- versus membrane-staining of CD3 on tissue sections. Only separation of cortical (CD1A+) in 61% (43/70) versus non-cortical subtypes of T-LB in 39% (27/70) was practicable. The role of Pax5 for lineage determination in LB will be discussed.

**Conclusion:** This study provides an extensive Herein we present the largest immunologically characterised cohort of childhood LB treated in a prospective, randomised trial. The data will help to design rational staining panels for diagnostic work-up of LB.

**Do-042****Combined over-expression of PAX5 and epigenetic treatment are unable to restore the B-cell phenotype in the Hodgkin cells**

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**Background and Aims:** The expression of the transcription factor PAX5 is significantly reduced in the tumour cells of classical Hodgkin lymphoma (cHL). Furthermore, almost all other B-cell associated genes are at least partially due to epigenetic mechanisms - silenced. Therefore we tested the hypothesis whether an induced strong expression of PAX5 in combination with demethylation and histone acetylation is able to restore the B-cell gene expression program in cHL.

**Methods:** The Hodgkin-derived cell line L428 with and without inducible ectopic PAX5 expression was treated with 5-aza-deoxy-cytidine (5-aza-dC; demethylation) and trichostatin A (TSA; acetylation). RNA and protein were extracted after 6 days of treatment and subjected to microarray analysis (Affymetrix U133A), real-time RT-PCR and Western blot analysis.

**Results:** PAX5 proved to be strongly expressed in L428 cells after induction with Mifepristone. However, a re-activation of the B-cell phenotype was not detectable. The additional treatment with 5-aza-dC and TSA led to the expected up-regulation of cancer testis antigens but not to a re-expression of B-cell characteristic genes.

**Conclusions:** Our results show that a forced strong PAX5 expression combined with promoter demethylation and histone acetylation does not suffice to restore the B-cell gene expression program in the Hodgkin cell line L428. This indicates that additional mechanisms are operative in suppressing the B-cell genes in cHL.

**Do-043**

**Uncompressed WSI in diagnostic virtual 3D microscopy – a dispensable quality**

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**Aims:** Lossy compression influences the quality of whole slide images (WSI) and may have an influence on the diagnostic accuracy. Anyway, compression artifacts should not be recognizable in JPEG2000 format below a compression ratio of 20:1. To verify this assumption in practice we carried out another comparative investigation.

**Methods:** Virtual 3D slides of gastric biopsies with or without *Helicobacter pylori* gastritis were constructed using uncompressed WSI or compressed WSI using compression ratios of 5:1, 10:1, and 20:1. The virtual 3D slides were diagnosed in a blinded manner by three pathologists according to the updated Sydney classification.

**Results:** Our results showed no significant differences using uncompressed or compressed WSI.

**Conclusions:** We conclude that uncompressed WSI or near-lossless compression is a dispensable quality in virtual microscopy, even in diagnostic applications.

**Do-044**

**Intelligent navigation in virtual slides of serial sections**

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**Aims:** Tracking a suspicious region through serial sections is a laborious and time consuming task in conventional microscopy. We present a method for computer assisted navigation within biopsy specimens based on virtual microscopy.

**Methods:** Automatic image analysis on virtual slides recovers alignment information of sections that is lost during cutting process of the specimen. In a first step segmentation of biopsy particles is performed in low image resolution. The location of tissue sections is represented as a so-called serialization curve. Expectation maximization classification is applied to cluster particles using shape features. Subsequent feature point extraction and matching refines the alignment of corresponding particles.

**Results:** Intelligent navigation is a new way to extend the functionality of a virtual microscope beyond those of conventional microscopy. The algorithms were tested on multiple types of biopsy slide series, including stomach and kidney. A demo is available at <http://virtuellemikroskopie.charite.de/dgp-2010nav/>.

**Conclusions:** The introduced tool enables pathologists using virtual microscopy to efficiently navigate through serial sections of the same slide. Moreover serial sections are traceable across different slides by retaining a relative position. The algorithm works independent of the histological origin of material and biopsy technique.

**Do-045**

**The European scanner contest implications and limitations**

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**Aims:** The vendors of scanner for virtual microscopy offers various scanning technologies with different advantages and disadvantages. A European Scanner Contest, supported by the COST Action IC06004 „Eurotelepath“, was established to get a general impression about the state of the art in scanner technologies.

**Methods:** In a pre-test the quality evaluation parameters were specified. The main test takes place during this conference. Different disciplines were defined: Fast routine scanning 400 randomly selected slides from a pathology department should be scanned as fast as possible with a default scanner setting. High quality scanning 20 special selected slides consist of biopsies, operating material should be scanned to get best quality for every slide the scanner setting can be optimized. Cytology Scanning 50 slides from different cytology areas. Special techniques Scanning 20 slides with immunofluorescence.

**Results:** The first preliminary results can be presented in a specialized result matrix. This test is the first contest to compare different scanner.

**Do-046**

**Automated selection of diagnostic fields of view in microscopical images**

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**Aims:** Virtual slide technology is on its step into routine diagnostic pathology. The application of to fully digital microscopy permits and increases the demand for supportive techniques. Within these automated selection of fields of view ranks in the first places of pathologists' requests. We developed new algorithms for automated selection of fields of view to display the diagnostic relevant areas only.

**Methods:** A) sliding technique: a fixed frame (10% of the image area) was shifted horizontally and vertically over the whole image. Each area was separately analyzed for gray-value thresholds, local gray value maxima and minima, transformation of gray values and stochastic geometry. B) Cluster analysis: Local gray value maxima served for cluster detection by graph theory approaches. All in all 2078 images of different magnifications (4x, 10x, 20x, 40 x) and different organs (stomach, colon, lung, pleura) have been analysed by both methods. The automatically selected areas were manually reclassified for their diagnostic value.

**Results:** In each classification algorithm the diagnostically relevant image areas were correctly selected in 92.3 to 100%. This correct selection was independent from the magnification the images were acquired in. All algorithms were robust and delivered reproducible results.

**Conclusions:** We have developed reliable algorithms for automated selection of fields of view in histological images with correct area-selection of at least 92.3%. Future analyses and applications including a quantitative mapping of image information will be applied on virtual slides.

**Do-047**

**Automated processing of whole slide images using open source image analysis software**

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**Aims:** Image analysis on whole slide images (WSI) is a growing challenge in scientific research on virtual slides. We present a framework that combines virtual microscopy and public domain image analysis software.

**Methods:** ImageJ is an open source image processing program developed at the National Institutes of Health having a large user community that contributes extensions (plugins) in a wide field of life science applications. We apply the open architecture of ImageJ to interactively explore, process and, analyse WSI.

**Results:** Image processing software usually works on standard-size images whereas virtual slides are very large. Based of the framework we developed an ImageJ plugin that 1) offers simple virtual microscope functionality and 2) allows batch processing of WSI. As image providers we use file system interfaces to virtual slide as well as web based WSI streaming servers. The introduced bridging technology has applications in rapid prototyping of scientific image analysis software and education. A demo is available at <http://virtuelle-mikroskopie.charite.de/dgp2010ioma/>.

**Conclusions:** The introduced framework enables computer scientists and pathologists to process and analyze whole slide images within ImageJ. The most prominent toolkit in open source medical image analysis with its wide range of available algorithms is now applicable to virtual microscopy.

#### Do-048

##### Automated quantitative analysis of Her2 expression in immunohistochemical data in a neoadjuvant breast cancer study

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The increased availability and use of immunohistochemical data from large patient collectives in the search for novel predictive disease markers and the study of pathomechanisms call for objective, standardized, automated and high-throughput analysis methods. While the field of medical image analysis has started to produce various computer-aided, automated segmentation and IHC analysis methods during the last years the question about what approach works best for what type of data is still open, and comprehensive, methodologically transparent analysis methods are rare. Therefore, most studies still rely on the „manual“ evaluation, i.e. classification and quantification of IHC data, which is time-consuming, subjective and consequently also reduces the comparability of studies published by different groups. In the approach presented here we combine virtual microscopy with a sequence of algorithms for signal channel separation, nucleus detection and classification and membrane stain quantification and compare its performance with manually analyzed data from a neoadjuvant breast cancer study assessing the impact of human epidermal growth factor receptor 2 (Her2) expression on patient survival. In summary, our approach allows for the automated and standardized quantitative analysis of cell membrane markers in large IHC image data sets.

#### Do-049

##### The current development of structured reports in pathology

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**Aims:** The College of American Pathologists will publish the next version of structured reports soon. In France and Spain the national societies of pathology support the process of report structuring and report unification with clear defined targets:

- usage of structured report templates as check lists
- improve the comprehensibility of pathology reports for the clinicians
- improve the interchangeability and comparability of reports on national and international level

**Methods:** In various meetings organized by IHE the requirements of structured reports were defined by a team of pathologists and computer scientists. The existing structured reports in different countries were compared. In an

unification and smoothing process the practicability and the scientific requirements as well as completeness were evaluated.

**Results:** First templates from American, French and Spanish societies are compared to the results of the German working groups. Tools for management of templates and establishment of structured reports will be presented.

**Conclusions:** It is a common sense on the international level that the structured reports can reflect the complexity of pathology as well as the clinical requirements and support clinical decision and evaluation related to quality assurance.

#### Do-050

##### Structured reports in german pathology state of the art

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**Aims:** Among all medical documents pathology reports are often highly critical for patient care. In terms of information technology and knowledge engineering presently these reports are widely lacking adequate structure to support their usage in knowledge retrieval technologies for medical decision making, research, epidemiology, quality management and medical education.

**Methods:** By means of PubMed surveys and by a questionnaire sent to vendors of Pathology Management Systems (PMS) the degree of structure in pathology reports in PMS sold in Germany was analyzed.

**Results:** Pathology reports are widely displayed, stored and exported as free text, identifiable by human beings, but without „observation identifiers“ of a coding system not readable by computer. A few systems allow textual information to be structured in sections partly identified by coding systems as ICD-O, SNOMED CT, etc. Within the sections the information is still unstructured. At the very beginning are attempts to structure the report as a list of coded items based on templates, which are controlled for identifiers, version, and the underlying concepts and representations. This is by far the most granular structure for computer readability and knowledge engineering.

**Conclusions:** Compared with the EU and North America the development of pathology informatics in Germany is losing ground at this most important field. Although standardization efforts have led to a series of reporting minimum requirements in tumour pathology, their implementation in structured data entry systems is still lacking. This task has to be led by pathology organizations.

#### Do-051

##### Quality control in pathology and surgery of rectal cancer by means of oncological datasets in structured reports

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**Aims:** Diagnostics and treatment of malignant diseases are confronted with an increasing demand for quality control as to assure a high level of guideline conformity. Structured pathology reports may provide a comprehensive data set for those purposes, too. For four years key quality control variables according to S3-guideline and to the Working Group Workflow Rectal Cancer II (WGWRCII) from three different surgical clinics were analysed.

**Methods:** Structured pathology reports for rectal tumour specimens were used in a mock-up solution for a retrospective analysis of 76 data elements, 38 of them derived from the DGP minimal requirements for colorectal cancer reporting, in all of each 50 rectal cancers in the years 2007 through 2009 and 2001.

**Results:** The key variables „tumour type“, „depth of invasion“, „lymph node status“, „number of lymph nodes studied“, „circumferential resection margin“, „venous invasion“, „grading“, „R-status“, „quality of mesorectal excision“, „coning in PME“, and „intraoperative perforation“ showed partly considerable variation in the time periods studied, between pathologists, and between the clinics with a trend of improvement in recent years. A few remained substandard in terms of the S3-guideline for colorectal cancer.

**Conclusions:** Quality control measures in diagnostics and treatment should detect weak points in the processes and outcomes. Structured pathology reports are a valuable tool for a timely control of process and product quality in both pathology and tumour surgery.

#### Do-052

##### **Process analysis and management in pathology as a tool for inducing quality assessment**

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**Aims:** Progressive changing of economic environment forces clinics and clinic-associated institutes or departments to improve efficiency and effectiveness. A possible and reliable approach is the technique of process analysis being integral element of the DIN ISO certification.

**Results:** According to the clinical situation major processes could be divided in receipt, treatment, diagnostic findings and completion of the received human tissues. The process visualisation with MS Visio could identify 92 different processes on the basis of the actual status of the Institute of Pathology Salzburg, whereby 23 (25%) processes showed adding value characteristics. Additionally, with visualized process analysis starting point of quality improvement (such as tissue tracking) could be identified.

**Conclusions:** Overall, process analysis with specialized visualisation software could identify critical processes for further quality assessments.

**Methods:** A complete four step method for process analysis was carried out (i) to detect and distinguish processes, to analyze the (ii) status quo and (iii) future challenges as well (iv) to develop quality assessment. Processes are described as primary processes having central adding value character and as secondary processes with supporting features, which could further differentiated into high and low frequency. The professional Microsoft (MS) Visio software packages were applied to visualize and identify the processes.

#### Do-081

##### **WSI Integration of digital pathology in clinical PACS systems**

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**Aims:** The storage of image data in picture archiving and communication systems (PACS) is required for all data in routine pathology. Besides conventional image data such as macroscopic pictures, data consuming whole slide images (WSI), as well as additional information and reports are intended to be integrated in PACS.

**Methods:** Common clinical PACSs use DICOM standard, which allows the storage of images and case related informations in a common open format. As for radiology, DICOM standard includes definitions for several other image types, adapted to the specific requirements of the departments.

**Results:** The use of DICOM standard for conventional image data in pathology is already applicable. However, for efficient use of WSI, adjustments of the DICOM standard are necessary. Although subtle attempts have been made by the DICOM committee to integrate WSI using fragmentation of the huge image data, certainly profound modifications of the DICOM standard are necessary to meet the requirements of WSI.

**Conclusions:** The current proposal of the DICOM committee limits the further development of PACS in Digital Pathology, as out of date methods coming from radiology have been recommended, which are not sufficient enough for WSI, and which might lead to a failure of WSI-PACS integration in the future. Therefore, modifications of the DICOM standard are urgently needed to prevent any cessation in the further development of Digital Pathology.

## Sitzung: AG Orthopädische Pathologie

#### Do-053

##### **Downregulation of CD34 is associated with malignancy in solitary fibrous tumors – analysis of 279 cases**

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**Purpose:** After the first detailed histologic description of solitary fibrous tumor (SFT) by Wagner in 1870 in Leipzig this tumor group become more popular. Genomewide expression profiling has identified a number of genes differentially expressed in soft tissue sarcomas. Our objectives in this study were: 1) to test whether differentially expressed genes are also distinct on the protein level 2) to evaluate these biomarkers in a series of well characterized solitary fibrous tumors 3) to correlate the expression with clinicopathological data. Methods: 279 intrathoracic and extrathoracic SFTs from the german consultation and reference center of soft tissue tumors were revisited and analyzed immunohistochemically with antibodies CD34, BCL2, CD99, SMA, S100, PanCK and Ki67. Furthermore SFTs were categorized into the new proposal of SFT designation: Fibrous variant, cellular variant, fat forming variant, giant cell-rich variant and malignant SFTs of these subgroups. The anatomical location, the size, mitotic index, necrosis, cellularity, collagenous ropes and growth pattern of the vessels are recorded. Results: 79% of the SFTs were categorized into the fibrous variant, 17% cellular, 2% fat forming, and 1% giant cell rich variant. Three percent were categorized in malignant SFTs. Interestingly in malignant SFTs downregulation of CD34 was observed on protein level and a statistically significant parameter.

**Conclusions:** The fibrous variant of SFTs is most frequent, following by the cellular variant. The fat forming and the giant cell rich variant of SFTs are very rare. Downregulation of CD34 is associated with malignancy in SFTs pertaining to the underlying biologic mechanism.

#### Do-054

##### **MYC high level gene amplification is a distinctive feature of angiosarcomas after irradiation or chronic lymphedema**

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**Aims:** Angiosarcomas (AS) are rare vascular malignancies that arise either de novo as primary tumors or secondary to irradiation or chronic lymph edema. The cytogenetics of angiosarcomas are poorly characterized.

**Methods:** We applied array-CGH as a screening method to identify recurrent alterations in 22 cases and FISH to confirm our findings in 61 angiosarcomas (28 primary and 33 radiation-induced).

**Results:** Recurrent genetic alterations were identified only in secondary but not in primary AS. The most frequent recurrent alterations were high level amplifications on chromosome 8q24.21 (50%), followed by 10p12.33 (33%) and 5q35.3 (11%). FISH analysis confirmed high level amplification of MYC on chr. 8q24.21 as a recurrent genetic alteration found exclusively in 55% of AS secondary to irradiation or chronic lymph edema, but not in primary AS. Amplification of MYC was not predisposing to high grade morphology or increased cell turnover.

**Conclusions:** In spite of their identical morphology, secondary AS are genetically different from primary AS and are characterized by a high frequency of high level amplifications of MYC. This finding may have implications both for the diagnosis and treatment of these tumors.

**Do-055****Combining three distinct histological features of synovitis into a 3-component grading system leads to greatly increased diagnostic accuracy**E. Slansky<sup>1</sup>, J. Li<sup>2</sup>, T. Häupl<sup>3</sup>, L. Morawietz<sup>4</sup>, V. Krenn<sup>5</sup>, F. Pessler<sup>1,6</sup><sup>1</sup>Klinik und Poliklinik für Kinder- und Jugendmedizin, TU Dresden<sup>2</sup>Dept. of Statistics, National Univ. of Singapore<sup>3</sup>Rheumatologie, Charite Universitätsmedizin, Berlin<sup>4</sup>Pathologie, Charite Universitätsmedizin, Berlin<sup>5</sup>Zentrum für Pathologie, Trier<sup>6</sup>Helmholtz Zentrum für Infektionsforschung, Braunschweig

**Aims:** Using multicategory and binary ROC analysis, to compare a 3-component synovitis score with each of its components (stromal cellularity, intimal hyperplasia, inflammatory infiltration) in the ability to differentiate among inflammatory and degenerative arthropathies and normal synovium.

**Methods:** The 3-component synovitis score was determined in 666 synovial specimens: normal synovium, n=33; post-traumatic arthropathy (PtA), n=29; osteoarthritis (OA), n=221; psoriatic arthritis, n=42; and rheumatoid arthritis (RA), n=341. The discriminatory abilities of the score and its components were quantified with the area under the curve (AUC) of binary ROC analysis and the hypervolume under the manifold (HUM) of multicategory ROC analysis.

**Results:** The 3-component score differentiated all arthropathies accurately from normal tissue (AUC: 0.87–0.98), and RA from OA or PtA (AUC: 0.85 for both). AUCs of the intimal hyperplasia and stromal cellularity components (r=0.94 and 0.91, respectively) correlated with the AUCs of the complete score markedly more strongly than the inflammatory infiltration component (r=0.60). Multi-category ROC analysis ranked the overall discriminatory ability of the 3-component score several-fold higher than that of any of its components, with HUMs of 0.062 (complete score), 0.014 (stromal cellularity), 0.0075 (intimal hyperplasia) and 0.0005 (infiltration).

**Conclusions:** Combining three distinct histological parameters into one 3-component score led to greatly increased overall diagnostic power. The discriminatory ability of the score stems more from measuring proliferative than infiltrative aspects of synovitis.

**Do-056****Detection of minimal infection in synovial tissue of patients with prosthesis loosening**M.Otto<sup>1,2</sup>, N. Arens<sup>1</sup>, R.P.H. Schmitz<sup>2</sup>, M. Lehmann<sup>3</sup>, S. Bertz<sup>2</sup>, J. Kriegsmann<sup>1,2</sup><sup>1</sup>Molekularpathologie Trier<sup>2</sup>ZHZMD Trier<sup>3</sup>SIRS Lab Jena

**Aims:** Minimal infections in synovial membranes can be diagnosed using standardized morphological criteria. On one hand culturing synovial fluid provides a high rate of false negative results, on the other hand until now the disadvantage of molecular analysis was a high amount of false positive results.

**Methods:** We have adapted a commercial sepsis test, validated for whole blood, for the use in synovial tissue specimens. The testing system detects 40 bacterial and fungal species as well as 5 types of resistances against antibiotics. A special enrichment-method of bacterial and fungal DNA, which eliminates the human background DNA, provides high specificity and sensitivity. Synovial tissue from 20 patients with degenerative joint disease and total joint replacement was collected and stored in sterile NaCl-solution and was analysed using our commercial testing system (VYOO, Jena, Germany). The tissue specimens were also analyzed using conventional culture techniques and histology.

**Results:** All infections and resistances detected by molecular analysis could be proved by conventional culture techniques or by the histological parameters of low grade infection. There were no false positive results based on morphological analysis.

**Conclusions:** Analysis of synovial tissue samples by multiplex-PCR for bacterial and fungal DNA is a reliable, highly sensitive tool for detection of „low

level“ infection. The major diagnostic problem of false positive results will be eliminated by a special adsorption technique, which improves the sensitivity/specificity of the test.

**Do-057****Adipose triglyceride lipase expression differs between leiomyosarcoma and leiomyoma**

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**Aims:** Lipid metabolism is now established as a key factor influencing the pathophysiology of malignant growth. Adipose triglyceride lipase (ATGL) is a recently discovered enzyme that performs the initial step in triglyceride hydrolysis. Immunohistochemical studies have demonstrated ATGL expression in smooth and striated muscle cells. In this study we evaluated the protein expression of ATGL in smooth muscle tumours (STM), leiomyosarcoma (LM) and leiomyosarcoma (LMS).

**Methods:** 33 cases of uterine and extra-uterine SMT, including 16 LMS, 3 myxoid LMS and 14 LM, were evaluated for histological features and ATGL expression. Cases were scored for staining intensity (weak, moderate, or strong) and pattern of staining (focal or diffuse).

**Results:** 13 (93%) LM showed diffuse strong granular cytoplasmic expression of ATGL. Diffuse and weak to moderate expression was observed in 1 case of LM. Fourteen (88%) of the 16 LMS were either negative or weekly/focally positive. All of the 3 myxoid LMS as well as 2 LMS, showed diffuse moderate to strong staining.

**Conclusions:** Our results show a remarkable difference in ATGL protein expression between benign and malignant SMT and between variants of LMS. This suggests that ATGL might be helpful in distinguishing LM from LMS. Its potential as a prognostic marker will be investigated further.

**Do-058****Simple bone cysts: do their walls really contain fibrin?**D. Baumhoer<sup>1</sup>, G. Jundt<sup>1,2</sup><sup>1</sup>Institut für Pathologie, Universitätsspital Basel, Schweiz<sup>2</sup>Knochentumor-Referenzzentrum am Institut für Pathologie, Universitätsspital Basel, Schweiz

**Aims and Methods:** Simple bone cysts (SBC) are tumor-like lesions of unknown cause that characteristically contain a cloudy-appearing, acellular material in their septae-like walls. In the literature this substance is regarded as (degenerated) fibrin without further proof. We analyzed 12 cases of SBC (8 male and 4 female patients, mean age 19.3 years) with special stains (van Gieson) and immunohistochemistry against fibrin, pro-collagen I as well as collagen I and III to characterize the cell-free material. Additionally, three cases were investigated by electron microscopy.

**Results:** All cases were evaluable and contained sufficient amounts of the respective wall component. In all cases the acellular substance stained strongly red in the van Gieson stain and showed marked immunoreactivity against collagen I. Furthermore, all cases showed a weak to moderate positivity for pro-collagen I and five cases additionally for collagen III. Immunohistochemistry against fibrin, on the other hand, constantly revealed negative results in all investigated cases. Electron microscopy demonstrated fibrils with a periodic banding of 61.3 to 63.5 nm lengths, typical of collagen.

**Conclusions:** The cloudy acellular material in the walls of SBC unequivocally represents collagen and not fibrin as stated in various textbooks.

## Do-059

### Identification of broadly discriminatory tissue biomarkers of synovitis with binary and multicategory receiver operating characteristic analysis

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**Aims:** Using conventional (binary) and multicategory receiver operating characteristic (ROC) analysis, to evaluate the accuracy of several immunohistochemical markers in synovial tissue classification.

**Conclusions:** Several immunohistochemical markers that are commonly used in pathology practice can be used in synovial tissue classification. Multicategory ROC analysis identified subintimal Ki-67, CD68 and CD15 as the three most broadly discriminatory markers for this purpose.

**Methods:** Expression of Ki-67, von Willebrand factor (vWF), CD15, CD3, CD38, CD20, and intimal and subintimal CD68 was measured immunohistochemically in synovial specimens from individuals with rheumatoid arthritis (RA, n=28), chronic septic arthritis (SeA, n=11), osteoarthritis (OA, n=31), early undifferentiated arthritis (n=10), „noninflammatory“ orthopedic arthropathies (n=23), and normal synovium (n=22).

**Results:** In binary ROC analysis, areas under the ROC curves (AUC) of >0.90, corresponding to highly accurate tests, resulted from 37/92 (40%) comparisons. CD15 had the highest number of AUCs >0.90 (7/12 comparisons). Subintimal CD68 distinguished best between OA and normal synovium (AUC, 0.92), while 6 of the 8 markers differentiated well between RA and OA (AUC, 0.91–0.97). Fold differences in mean expression correlated only modestly with AUCs. Indeed, vWF, whose greatest mean expression difference was only 3-fold (SeA vs. normal), possessed AUCs >0.90 in 3/12 comparisons. Multicategory ROC analysis identified Ki-67, subintimal CD68, and CD15 as having the largest hypervolumes under the ROC manifold, i.e. the best overall discriminatory abilities.

## Sitzung: AG Urologische Pathologie

### Do-060

#### Tubular Gb3-deficiency blocks verotoxin-mediated renal-failure but not cerebral thrombotic microangiopathy

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**Background:** Verotoxins are one of the etiological agents of the hemolytic-uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP). In vitro experiments have implicated the glycosphingolipid globotrihexosylceramide (Gb3, CD77) as the receptor mediating the verotoxin toxicity.

**Aims:** (1) To test if Gb3 is the exquisite receptor responsible for verotoxin toxicity. (2) To test which cells contribute to the systemic disease of verotoxin-induced HUS-TTP.

**Methods:** Generation of mice with (1) global Gb3-deficiency and (2) tissue-specific Gb3-deficiency in renal tubular cells. Administration of verotoxin 2 followed by survival analysis, organ histology and biochemical analysis of blood and urine.

**Results:** (1) After administration of verotoxin 2 all wild-type (WT) mice died between day 2 and 4 because of renal tubular dysfunction (potassium retention, sodium and water loss) with ensuing uremia, but mice with global Gb3-deficiency survived and did not show biochemical or histological alterations. (2) Renal tubular Gb3-deficiency fully protected the mice from uremia and water loss. However 4 to 7 days after verotoxin injection these mice demonstrated cerebral thrombotic microangiopathy leading to death in 50%.

**Conclusions:** Gb3 is the exquisite mediator of verotoxin toxicity. In the murine verotoxin-toxicity model the dominant renal pathophysiologic mechanism is toxicity towards the tubular epithelium with resultant sodium and

water depletion followed by kidney failure. Gb3 (CD77) mediates cerebral thrombotic microangiopathy.

### Do-061

#### Erythropoietin does not prevent albuminuria in 5/6 nephrectomized rats

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**Aims:** Chronic kidney diseases progress over time irrespective of the primary insult. Erythropoietin not only promotes erythrocytes production, but also acts as an anti-apoptotic cytokine. We hypothesize that treatment with erythropoietin in subtotally nephrectomized rats may slow down the progression of renal damage.

**Methods:** 12-weeks old male Sprague-Dawley rats were randomized to 5/6 nephrectomy (SNX) or sham operation and subsequently received murine erythropoietin (2.5 µg/kg/week), enalapril (12 mg/kg/day), erythropoietin plus enalapril, erythropoietin plus dihydralazine (25 mg/kg/day), or vehicle for 16 weeks.

**Results:** The albumin excretion was significantly higher in untreated SNX (48.6±43.0 mg/24 h) compared to sham-op (0.8±0.7), and this was reduced in SNX treated with enalapril (3.6±3.1) and erythropoietin plus enalapril (12.7±10.2) but was not changed in SNX treated with erythropoietin (50.1±34.7) and erythropoietin plus dihydralazine (85.2±59.9). Systolic blood pressure was higher in SNX treated with erythropoietin but not in SNX treated with erythropoietin plus enalapril and erythropoietin plus dihydralazine compared to untreated SNX. Treatment of SNX rats with erythropoietin resulted in lower expression in the kidney of p47phox NADPH oxidase, glutathione peroxidase 3, eNOS, iNOS, and β2 subunit of the soluble guanylyl cyclase.

**Conclusions:** Treatment with erythropoietin failed to decrease albuminuria in 5/6 nephrectomized rats regardless of the blood pressure.

### Do-062

#### Cardioprotection in chronic kidney disease the role of erythropoietin

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**Aims:** Cardiovascular disease is the primary cause of mortality in patients with chronic kidney disease (CKD). Heart remodeling develops in CKD comprising of interstitial fibrosis and capillary loss.

**Methods:** 2-weeks old male Sprague-Dawley rats were randomized to 5/6 nephrectomy (SNX) or sham operation and subsequently received murine erythropoietin (2.5 µg/kg/week), enalapril (12 mg/kg/day), erythropoietin plus enalapril, erythropoietin plus dihydralazine (25 mg/kg/day), or vehicle for 16 weeks. Volume density of capillaries, interstitium, and fibrocytes as well as length density of capillaries were analysed in the myocardium using stereology.

**Results:** Left ventricle fractional shortening (by echocardiography) was reduced in vehicle treated SNX (66.3%) compared with sham-op (81.2%) and this was ameliorated by erythropoietin (72.6%) and prevented by enalapril (80.6%). Capillary length density was lower in vehicle treated SNX (3631±466 mm/mm<sup>3</sup>) compared to sham-op (4264±442), and the capillary rarefaction was prevented in SNX treated with erythropoietin plus enalapril (4298±576) and reduced in SNX treated with enalapril (3908±383), and erythropoietin plus dihydralazine (3949±355), but not with erythropoietin (3697±565; ANOVA p<0.02). In parallel expression of the p47phox NADPH oxidase was higher in untreated SNX and most effectively reduced in SNX treated with erythropoietin plus enalapril. In basal condition, there was no

difference between the groups regarding myocardial hypoxia, reflected by pimonidazole staining.

**Conclusions:** Erythropoietin in combination with enalapril additively reduces cardiac fibrosis and microvessel disease in 5/6 nephrectomized rats presumably by decreasing myocardial oxidative stress.

#### Do-063

##### **Blocking Fas Ligand in acute lethal cisplatin induced renal failure prolongs survival in mice via two different mechanisms**

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**Aims:** Fas Ligand expression on both tubular cells and infiltrating T-Lymphocytes has been suggested in the pathophysiological course of cisplatin induced nephrotoxicity (CIN). Here we show for the first time that blocking murine Fas Ligand by the monoclonal antibody MFL3 protects wildtype mice from acute renal failure and from lethal CIN in a dose dependent manner.

**Methods:** To investigate the in vivo relevance of non-T/non-NK-cell mediated Fas Ligand dependent cisplatin nephrotoxicity we treated SCID-Beige mice with cisplatin.

**Results:** Whereas all wildtype mice died from CIN within 5 days, SCID-Beige mice show 100% survival at day 5, but develop significant renal failure at that time as shown by highly elevated serum creatinine and serum urea levels. At day 10 after Cisplatin injection a mortality rate of 55,6% was registered in SCID-Beige mice (n=9). Interestingly, treating SCID-Beige mice with 200 µg MFL3 completely protected from lethal CIN (100% survival rate at day 16 after Cisplatin-injection). Above this we show that kidney expressed Fas Ligand, independently of infiltrating immune cells, is of functional relevance and has to be blocked to completely reverse lethal CIN.

**Conclusions:** We provide a model in that infiltration of Fas Ligand positive immune cells accelerates and deteriorates CIN and conclude that Fas Ligand expressed by kidney cells is of functional relevance for renal apoptosis, renal failure and death from CIN.

#### Do-064

##### **No evidence for an association of p53-R72P single nucleotide polymorphism with cancer risk or histopathologic features in renal cell carcinoma patients**

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**Aims:** The single nucleotide polymorphism (SNP) p53-R72P affects numerous carcinogenesis related cell functions. P53-R72 was found to be more effective at protecting stressed cells from neoplastic development. Significant associations of this variant to cancer development were found in various cell types. No data are available for renal cell carcinoma (RCC).

**Methods:** DNA extracted from formalin-fixed, paraffin-embedded normal tissue of 296 RCC patients (among them 157 affected by clear cell, 54 by papillary and 70 by chromophobe RCC) and 196 controls was examined by restriction fragment length polymorphism (RFLP) using restriction enzyme BstUI after PCR. Fragment length was determined by gel electrophoresis. Comparison of genotype distribution of both groups and associations of any genotype to histologic subtype, stage, nuclear grade, age at diagnosis were assessed using Pearson's  $\chi^2$  or two sided Fisher's exact tests where appropriate.

**Results:** Genotype distribution of RCC carriers and controls did not differ significantly; there was no correlation of genotype and gender, age group, subtype, stage or nuclear grade.

**Conclusions:** The p53-R72P polymorphism does not seem to affect the risk for RCC or histopathologic features.

#### Do-065

##### **EGFR analysis in various kidney cancers**

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**Aims:** Epidermal Growth Factor Receptor (EGFR) is a protein involved in progression of many cancer types and serves as an important therapeutic target. The aim of this study was to determine its role in kidney cancer.

**Methods:** Tissue samples from 1392 kidney cancers were brought into a tissue microarray format and analyzed by immuno-histochemistry and FISH-analysis. In addition a subset of 60 cancers was sequenced for EGFR exon 18–21 mutations.

**Results:** Detectable EGFR expression was found in 85,5% of clear cell cancer, 68,2% of papillary cancers, 76% of chromophobe cancers and 51% of oncocytomas. Within clear cell cancers, EGFR expression was associated with high grade, tumor stage, nodal status but not with survival. FISH analysis showed high polysomy in 6,1% and amplification in 0,1% of cases. No exon 18–21 mutations were found in 60 sequenced cancers.

**Conclusions:** Increased EGFR expression occurs in a fraction of kidney cancers with unfavourable histological phenotype. EGFR copy number gain represents one possible cause for overexpression. The potential utility of anti-EGFR medications might be worth further investigation in kidney cancer, since a fraction of tumors showed high polysomy (predictive for response to anti-EGFR inhibitors).

#### Do-066

##### **VHL mutations and their impact on VHL protein function in sporadic clear cell RCC**

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**Aims:** Mutations of the VHL gene are an early event in sporadic and hereditary clear cell renal carcinoma (ccRCC). The significance of different VHL mutations for the biologic activities of VHL protein (pVHL) in sporadic ccRCC is still unclear. Our goal was to analyze the effects of VHL mutations on the stability of the pVHL by using the in silico predictive tool SDM. Appropriate in vitro analyses allow testing the impact of identified mutations to the functional properties of pVHL.

**Methods:** The VHL gene was sequenced in 256 ccRCCs. As nonsense and frameshift mutations predict loss of function of pVHL, VHL missense mutations were analyzed using SDM which predicts the thermodynamic stability change of pVHL. In vitro assays addressing different functions of pVHL, including ability to degrade HIF and to bind to various adaptor proteins, serve as validating tools for the results obtained in silico.

**Results:** In 181 of 256 ccRCC (71%), a VHL gene mutation was identified. Forty-nine of them had point mutations leading to single amino acid exchanges. By using in silico prediction of pVHL stability, 42 of the missense mutations affected HIF binding. The remaining seven missense mutations negatively influenced VHL binding of partners different to HIF (i.e., KIF2A and aPKC). All mutations will be analyzed using in vitro assays for stability and ability to bind adaptor proteins.

**Conclusions:** Knowledge of the biologic impact of a specific VHL mutation on pVHL function may lead to a molecular re-classification of sporadic ccRCC and might help to design a risk profile to predict patient outcome.

**Do-067****Renal cell carcinoma regression after targeted neoadjuvant therapy**

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**Aims:** Treatment management of renal clear cell carcinoma (RCC) has fundamentally changed within the last years since target therapeutics are available. However, until now there are only few experiences regarding the benefit of neoadjuvant therapy with tyrosine kinase inhibitors.

**Methods:** A 63-yr-old patient with locally advanced renal cell carcinoma including an atrial thrombus underwent two cycles of neoadjuvant therapy with the multi-kinase inhibitor Sunitinib (Sutent<sup>®</sup>) followed by tumor-surgery. Histopathological examinations in primary tumor after tyrosine kinase inhibitor therapy were evaluated in comparison to pretherapy tumor biopsy material.

**Results:** Primary surgery therapy had to be delayed because of suspicion of bronchial carcinoma and additional diagnostics. After neoadjuvant therapy with downsizing of the tumor thrombus and exclusion of an additional malignancy, tumor operation was done via abdominal access, no sternotomy was necessary. Microscopic examination demonstrated different histological parts: There was a typical renal clear cell growth structure, beside hemorrhagic necrotic areas and microvascular invasion. Typical tumor necrotic areas were located next to areas which showed hyperplastic vacuolar cytoplasm cells. Microvascular invasion was ascertained. Immunohistological detection of VEGF was not possible in the biopsy specimen as well as in surgical specimen after treatment invention.

**Conclusions:** Neoadjuvant therapy with Sutent<sup>®</sup> may represent a favorable treatment option in cases of locally advanced clear cell RCC with extended tumor thrombus.

**Do-068****MDR1 and ERCC1 expression levels predict outcome in patients with advanced bladder cancer and adjuvant chemotherapy with M-VEC or CM**

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**Aims:** MDR1 and ERCC1, genes which have been linked to resistance against platinum based chemotherapy, were tested for their association with outcome in patients with locally advanced bladder cancer enrolled for adjuvant chemotherapy with M-VEC or CM (AUO-AB 05/95 Phase III Trial).

**Methods:** Tumor samples from 108 patients with locally advanced bladder cancer receiving adjuvant therapy with CM or M-VEC were analyzed for MDR1 and ERCC1 mRNA expression by quantitative real-time RT-PCR using formalin-fixed and paraffin embedded tumor tissue.

**Results:** High expression levels of both genes were significantly correlated with worse overall and progression-free survival ( $p=0.002$ ;  $p=0.0002$ ). For MDR1 a significantly stronger association ( $p=0.03$ ) with an unfavorable prognosis was found in the M-VEC group. A combined expression score of both genes showed a sensitivity of 94.7% and a specificity of 40.0% to predict overall survival.

**Conclusions:** Our results suggest a predictive value of ERCC1 and MDR1 expression levels for therapy response and outcome in patients with locally advanced bladder cancer which allows detection of patients who are likely to benefit from adjuvant cisplatin-based chemotherapy. Furthermore we demonstrate that qPCR is feasible in studying paraffin-embedded material from clinical bladder cancer trials.

**Do-069****ERCC1 protein expression detected by immunohisto-chemistry predicts poor survival in patients with advanced bladder cancer after cystectomy and chemotherapy**

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**Aims:** To evaluate the impact of classical histological and new immunohistochemical markers on prognosis of patients with locally advanced bladder cancer undergoing cystectomy (pT3a-4a and/or pN+) and response to chemotherapy within a randomized, multicenter, phase III trial (AUO-AB 05/95).

**Methods:** Clinico-pathological parameters were assessed retrospectively in 204 patients randomized to adjuvant systemic chemotherapy with cisplatin and methotrexate (CM) vs. methotrexate, vinblastine, epirubicin, and cisplatin (M-VEC) after radical cystectomy. Tissue microarrays were constructed to study the immunohistochemical expression of ERCC1, BSG (Emmprin/CD147) CAV1, phospho-CAV1 (Y14), RRM1, BRCA1, BRCA2, BIRC5 (Survivin), and KISS1.

**Results:** ERCC1 and RRM1 expression correlated significantly with shorter overall survival in both therapy arms by univariate analysis. Multivariate analysis showed that ERCC1 expression was an independent prognostic factor for shorter overall survival (HR=2.3, 95%CI=1.18–4.43,  $p=0.014$ ). Positive nodal status (pN1–3), histologic blood vessel invasion (V1), and positive BIRC5 immunoreactivity were significant as well.

**Conclusion:** Our data suggest the use of standard histological variables in combination with immunohistochemical prognostic markers for muscle invasive bladder cancer patients, irrespective of the mode of chemotherapy.

**Do-070****Identification of differentially expressed proteins in LG versus HG pTa bladder tumors**

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**Aims:** By incidence, bladder cancer is the ninth most common cancer worldwide. The shortcomings of the gold standard diagnostic tools (cystoscopy and cytology) lead to an ongoing search for more convenient as well as reliable biomarkers. In order to discover new potential markers, especially in case of tumor progression to high grade tumors, papillary low grade (LG) and high grade (HG) bladder tumors were analysed for protein expression differences. Furthermore these detected proteins should reveal biological information about the protein alteration in tumor progression.

**Methods:** Tumor cells (4000 cells) from LG (n=7) and HG (n=7) papillary bladder tumors were manually microdissected using a sterile injection needle and 15 µm haemalaun stained serial sections. Cells were lysed in 10 µl lysis buffer (Tris HCl 30 mM; thiourea 2 M; urea 7 M, CHAPS 4%; pH 8.0). Afterwards lysates underwent Difference In Gel Electrophoresis (DIGE) saturation labelling (Cy5) in comparison to the appropriate internal standard (Cy3). Protein differences were analysed using the wilcoxon mann whitney test for statistical analysis (Delta 2D software). Results were validated by western blot analysis and immunohistochemistry (IHC).

**Results:** We found 15 differentially expressed proteins, whereof 14 proteins were up-regulated in HG tumors and only one was down-regulated. We identified various members of the 14–3–3 protein family, which are associated with inhibition of Raf-1 kinase activity and the interference of BAD (Bcl-xL/Bcl-2-associated death promoter)-induced apoptosis. Proteins could be validated by western blot and IHC and were additionally analysed in bodyfluids.

**Conclusions:** DIGE is a promising tool for identification of tumor specific protein differences during tumor progression and could supplement diagnostics and course prediction in bladder cancer.

#### Do-071

##### **Proteomics in grading non invasive papillary bladder cancer**

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**Aims:** Bladder cancer is a common and potentially dangerous disease with a high recurrence rate. Gold standard for diagnosis and follow up is the costly and invasive cystoscopy. In 2004 a controversial issue came up as the prior WHO grading system (1974) based on three grades (G1, G2 and G3) was displaced by a two grade system (low grade (LG) and High grade (HG)). Especially the classification of the intermediate G2 was leading to disagreement among the pathologists. To search for a more objective method validating the histopathologic classification is the major aim of our work.

**Methods:** We used MALDI- TOF- MS (Matrix-Assisted-Laser-Desorption/Ionization-Time-of-Flight-Mass-Spectrometry) imaging to detect proteomic patterns specific for G3 and G1 pTa tumours, referring the LG and HG group. From well defined urothelial areas, containing characteristic histological patterns of the respective grade, we obtained 120 mass spectra of each (seven G3 and ten G1) frozen tissue sample. On the basis of their mass spectra, an algorithm (Support vector machine) was automatically classifying seventeen G2 tumour samples into either the LG or the HG group. Those results were compared with the histological diagnosis of several pathologists.

**Results:** Referring to a Wilcoxon rank-sum test, we found 23 peaks being significant in mass and intensity to differentiate between the LG and the HG group. Therefore we achieved a cross validation of 86.6% and 80.4%, respectively. We compared the histopathologic with the proteomic classification of G2 tumours and obtained an overall conformance of 94.1%.

**Conclusions:** The proteome changes with the differentiation grade of the urothelium. Maldy imaging can be used to verify the pathologists' diagnosis as an assisting tool for bladder cancer grading.

#### Do-072

##### **Metabolite profiling of prostate tissues to identify candidates for potential metabolic biomarker**

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**Aims:** With the discovery of DNA the focus of the scientific mainstream has changed from metabolic analyses to genetic alterations. However, modern techniques have recently led to a revival of this old field. Metabolic alterations are a hallmark of malignancy, yet the metabolome of normal prostate tissues and prostate cancer is only insufficiently characterized. We aimed to identify metabolic biomarker candidates by mass spectrometry.

**Methods:** Frozen tissues from 107 prostate cancer samples with matched adjacent normal tissue were histologically verified and then extracted, fractionated and analyzed using Gas Chromatography/Liquid Chromatography Mass Spectrometry. Statistical analyses included paired t-tests and multivariate models on log<sub>10</sub> transformed data.

**Results:** A high number of significantly changed metabolites were found in prostate cancer. In principle component analyses tumor and normal tissue were still difficult to discriminate due to a high number of non-regulated metabolites. Carbohydrates destined to catabolism were generally decreased in tumor tissues whereas carbohydrates from anabolic pathways, fatty acids, cholesterol, glycolipids, phospholipids, sphingolipids and also vitamins and co-factors were consistently and strongly increased.

**Conclusions:** The metabolome of prostate cancer shows strong and biologically meaningful alterations for further study.

#### Do-073

##### **Cohort design and localization is critical for the understanding of the clinical implications of prostate cancer with ERG rearrangement**

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**Aims:** TMPRSS2-ERG gene fusions are the most frequent rearrangements in prostate cancer (PCa) with a great variability in its reported prevalence ranging from 15–78% depending on the study cohorts. The reason for this variability in different study cohorts is unknown. The aim of our study was to elucidate the differences in ERG rearrangement prevalence.

**Methods:** We assessed the frequency of ERG rearrangement by FISH in three clinical cohorts. The first cohort comprises the index tumor focus from the peripheral zone of 109 partially PSA-screened prostatectomy samples. The second cohort includes 105 PCa samples incidentally diagnosed by TUR-P, and the third cohort includes 71 PCa samples incidentally identified in the course of cystoprostatectomy. Within the cystoprostatectomy PCa cases, 9 cases had tumor foci within the transition zone.

**Results:** Out of 109 prostatectomy samples, 49.5% harbored the ERG rearrangement. 29.5% of the PCa cases incidentally identified by TUR-P and 33.8% of the PCa cases incidentally detected in the course of cystoprostatectomy revealed ERG rearrangement. Of note, 32.2% of the PCa cases diagnosed by TUR-P and 23.8% of the PCa identified in cystoprostatectomy specimen showed interfocal heterogeneity with regard to the rearrangement status. Two of the 9 (22.2%) transition zone PCa foci revealed the ERG rearrangement. Overall, the prostatectomy cohort had a higher Gleason grade as compared to the incidentally diagnosed cohorts.

**Conclusions:** We compared the ERG rearrangement frequency in incidentally detected cohorts and a prostatectomy PCa cohort. We could confirm that the ERG rearrangement appears in approximately half of the cases in the prostatectomy cohort but was observed significantly less frequently in incidentally diagnosed PCa cohorts. Our observations support the hypothesis that the ERG rearrangements may be more frequent in peripheral zone tumors than transition zone tumors, a finding consistent with the potentially more aggressive nature of TMPRSS2-ERG fusion PCa cases.

#### Do-074

##### **ERG rearrangement is specific to prostate cancer and does not occur in any other common epithelial tumour**

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**Aims:** The ERG rearrangement, a genetic alteration that is known from hematological diseases and Ewing's sarcoma, has recently been described in prostate cancer. With a frequency of 40 up to 78% reported in prostate resection material, it is the most common gene rearrangement in prostate cancer. The ERG rearrangement in prostate cancer might be an adjunct diagnostic marker in specific settings. The aim of this study was to assess, if the ERG rearrangement occurs in other common epithelial and non-epithelial tumors.

**Methods:** We assessed 3033 tumor samples for their ERG rearrangement status using an ERG break-apart FISH assay. There were 2942 samples from common epithelial and non-epithelial tumors. We also evaluated 91 prostate cancer samples.

**Results:** Seventy-seven percent (2325) of the 3033 cases were assessable by FISH. We found ERG rearrangement in 24 of 63 (38%) prostate adenocarcinoma samples. Of note, none of the epithelial and non-epithelial tumors assessed revealed an ERG rearrangement. This included lung, colon, kidney, breast, endocrine, and hematopoietic malignancies among others.

**Conclusions:** We were able to confirm ERG rearrangement in approximately 40% of the prostate cancers tested. This is largest survey of non-prostate cancer tumors for the presence of ERG rearrangements. Although Ewing's sarcoma (EWS-ERG) and AML (FUS-ERG) have known rearrangements involving ERG, the current finding supports the hypothesis that ERG rearrangement is a highly prostate cancer specific alteration. This study does not exclude rearrangements of other ETS transcription factors in these other tumor types.

#### Do-075

##### **VEGF-C protects prostate cancer cells from oxidative stress by the activation of mTORC-2 and AKT-1**

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**Aims:** Recurrence and subsequent metastatic transformation of cancer develops from a subset of malignant cells, which show the ability to resist stress and to adopt to a changing microenvironment. Long-term therapeutic success can only be achieved by identifying and targeting signaling cascades that help these cells to survive during stress. Here, we evaluate the role of the lymphangiogenic growth factor VEGF-C in reactive oxygen stress resistance of prostate cancer cells in vitro.

**Methods:** To induce reactive oxygen stress in prostate cancer we added hydrogen peroxide to cancer cells in vitro. Cell death was measured by immunofluorescence (PI staining). Immunoblot was used to detect the activation status of Akt and its downstream molecules. Immunoprecipitation experiments detected the status of mTOR Complex 2.

**Results:** We have discovered that VEGF-C acts directly on prostate cancer cells to protect them against oxidative stress. VEGF-C increased the survival of prostate cancer cells during hydrogen peroxide stress by the activation of AKT-1/PKBa. This activation was mediated by mTOR complex 2. Finally, the transmembrane non-tyrosine kinase receptor Neuropilin-2 was found to be essential for the VEGF-C-mediated AKT activation.

**Conclusions:** Our findings suggest a novel function of VEGF-C in protecting cancer cells from stress-induced cell death. This is different from the known function of VEGF-C in lymphangiogenesis.

#### Do-076

##### **Calcium activated nucleotidase 1 and hepatocyte nuclear factor 3 alpha promote prostate cancer progression**

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**Aims:** The aim of this project was to characterize the function of three newly identified prostate cancer biomarkers.

**Methods:** The overexpression of GOLPH2, HNF3alpha and CANT1 was confirmed by immunohistochemistry in a large prostate cancer cohort (n=640). The influence of these proteins on cell proliferation and migration was assessed in vitro in two different prostate cancer cell lines by siRNA knockdown, each expressing two or three of the target proteins.

**Results:** GOLPH2 and CANT1 are significantly overexpressed in malignant glands, suggesting a diagnostic use. Functionally, the proliferation of LNCaP

cells was reduced by knockdown of HNF3alpha, whereas downregulation of CANT1 reduced proliferation of both, LNCaP and PC-3 cells. Moreover, a considerable decrease of cell migration upon knockdown of both, CANT1 and HNF3alpha, was observed in LNCaP as well as PC-3 cells. CANT1 loss in LNCaP cells is further characterized by morphological changes of the cells. GOLPH2 knockdown neither influences the proliferation or migration behaviour of LNCaP cells nor cell morphology.

**Conclusions:** These data clearly suggest a pro-tumorigenic function for HNF3alpha and especially CANT1 in prostate cancer. In future experiments the mechanism how CANT1 and HNF3alpha exert their effects on proliferation and migration will be elucidated to better understand their role during prostatic carcinogenesis.

#### Do-077

##### **A large RNAi screen identifies multiple synthetic lethal interactions with the PTEN tumor suppressor gene**

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**Aims:** Loss of the tumor suppressor PTEN is highly prevalent in many cancer subtypes but an understanding of the vulnerabilities of these cancers is lacking.

**Methods:** Using the human prostate cancer cell line DU145 with shRNA mediated PTEN knockdown, a large-scale in vitro siRNA screen to identify synthetic lethal interactions with the PTEN tumor suppressor was conducted. MTT has been used to test cell viability. The percentage of apoptotic cells was assessed via caspase activity using a fluorescently labeled poly-caspase Inhibitor.

**Results:** A set of proteins was found whose depletion selectively impaired the viability and increased the rate of apoptotic cells upon PTEN knock down.

**Conclusions:** The work presented here describes an approach for the rational identification of functional co-dependencies of cancer pathways and the identification of novel therapeutic targets for the treatment of cancer with a functional deficiency of the tumor suppressor PTEN.

#### Do-078

##### **KPNA2 expression is an independent adverse predictor of biochemical recurrence after radical prostatectomy and a marker for castration-resistant prostate cancer**

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**Aims:** To analyze rates of expression of KPNA2 (karyopherin alpha 2) in different prostate tissues and to evaluate the prognostic properties for patients with primary prostate cancer.

**Methods:** Tissue microarrays contained 798 formalin-fixed, paraffin-embedded prostate tissues from two independent institutes of pathology.

**Results:** KPNA2 expression was significantly upregulated in carcinomas of the prostate, especially in metastatic and castration-resistant prostate cancer samples. Positive nuclear KPNA2 immunoreactivity was identified as a novel predictor of biochemical recurrence after radical prostatectomy, which was independent of the well-established predictive factors preoperative PSA, Gleason score, tumor stage, and surgical margin status. To validate these results, we compared a second cohort from Regensburg, Germany, with the

present results and could also find a significant correlation with shorter recurrence-free survival.

**Conclusions:** Correlation of KPNA2 expression with increased relapse rates raises the possibility of identifying patients who need more aggressive treatment and are not suited for surveillance regimens.

#### Do-079

##### Clinically relevant prostate cancer is found more frequently in thorough versus standard histopathological processing in radical cystoprostatectomy

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**Aims:** Incidental prostate cancer (PC) in cystoprostatectomy (CPX) for bladder cancer is reported in up to 50% in dependence of histopathological processing. Significant tumour has an impact on follow-up strategies. In prostatectomy specimen for PC whole mount sections improve diagnostic accuracy. The present analysis compares detection of incidental PC in complete to routine processing.

**Methods:** All CPX specimens were consecutive, unselected and unsuspected for PC. Between 01/1995 and 01/2004 (Period I) n=155 underwent conventional processing, i.e. removal of ink stained apical margins (35 mm), serial sectioning perpendicular to the ink, embedding of three sections of each side in conventional blocks and one transverse slice cut perpendicular to the rectal surface. Between 01/2004 and 03/2009 (Period II) n=166 underwent thorough processing, i.e. removal of all ink stained surgical margins and processing of the entire gland for whole-mount sections with 4 mm slices.

**Results:** Detection rate of PC was 16% and 40% ( $p < 0.0001$ ), of PC  $\geq$  pT2b 8% and 16% ( $p = 0.046$ ), of positive surgical margins 1% and 4% and of PC  $\geq$  Gleason 7 3% and 9% ( $p = 0.014$ ) in Period I and Period II, respectively.

**Conclusions:** Thorough processing of cystoprostatectomy specimen does detect significantly more clinically relevant prostate cancer. The present data warrant further studies and suggest consideration of thorough processing in younger men.

#### Do-080

##### Prostate carcinoma. The significance of second opinion of histology

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**Aims:** The significance of a second opinion of histology of prostate carcinomas as well as suspicious lesions on core needle biopsy specimens was studied on cases of our uropathological consultation service of one year (2008).

**Methods:** 922 cases of core needle biopsy specimens of the prostate with suspicious lesions but not diagnostic for carcinoma, suspicion of carcinoma, and what kind of Gleason pattern and scores were re-diagnosed with histological and immunohistochemical methods for second opinion. The modified Gleason grading and the combined histological and cytological grading system of Mostofi (WHO) and Helpap were used.

**Results:** In 43.5% of suspicious lesions (ASAP) adenocarcinomas of the prostate were found. In 53.2% the diagnoses ASAP, HGPIN or AAH were confirmed. The suspicion of prostatic carcinoma could be confirmed in 61% by the diagnosis of adenocarcinoma. After Gleason grading 80% of all diagnosed carcinomas had scores 6 and 7a (3+4). The corresponding values after the combined histological and cytological grading systems of Mostofi (WHO) and Helpap were 3-4 and 2a in 42% and 5-6 and 2b in 49.4%, respectively. High grade carcinomas were without diagnostic problems.

**Conclusion:** A second opinion of histological analysis of suspicious lesions of the prostate as well as of confirmation of Gleason grading is a very important point of quality management of diagnostic steps of prostate carcinomas for patients, urologists and pathologists and may be helpful for the different therapeutic strategies.

## Poster: Gastroenteropathologie

### Oberer GI-Trakt

#### Fr-001

##### Identification of p21<sup>WAF1</sup> as novel ATF-2 target gene after oxidative stress-induced DNA damage in the human oesophageal carcinoma cell line TE-7

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**Aims:** To gain a more detailed insight into the oxidative DNA damage response of p53<sup>-/-</sup> tumor cells, this study aimed to find novel molecular mechanisms using the p53<sup>-/-</sup> human oesophageal carcinoma cell line TE-7.

**Methods:** DNA damage was visualized by  $\gamma$ -H2AX, 8-OHdG staining, and comet assay. H<sub>2</sub>O<sub>2</sub>-induced G<sub>2</sub>/M arrest was detected by FACS analysis. Transcriptional up-regulated genes were determined by cDNA- and PCR-Array and activation of signal transducers and cell cycle regulators was detected by Western Blotting. Verification of the novel ATF-2 target gene was performed by MAPKs inhibition, ATF-2-siRNA, and CHIP.

**Results:** Oxidatively induced DNA damage caused early transcriptional up-regulation and activation of MAPKs and sub-sequently of the downstream transcription factor ATF-2. Inhibition of JNK's and ATF-2 knockdown showed abrogated p21<sup>WAF1</sup> expression. Importantly, we demonstrate that ATF-2 binds directly on the promoter of the cell cycle regulator p21<sup>WAF1</sup> to induce its transcription and thus G<sub>2</sub>/M arrest.

**Conclusions:** For the first time, we could show that the important oxidative stress-induced DNA damage response molecule ATF-2 induces p21<sup>WAF1</sup> transcription independent of p53 through direct promoter binding.

#### Fr-002

##### Incidence of Helicobacter Pylori in Cambodia – Preliminary study on a National Survey

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**Aims:** There are some studies about incidence of TB, Malaria, Dengue fever and HIV in Cambodia, however figures about incidence of Helicobacter Pylori infection is still missing. This investigations are important on the background of increasing Tourism industries, because the most common disease in tourists are Gi Infections therefore the authors started together with the Cambodian Society of Hepato-Gastroenterology a preliminary study on a national survey of H. P infection.

**Methods:** In a retrospective study 600 gastric biopsies stained in H&E and Giemsa were reviewed by 2 pathologists and classified according Sydney classification additional findings were included together with clinical history, age, gender, living area and detailed endoscopic findings.

**Results:** The study showed following results: 1. 40% of a patients with epigastric symptoms show no Pathological findings, 30% showed HP gastritis and 30% C gastritis. There was a significant predominance of B- gastritis in female and a predominance of C gastritis in male 2. Age distribution showed an increase of B- gastritis from 8% in pt <30 to 36% in pt >61, there was a constant rate of 30% of C- Gastritis in all ages. 3. There was no difference B- vs C- gastritis between rural and urban areas.

**Conclusion:** Further investigations will be done 1. To complete the study 2. To focus on touristic areas 3. To compare the results especially with the national study on HP infection in Thailand.

#### Fr-003

##### **Detection of Helicobacter pylori in gastric biopsies by PCR**

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**Aims:** Conventional histology is considered the gold standard for the diagnosis of H. pylori infection in gastric biopsies. We previously showed that in 20% of histological negative biopsies, H. pylori infection can be detected by PCR. (Zsikla et al.; Am J Surg Pathol 2006, 30:242). To further validate this data, we prospectively analyzed all biopsies for the presence of H. pylori by PCR, which were considered by the signing out pathologist having an inflammatory pattern compatible with Helicobacter infection but lacking bacteria by histology.

**Methods:** Biopsies analyzed between 1. 1. 2003 and 31.12.2007 by PCR for Helicobacter bacteria at our Institute were re-evaluated and inflammation graded according to the revised Sidney classification. Nested PCR for H. pylori was performed as previously described, including a control PCR to verify adequate DNA quality.

**Results:** Of the total of 346 biopsies tested, H. pylori was detected by PCR in 157 (45.4%) biopsies. An increased total inflammatory score (Score 3–6) was significantly associated with a positive PCR compared to a low inflammatory score (2+3) (55.4% versus 31.2%;  $p < 0.0001$ ) and the mean total score of inflammation was significantly increased in PCR positive biopsies (2.90 versus 2.24;  $p < 0.0001$ ). In addition, additional histological parameters including the presence of neutrophils, lymphoid aggregates or follicles and an increase in plasma cells were significantly associated with PCR positive biopsies.

**Conclusions:** Our data show that in gastric biopsies with an appropriate inflammatory pattern but lack of Helicobacter by histology, PCR detects H. pylori in about 45% of cases, confirming our previous data and validating this procedure in the setting of clinical practice. PCR positivity correlates furthermore with the presence of additional histological signs of Helicobacter infection. However, even in higher inflammatory scores, in a considerable number of biopsies the etiology of the inflammation remains unclear.

#### Fr-004

##### **Gastric carcinogenesis in Helicobacter pylori infected INS-GAS mice. Comparison between wild type and CTSX<sup>-/-</sup>**

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**Aims:** H. pylori infection is an important independent risk factor for gastric carcinomas. To explore the cathepsin X (CTSX) function in the context of immune response in H. pylori-dependent gastric carcinogenesis, we used CTSX<sup>-/-</sup> transgenic mice in a gastritis model and crossed these CTSX<sup>-/-</sup> mice with hypergastrinemic INS-GAS mice to create a corresponding gastric cancer model.

**Methods:** Eight-week-old male and female CTSX<sup>-/-</sup>, INS-GAS and CTSX<sup>-/-</sup>/INS-GAS mice were infected by oral gavage with 0.2 ml H. pylori strain SS1 three times per week. The mice were sacrificed at 24, 36 and 50 weeks post infection, and blood was immediately collected for gastrin-17 ELISA. The stomach was removed, and gastric strips were snap-frozen or embedded and stained with H&E. Tissue sections were scored for gastric lesions and immune cell immigration. Ki-67 immunostaining was used to measure epithelial proliferation.

**Results:** H. pylori infection of INS-GAS mice led to the development of gastric metaplasia, dysplasia and carcinoma in situ (CIS) at 24 wpI as well as to intramucosal carcinoma with submucosal invasion (36wpI). Infected CTSX<sup>-/-</sup> exhibited only moderate hyperplasia and atrophy but no preneoplastic lesions. CTSX<sup>-/-</sup>/INS-GAS mice developed less severe epithelial lesions with a lag of several weeks compared to INS-GAS mice.

**Conclusions:** The lack of cathepsin X in gastric mucosa leads to an alleviated morphologic picture in conjunction with less severe inflammation and gastric lesions, revealing CTSX as one mediator of gastric carcinogenesis.

#### Fr-005

##### **Primary mouse epithelial cells of CTSX<sup>-/-</sup> mice in co-culture with macrophages to examine the functional properties of cathepsin X over-expression in gastric carcinogenesis**

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**Aims:** We have shown an association between H. pylori infection, the up-regulation of cathepsin X (CTSX) and the development of gastric cancer. Functional properties of this CTSX over-expression in H. pylori-infected gastric epithelial cells and associated macrophages have not been proposed.

**Methods:** Gastric epithelial cells were isolated from stomachs of wild-type C57BL/6 and CTSX<sup>-/-</sup> mice and compared with the conventional mouse gastric cancer cell line CLS103. Indirect co-cultures of epithelial cells and macrophages were infected with H. pylori strain SS1 and analyzed for the expression of other cathepsins, cytokines and adhesion factors like integrins. Cellular interactions, morphological changes, migration capability and adherence of H. pylori were assessed using time-lapse video microscopy.

**Results:** Isolated primary epithelial cells from wt and ko mice revealed qualities and expression profiles similar to those of corresponding tissue samples. Adherence of H. pylori is comparable in primary and commercially cancer cells. However, induction of cathepsins, cytokines and especially adhesion proteins was detected solely in primary cells and co-cultured macrophages. cDNA microarray and migration experiments indicated that CTSX is involved in B- and T-cell proliferation/migration and adhesion of macrophages at sites of H. pylori attack.

**Conclusions:** Primary epithelial cells from stomach of CTSX<sup>-/-</sup> mice represent an excellent model of H. pylori-gastritis to elaborate the special functions of CTSX in regulating the immune response to H. pylori.

#### Fr-006

##### **Investigation of candidate biomarkers for the prediction of clinical outcome of patients with advanced adenocarcinoma of the stomach and gastro-esophageal junction treated with cetuximab and chemotherapy**

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**Aims:** The activity of the therapeutic antibody cetuximab against the epidermal growth factor receptor (EGFR) and chemotherapy was assessed in first-line metastatic gastric and gastro-esophageal junction cancer in a phase II study of the Arbeitsgemeinschaft Internistische Onkologie (AIO) with objective tumor responses in 65% of patients. The aim of the study was to investigate whether candidate biomarkers predict the clinical outcome of the patients.

**Methods:** Patients were a subset from the phase II trial of cetuximab plus weekly oxaliplatin, 5-fluorouracil and folinic acid (n=39). We performed FISH analysis, immunohistochemistry and mutation analysis.

**Results:** We analysed the association of clinical outcome with (1) EGFR gene copy number, (2) expression levels of EGFR, phosphorylated EGFR (pEGFR), phosphorylated mitogen-activated protein kinase (pMAPK), phosphorylated Akt (pAkt) and E-cadherin and (3) the mutation profile of selected exons of the E-cadherin gene CDH1, KRAS and BRAF.

**Conclusions:** The most important candidate biomarkers to predict clinical outcome were EGFR gene copy number and pEGFR expression. The frequency of genomic mutations in the selected genes was low (<10%).

**Fr-007****Expression of class I Histone Deacetylases in esophageal adenocarcinomas – an immunohistochemical study**

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**Aims:** To investigate the potential prognostic and predictive value of the expression of class-I Histone Deacetylase (HDAC) isoforms 1 and 2 in esophageal adenocarcinomas.

**Methods:** 132 primary resected tumors and 75 tumors treated by a 5-FU/cisplatin based neoadjuvant chemotherapy (CTX) were analyzed. The expression of HDAC1 and 2 was determined by immunohistochemistry, applied on a tissue microarray and on pre-therapeutic biopsies. Expression patterns were correlated with pathologic features (pT, pN, G, tumor regression after CTX) and patients survival.

**Results:** High expression of HDAC1 was found in 108/207 (52%) and of HDAC2 in 124/202 (61%) of the cases. High HDAC2 levels were associated with lymphatic tumor spread (pN category;  $p=0.046$ ) and lower tumor differentiation grade ( $p=0.008$ ) in primary resected tumors and there was also a trend for an association with tumor regression after CTX in neoadjuvant treated tumors ( $p=0.06$ ). HDAC1 levels were neither associated with pT- or pN category or tumor differentiation grade nor with regression after CTX. Survival analysis failed to show any prognostic impact of HDAC1 or HDAC2 expression.

**Conclusions:** High HDAC2 expression is associated with aggressive tumor behaviour, reflected by the association with lower tumor differentiation and the presence of lymphatic tumor spread in esophageal adenocarcinomas. Although we could not demonstrate a significant prognostic or predictive value for HDAC1 and HDAC2 expression, immunohistochemical determination of HDACs may be useful for prediction of response to specific HDAC-inhibitors.

**Fr-008****Histone deacetylase 1 and 2 expression in neoadjuvant treated locally advanced gastric cancer: prognostic and predictive value**

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**Aims:** To investigate the prognostic and predictive impact of HDAC1/2 expression in locally advanced gastric carcinomas (GC) treated by a platinum/5FU based neoadjuvant chemotherapy (CTX) and to evaluate the effect of HDAC inhibitors in vitro as an alternative treatment option.

**Methods:** HDAC1/2 expression was evaluated immunohistochemically in 127 pretherapeutic FFPE biopsies. Expression patterns were correlated with histopathological and clinical response and with patients' survival. In vitro chemosensitivity of GC cell lines for cisplatin and HDAC inhibitors was determined using XTT-assays.

**Results:** High expression of HDAC1 and HDAC2 was found in 69/127 (54%) and 108/129 (85%) of gastric carcinomas respectively. Overall, HDAC1/2 expression was not associated with histopathological or clinical response or survival. In the subgroup of responding patients high HDAC1 was associated with worse overall survival ( $p$ -values  $\leq 0.005$ ). In vitro analysis displayed a significant reduction of cell proliferation in the cell line AGS by sequential or concurrent treatment with SAHA and cisplatin ( $p < 0.05$ ) compared to cisplatin as a single agent.

**Conclusions:** HDAC 1/2 expression is not a suitable marker to predict response and survival for neoadjuvant treated GC patients, but HDAC1 expression may be used for risk stratification in the subgroup of responding patients. In vitro, a pretreatment with the HDAC inhibitor SAHA enhanced the antiproliferative effect of cisplatin, which points to an alternative treatment option.

**Fr-009****Aneuploid esophageal cancer cells show distinct Aurora-A, Aurora-B and p53 patterns**

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**Aims:** Aneuploidy is a hallmark of esophageal squamous cell carcinoma (ESCC) and Barrett's adenocarcinoma (BAC). Here, we investigated Aurora-A/-B kinases, p53 alterations, mitotic features and aneuploidy in ESCC and BAC.

**Methods:** ESCC (OE21, Kyse-410) and BAC (OE33, OE19) cell lines were analyzed for ploidy (metaphase spreads). Morphological examination of mitoses (HE) was done in double-thymidine synchronized cells. Aurora-A/-B gene copy numbers and mRNA/protein expression were analyzed by FISH, qRT-PCR, Western Blot and Immunofluorescence (IF). p53 mutations and protein localization were identified by direct sequencing and IF.

**Results:** All cell lines were aneuploid, with frequent multipolar mitoses in OE21 (10%) and OE33 (30%) cells. Kyse-410 cells displayed Aurora-A gene amplification, the others chromosome 20 polysomy. All cell lines had stable Aurora-A mRNA/protein expression, whereby an altered N-terminal domain in Kyse-410 cells indicates a splice variant. BAC cells showed 50% Aurora-B gene copy numbers compared to chromosome 17, with lowest Aurora-B mRNA/protein expression. p53 mutations caused nuclear p53 accumulation (Kyse-410, OE33, OE19) or cytoplasmic retention (OE21, loss of nuclear localization signal).

**Conclusions:** Within aneuploid esophageal cancer cell lines, distinct patterns of Aurora-A/-B kinase, p53 and mitotic (dys-)regulation occur. This may reflect heterogeneity of esophageal carcinomas and hence impact (Aurora kinase-) targeted therapies.

**Fr-010****Multilayer analysis of Signal Transduction and Cell Cycle Control in GISTs reveals prognostically relevant interactions between mRNA, miRNA and protein**

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**Aims:** Changes in mRNA and protein expression of signal transduction pathways and cell cycle control networks are already established prognostically relevant events enabling accelerated cell proliferation and tumor progression in GISTs. Our aim was to include data on miRNA expression, and to combine these three layers of gene expression to construct functionally relevant regulation networks in GIST.

**Methods:** mRNA and protein expression of 50 genes from KIT/PDGFR signaling and cell cycle control was analysed in 48 primary GISTs. This data was further compared to miRNA expression data from a microarray expression analysis of 735 miRNAs in 12 of the 48 primary GISTs.

**Results:** Computational analysis revealed several predicted miRNA/mRNA interactions with probable impact on protein expression. Mostly, the miRNA expression lead to further enhancement of regulation already known from mRNA analysis, while for some genes, adverse regulation on miRNA and mRNA level was seen. Four distinct signaling loops were identified with strong differences comparing GISTs with KIT and PDGFRA mutation.

**Conclusions:** Multilayer analysis leads to a more comprehensive insight into regulation mechanisms of tumor progression in GISTs. These observations emphasize that GISTs with KIT and PDGFRA mutation are distinct on a molecular genetic level.

**Fr-011****Differently activated pathways to cell proliferation in mesenchymal tumors of the gastrointestinal tract**K. Köhler, A. Agaimy<sup>1</sup>, L. Füzesi<sup>2</sup>, F. Haller

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**Aims:** The tumor-initiating events in GISTs are activating mutations in the receptor tyrosin kinases KIT and PDGFRA. In contrast, little is known about tumor initiation in true smooth muscle tumors of the gastrointestinal tract. While immunohistochemical expression of KIT is restricted to GISTs, the diagnostic utility of PDGFRA expression in the comparison of GISTs with smooth muscle tumors has not been analysed yet.

**Methods:** The expression of KIT and PDGFRA, their downstream signaling targets ERK and AKT, and of cell cycle regulators from different phases of the cell cycle was determined quantitatively by immunohistochemical staining on tissue microarrays in 80 GISTs, 15 leiomyomas and 10 leiomyosarcomas of the GI tract.

**Results:** KIT expression was found exclusively in GISTs, while leiomyosarcomas had a significant expression level of PDGFRA. ERK and AKT expression was higher in GISTs compared to smooth muscle tumors. While cyclin D was higher expressed in GISTs, E2F1 and cyclin B showed the highest expression in the leiomyosarcomas.

**Conclusions:** PDGFRA is significantly expressed in leiomyosarcomas, but not in leiomyomas. In GISTs, a sequential activation of intracellular pathways leading to accumulation of cyclin D exists. In contrast, yet unknown genetic events lead to upregulation of E2F1 and late G1-phase cyclin B in leiomyosarcomas, probably without previous accumulation of cyclin D.

**Fr-012****Intra-tumoral lymphatics and D2-40/podoplanin expression in gastric GISTs with and without lymph node metastasis: An immunohistochemical study with special reference to the Carney triad**A. Agaimy, J. Aidan Carney<sup>1</sup>

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**Aims:** The mechanisms responsible for the high frequency of regional node metastasis in gastric GISTs in children and young adults specially those with the Carney triad ( $\geq 20\%$ ) compared to sporadic GISTs in adults ( $\sim 2\%$ ) are unknown.

**Methods:** We investigated the distribution of intratumoral and peritumoral lymphatics, and expression of D2-40/podoplanin in 16 syndromic/pediatric GISTs of the stomach (11 had Carney triad, 2 had Carney-Stratakis syndrome and 3 had pediatric GISTs suspicious for the Carney triad) with histologically verified regional lymph node metastasis and compared the results to those found in 35 sporadic gastric GISTs lacking nodal spread, using D2-40/ podoplanin antibody.

**Results:** Intratumoral lymphatics were detected with comparable low frequency in both groups (25% and 17%;  $P=0.705$ ). Peritumoral lymphatics were uniformly present in all tumors from both groups. Moderate to strong cytoplasmic or membranous staining for D2-40/podoplanin was frequent in tumor cells of syndromic/pediatric GISTs (87%), and infrequent and weak in sporadic GISTs (3%;  $P < 0.001$ ).

**Conclusion:** Intra-tumoral lymphatics are probably not involved in regional lymphatic spread in syndromic/pediatric GISTs. Expression of D2-40/podoplanin by tumor cells of syndromic/ pediatric GISTs might play a role in invasiveness and lymphatic spread, but this needs further validation in future studies.

**Fr-013****Spectrum of peripheral nerve sheath tumors of the gastrointestinal tract: A multicenter study of 59 patients**A. Agaimy, B. Märkl<sup>1</sup>, J. Kitz<sup>2</sup>, P.H. Wünsch<sup>3</sup>, H. Arnholt<sup>1</sup>, L. Füzesi<sup>2</sup>,A. Hartmann, R. Chetty<sup>4</sup>

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**Aims:** The frequency and spectrum of peripheral nerve sheath tumors (PNSTs) of the GI tract from a consecutive case material have not been studied before.

**Methods:** We reviewed all mesenchymal GI tumors at our departments according to current diagnostic criteria.

**Results:** PNSTs represent the third group of mesenchymal GI tumors ( $\leq 5\%$ ) after GISTs ( $\geq 50\%$ ) and smooth muscle neoplasms ( $\sim 30\%$ ). Granular cell tumors (GCT) (n=31) and schwannomas (n=21) predominated. Rare tumors included NF1-associated benign PNSTs (n=4), 1 benign gastric perineurioma and 1 solitary polypoid ganglioneuroma. One gastric low-grade perineurial neoplasm (perineurial MPNST) metastasized 7 yrs later. Schwannomas were commonly misdiagnosed as GIST, leiomyoma or neurofibroma. Unusual variants included GCT with prominent lipomatous component, reticular schwannoma, NF1-associated gastric schwannoma (the first such case to date), psammomatous colonic schwannoma unrelated to Carney complex and intramural rectal GCT mimicking GIST (one case each). Contrasting with GCT, schwannomas were negative for calretinin and  $\alpha$ -inhibin suggesting a histogenetic relationship to neurofibroma.

**Conclusion:** PNSTs of the GI tract are rare nearly always benign tumors that are probably under-recognized. Awareness of them will help to prevent confusing them with GIST.

**Fr-014****Gastrointestinal stromal tumour (GIST) presenting as a pelvic mass clinical, molecular and molecular-cytogenetic characterization of a case**M. Daniels<sup>1</sup>, A.F. Pelz<sup>2</sup>, M. Evert<sup>3</sup>, H.U. Schulz<sup>4</sup>, P. Lüders<sup>5</sup>, G. Müller<sup>6</sup>,J. Lasota<sup>7</sup>, K.-H. Pollak<sup>6</sup>, M. Miettinen<sup>7</sup>, P. Wieacker<sup>2</sup>, A. Roessner<sup>3</sup>, A. Agaimy<sup>1</sup>,R. Schneider-Stock<sup>1,3</sup><sup>1</sup>Institut für Pathologie, Universität Erlangen<sup>3</sup>Institut für Pathologie, Universität Magdeburg<sup>2</sup>Humangenetik, Universität Magdeburg<sup>4</sup>Allgemeine Chirurgie, Universität Magdeburg<sup>5</sup>Pathologische Praxis Stendal<sup>6</sup>Innere Medizin, Johanniter-Hospital Stendal<sup>7</sup>AFIP, Washington, USA

**Aims:** GISTs presenting as pelvic mass are rare and may cause diagnostic problems, being often initially misdiagnosed as urogenital tumours. To date molecular analyses have not been performed.

**Methods:** A 39-year-old female presented with tumour mass between rectum and vagina that turned out to be a high-risk GIST. Local recurrence (Rec) was diagnosed 11 months after tumour resection and imatinib therapy was initiated (400 mg/d) which was stopped by the patient 8 months later. A second Rec developed within 4 months which was locally excised in combination with another cycle of imatinib which was stopped again by the patient. A third relapse was then seen. After renewed imatinib therapy MRT demonstrated a partial response with tumour shrinkage. No distant metastasis was detected as yet.

**Results:** Available tumour tissue from both the primary tumour and the third Rec showed a somatic 6-bp deletion in exon 11 of the c-kit gene (W557-K558del). In addition, an exon 17 mutation (N822 K) was found in the Rec. The CGH karyotype of the primary tumour was rev ish enh(1q),dim(14q). Recurrent tumour showed a complete loss of nuclear p16 expression.

**Conclusions:** Molecular studies and p16 status confirmed the typical characteristics of GIST with an aggressive phenotype. GISTs at this unusual location deserve a special interdisciplinary treatment.

#### Fr-015

##### **Non-nodular gastrointestinal stromal tumors with unusual circular growth pattern and different molecular pathogenesis**

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**Aims:** Gastrointestinal stromal tumors (GISTs) typically develop in the outer layer of the gastrointestinal wall and grow as solid nodules towards the serosal surface. Recently, we observed three very unusual cases presenting as circular growing lesions surrounding the whole circumference of an intestinal or colonic segment, respectively. Here, we describe clinical features, macro- and microscopic aspects and the molecular background.

**Patients:** Case 1 (58-years old, female) had circular CD117-positive spindle cell proliferations in close relation to sigmoideal diverticles. Case 2 (44-years old, female) woman presented with a circular stenosis in the small bowel. Case 3 (67-years old, male) had a diverticle-like lesion in the rectum.

**Results:** In all cases, the spindle tumor cells replaced at least parts or even the whole outer layer of the muscularis propria and lead to stenosis of the respective intestinal segment. The tumor in case 1 carried a 27-bp deletion in exon 11 of KIT. In Case 2, a duplication in exon 9 of KIT could be detected. No KIT mutation was found in case 3. Normal tissue showed wild type sequences in all cases.

**Conclusions:** Rarely, GISTs can grow in a non-nodular manner surrounding the tubular GI tract. This pattern is well known from familial and syndromic GISTs as well as from GIST mouse models with KIT germline mutation but has not been described in sporadic GISTs so far.

#### Unterer GI-Trakt I

#### Fr-016

##### **Smoothelin is a specific marker to distinguish muscularis propria and muscularis mucosae in the gastrointestinal tract**

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**Aims:** The unequivocal recognition of anatomical structures relevant for staging is increasingly challenging as tumor specimens and biopsies become ever smaller. So far no marker is available to discriminate reliably between muscularis propria (MP) and muscularis mucosae (MM) of the gastrointestinal tract. Smoothelin has been proposed to differ in MP and MM of the urinary bladder and to aid in diagnostics. We aimed to analyze the expression of smoothelin in MP and MM in order to define a novel diagnostic tool to help identifying MM bundles in the gastrointestinal tract.

**Methods:** Expression of smoothelin and  $\alpha$ -smooth muscle actin was analyzed immunohistochemically in gastrointestinal specimens from colon, stomach and esophagus (n=107). For statistics, a two sided Wilcoxon rank test to compare expression levels was used.

**Results:** In contrast to  $\alpha$ -smooth muscle actin which stained MM and MP equally strong, smoothelin expression in MM was either absent or significantly weaker, which was particularly valid in gastric and colon specimens. In samples of the esophagus, however, smoothelin was consistently positive even in the MM, although in direct comparison to MP a slight decrease of intensity was noted.

**Conclusions:** The combination of smoothelin and SMA represents a robust marker combination to discriminate MM from MP in the gastrointestinal tract, since smoothelin expression in MM is absent or weaker than in MP.

#### Fr-017

##### **Small intestinal mucosa expression of chaperone fls485**

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**Aims:** The putative chaperone fls485 has been recently suggested as a protein involved in maturation of enterocytes along the intestinal crypt-villus axis. The aim of the present study was to substantiate this assumption.

**Methods:** Expression and synthesis of fls485 in purified normal or intestinal mucosa affected with celiac disease was investigated with a molecular approach including qRT-PCR, Western blotting, and expression strategies. Molecular data were corroborated with several in situ techniques using a panel of newly synthesized mouse monoclonal antibodies.

**Results:** fls485 mRNA expression was preferentially found in enterocytes and chromaffin cells of human intestinal mucosa as well as in several intestinal epithelial cell lines. Western blot analysis with our new anti-fls485 antibodies revealed at least two fls485 proteins. In a functional CaCo2 model, an increase in fls485 expression was paralleled by cellular maturation stage. Immunohistochemistry demonstrated fls485 as a cytosolic protein with a slightly increasing gradient along the crypt-villus axis which was impaired in celiac disease Marsh IIIa-c.

**Conclusions:** Expression and synthesis of fls485 are found in surface lining epithelia of normal human intestinal mucosa and deriving epithelial cell lines. An interdependence of enterocyte differentiation along the crypt-villus axis and fls485 chaperone activity might be possible.

#### Fr-018

##### **Enterocyte survival after substrate induced expression and synthesis of acyl-CoA-Synthetase 5 (ACSL5)**

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**Aims:** ACSL5 is essential for enzymatic activation of long chain fatty acids in enterocytes. It has been demonstrated in transgenic cell culture systems that enterocyte survival is repressed by ACSL5 activity. The aim of the present study was to analyze effects of extrinsic applied ACSL5 substrates on expression, synthesis, and function of the enzyme and the consequences for cell survival.

**Methods:** Several colorectal cell lines were incubated with ACSL5 substrates like oleic acid in different concentrations and time periods. ACSL5 expression, synthesis, and enzymatic function were analysed with qRT-PCR, Western blotting, and enzyme activity assays. Effects in cell survival were studied with apoptosis assays. In a functional approach, a biopsybased tissue culture model with human intestinal mucosa was used.

**Results:** A substrate-dependent increase in ACSL5 mRNA expression, protein synthesis, and enzymatic function was found after incubation of CaCo2 cells with oleic acid over a time period of 24 hours. This increase in ACSL5 expression was loosely associated with disturbed cell survival in our cell and tissue culture models.

**Conclusions:** In enterocytes, extrinsic applied ACSL5 substrates are able to induce expression, synthesis, and enzymatic function with consequences for cell survival. This putative pathway could contribute to lipid-dependent modification of enterocytic homeostasis.

### Fr-019

#### Selection of coding microsatellite mutations in MSI-H small bowel adenocarcinoma

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**Aims:** To determine the frequency of microsatellite instability in a large unselected cohort of small intestinal adenocarcinomas and to generate a profile of target gene selection in MSI-H small bowel cancer.

**Methods:** 65 consecutively collected small bowel adenocarcinomas were analysed for microsatellite instability. MSI-H cancers were investigated for instability at coding microsatellites in 24 genes known to be mutated in varying frequencies in MSI-H cancer of colon, stomach and endometrium. The MSI-target MARCKS was analysed for expression.

**Results:** 13 MSI-H small bowel carcinomas were identified (20%). MSI-H was associated with tumor development in celiac disease (3/3), only a weak association was found for early age of onset cancers. Coding MSI analysis revealed a similar selection as seen in colon and gastric cancer. MARCKS was identified as a common cMSI target with a high frequency of biallelic mutations. Immunohistochemistry showed loss of MARCKS expression in 80% of MSI-H and 10% of MSS-carcinomas.

**Conclusions:** Frequency of MSI and MSI-driven mutational selection is similar in carcinomas of the small and the large bowel. Celiac disease is a risk factor for MSI-associated carcinogenesis. Frequent biallelic mutations of MARCKS indicate a pivotal role of MARCKS inactivation for tumor development and progression.

### Fr-020

#### Expression of ALCAM (CD166) in inflammatory bowel diseases

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**Aims:** The activated leukocyte cell adhesion molecule (ALCAM), which is expressed on activated lymphocytes and monocytes, and on various epithelial cell types, participates in cell migration / clustering, and co-stimulation of T lymphocytes through homophilic ALCAM-ALCAM and heterophilic ALCAM-CD6 interactions. While ALCAM has been implicated in carcinogenesis and inflammatory processes, such as rheumatoid arthritis, its role in inflammatory bowel disease (IBD) remains unknown.

**Methods:** In this study, we investigated the intestinal compartment-specific expression of ALCAM in ulcerative colitis and Crohn's disease employing laser capture microdissection, followed by subsequent RNA isolation and PCR analysis. Expression of ALCAM was further assessed by immunohistochemistry and immunofluorescence as well as in isolated mononuclear cells via flow cytometry and quantitative PCR analysis.

**Results:** While total gene transcription of ALCAM is lower in whole wall tissue specimens from IBD patients when compared to normal gut tissue, compartment-specific expression analysis reveals an up-regulation of ALCAM in mononuclear cells in the lamina propria and a down-regulation in epithelial cells in IBD compared to healthy controls. Up-regulation of ALCAM expression in lamina propria cells occurs mostly in cells of the monocyte/macrophage and T cell lineage. It can be induced by tissue damage as revealed by a human organ culture model of intestinal inflammation.

**Conclusions:** Given the prominent role of ALCAM-CD6 interactions for T cell activation ALCAM may represent a potential target for therapy studies in IBD.

### Fr-021

#### The T-bet and GATA3 expression pattern of lymphocytic colitis is different from that of coeliac disease

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**Aims:** Lymphocytic colitis (LC) is a disease of unknown aetiology. Among other pathogenetic possibilities, an abnormal reaction to a luminal antigen has been discussed. To clarify this fact we characterized the inflammatory infiltrate in LC and compared it with the Th1-response related coeliac disease (CD).

**Methods:** Biopsies from 10 LC and 10 CD patients were analyzed by immunohistochemistry for detection of T-bet, the master regulator of Th1-response and its antagonist GATA-3 in T cells employing double labellings.

**Results:** In LC 10–20% of intraepithelial lymphocytes (IELs) expressed GATA-3 and the remaining T-bet, whereas in CD all IELs were T-bet-positive. The T cells in the lamina propria of LC (65–70% CD4+; 30–35% CD8+) showed a mixed expression pattern of T-bet and GATA-3. The majority of the CD4+ T cells were GATA-3+, while T-bet and GATA-3 were expressed at a similar frequency by the CD8+ T cells. Most of the T cells in the lamina propria of CD specimens were CD4+ showing a predominant T-bet expression. Also most of the CD8+ lamina propria T cells in CD were T-bet+.

**Conclusions:** We conclude that in contrast to CD, which exhibits immunophenotypical features of a Th1-response, LC shows features of a mixed Th1/Th2 immune response.

### Fr-022

#### EBV Colitis a new diagnostic entity?

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**Aims:** Histological evaluation of colonic mucosa specimens by routine H&E sections with erosive colitis showed nuclear alterations, especially in endothelial cells. A special molecular pathological testing method allows simultaneous detection of CMV as well as other viral infections, i.e. HSV, VZV HHV 6 and EBV. Most unexpected in some cases with erosive colitis EBV was the only infection detected by this method.

**Methods:** We performed a nested multiplex PCR using amplification of a specific fragment of the DNA-Polymerase alpha. Using this analysis we simultaneously detect viral infections by HSV, EBV, CMV, VZV and HHV 6-viruses.

**Results:** Two cases with clinical diagnosed colitis showed an unequivocal expression of EBV-DNA implying a new entity of viral colitis, as a result of EBV infection.

**Conclusions:** To our knowledge these are the first cases with detection of EBV in colitis, we suggest the "new" diagnosis of EBV colitis which until now was underestimated in conventional histopathology, lacking specific morphological features. The molecular analysis of lesions with signs of infection may be helpful in differential diagnosis of clinically obscure colitis.

## Fr-023

**Oral administration of the anti-proliferative substance Taurolidine has no impact on Azoxymethane-Dextran Sulfate Sodium-induced, colitis associated carcinogenesis**

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**Aims:** Ulcerative colitis (UC) bears a significantly increased risk of developing colorectal dysplasia and colorectal cancer. Hence, different substances like Taurolidin (TRD; derivate of the aminosulfoacid taurine with anti-inflammatory, anti-proliferative and anti-neoplastic properties) are currently under investigation for their chemopreventive capacity. Up to now it is unknown, whether TRD can decrease dysplasia in a well characterized experimental mouse model for UC-associated carcinogenesis.

**Methods:** The Dextran Sulfate Sodium Azoxymethane model of carcinogenesis was applied in female inbred C57BL/6 mice. Half of the mice were treated with TRD, the other half served as control. After 100 days macroscopical, histological and immunohistochemical ( $\beta$ -Catenin, E-Cadherin, SOX-9, Ki-67, and Cyclin D1) examination of the entire colon was performed.

**Results:** Incidence (66.7% vs. 55.6%), multiplicity, grading and growth pattern of adenomas did not differ significantly between the two groups. Compared to normal mucosa, adenomas of both groups displayed an increased expression of  $\beta$ -catenin, SOX9 and Cyclin D – without significant difference between TRD and control group.

**Conclusions:** Oral administration of TRD has no impact on DSS-induced colitis associated carcinogenesis. Up to now, SOX9 and Cyclin D1 representing key members of the Wnt pathway, have not been described in the DSS-AOM model of carcinogenesis hinting at the role of this oncogenic pathway in this tumour model.

## Fr-024

**Cutting edge: Chk1 directs senescence and mitotic catastrophe in recovery from G<sub>2</sub> checkpoint arrest**

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**Aims:** The aim of this study was to investigate cell fates focussing on recovery from oxidative stress-induced cell cycle arrest by checkpoint override to monitor cell cycle re-entry in colorectal cancer cells in line with chemotherapy.

**Methods:** G<sub>2</sub> arrest was detected by FACS analysis, while cyclin B1 localization was attributed to G<sub>2</sub> arrest modality. DNA damage was visualized by comet assay and  $\gamma$ -H2AX staining. Chk1 knockdown was performed using Chk1 siRNA. Senescent cells were stained for  $\beta$ -galactosidase activity.

**Results:** DNA damage caused G<sub>2</sub> checkpoint activation via Chk1, while overriding G<sub>2</sub> checkpoint led to (i) mitotic slippage, cell cycle re-entry in G<sub>1</sub> and subsequent G<sub>1</sub> arrest associated with senescence, or (ii) premature mitotic entry in the absence of p53/p21<sup>WAF1</sup> causing mitotic catastrophe. G<sub>2</sub> arrest correlated with downstream senescence, but late G<sub>2</sub> arrest led to mitotic catastrophe, while both cell cycle re-entries were linked to upstream Chk1 signaling. Chk1 knockdown deciphered that Chk1 directs long-term DNA damage responses.

**Conclusions:** We propose that recovery from H<sub>2</sub>O<sub>2</sub>-induced G<sub>2</sub> arrest requires Chk1, navigating cells to senescence or mitotic catastrophe. Thus, Chk1 is an important determinant of both checkpoint initiation and recovery.

## Fr-025

**Impact of oxidative stress on inflammation-induced transformation of human colon epithelial cells**

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**Aims:** The relationship between inflammation-associated oxidative stress and the pathobiology of colorectal cancer especially in the initiation phase is poorly understood, particularly due to the lack of immortalized normal epithelial cell lines. Thus, using human colon epithelial cells (HCEC), we aimed to investigate mechanisms by which ROS function in both normal physiological and disease states.

**Methods:** Inflammation was simulated by repeated H<sub>2</sub>O<sub>2</sub> cycles with inter-jacent recovery phases. The cell morphology of the generated cell lines was characterized after FITC-Phalloidin staining. Western Blot analysis, detection of cell cycle progression/apoptosis, and JNK inhibition were performed to investigate signaling mechanisms.

**Results:** H<sub>2</sub>O<sub>2</sub> induced cytoskeletal rearrangements of HCEC cells, which were reversible under JNK inhibition. Moreover, JNK inhibition caused a switch from H<sub>2</sub>O<sub>2</sub>-induced G<sub>2</sub> arrest to apoptosis. Thus, JNK activation seems to mediate cell survival upon oxidative stress. Consistently, H<sub>2</sub>O<sub>2</sub> induced down-regulation of important cell cycle regulators and apoptotic molecules, while the down-regulation may underlie upstream MAPK signaling. Thus, we suggest that H<sub>2</sub>O<sub>2</sub> stimulates rather proliferation of HCEC cells than apoptosis.

**Conclusions:** HCEC cells provide novel insights into oxidative stress transforming processes in cells of the intestinal tract, because these cells are more likely to represent the potential target for tumor initiation in vivo. We propose that H<sub>2</sub>O<sub>2</sub> is an universal inflammatory mediator in colorectal carcinogenesis.

## Fr-026

**AIM2 mediates IFN-gamma induced expression of HLA-DR and other interferon-responsive genes in colorectal cancers**

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**Aims:** Absent in melanoma 2 (AIM2), a member of the interferon- (IFN) inducible HIN-200 protein family, is associated with both, tumor pathology and innate immunity. We have previously demonstrated a high frequency of AIM2 mutations and gene silencing in microsatellite unstable (MSI-H) colorectal cancers (CRC). Because these tumors are often infiltrated by IFN-gamma-secreting lymphocytes resulting in better prognosis of patients, we here addressed the role of AIM2 in tumor cell fate.

**Methods:** We generated several constructs for stable or inducible expression of AIM2 fusion proteins in different cancer cell lines to study effects of AIM2 expression on cell proliferation, apoptosis, cell cycle, migration/invasion and gene expression pattern by using colony forming and WST1-proliferation assays, flow cytometry, invasion chambers, Affymetrix microarrays, quantitative real-time PCR, immunoblotting, and luciferase reporter assays.

**Results:** Restoration of AIM2 in CRC cells considerably decelerated cell growth by inducing G<sub>2</sub>/M cell cycle arrest and stimulated migration through extracellular matrix coated membranes. Down-stream effects of AIM2 expression included significant upregulation of 107 genes by more than 40%, among them a group of invasion/extracellular matrix regulating genes and a group of interferon-responsive genes (HLA-DRA, HLA-DRB, IFIT1, IFIT2, IFI6, ISG15, IRF7 and TLR3), all confirmed by real-time PCR and/or immunoblotting in different AIM2-expressing and IFN-gamma treated CRC cell lines. Our ongoing studies point to AIM2-mediated induction of MHC class II activator (CIITA) expression to be involved in induction of these IFN-responsive genes.

**Conclusions:** Our data suggest that AIM2 reduces CRC cell proliferation by cell cycle arrest, thereby inducing migration through ECM. At the same time, induction of IFN-gamma responsive target genes including HLA-DR is stimulated by AIM2 expression. Thus, AIM2 might alter the gene expression profile towards a phenotype that renders CRC cells more susceptible to anti-tumor immunity thereby providing a link between tumor infiltrating lymphocytes, IFN-gamma, and prognosis of patients.

#### Fr-027

##### **Prevalence of HPV-infection in colorectal cancer**

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**Aims:** According to the Vogelstein-Model, p53 inactivation plays a key role for the progression from colorectal adenoma to carcinoma. However, inactivation of p53 due to gene mutation is found in less than 50% of patients. Since in carcinomas of the oral cavity, TP53 gene mutation and p53 impairment by binding of human papilloma viral E6 protein are regarded as alternative mechanism for p53 inactivation, we investigated whether HPV infection may also play a role in colorectal carcinoma.

**Methods:** Primary colorectal adenocarcinoma from 62 patients (41 colon, 21 rectum; 22 stage II, 20 stage III, 20 stage IV) were screened for HPV-DNA using three independent multiplex PCR assays. Patient with known immunosuppression were excluded. DNA derived from 10 cervix carcinomas, 4 anal carcinomas, and 23 carcinomas of the oral cavity were used for comparison.

**Results:** Carcinoma of the control groups were positive for HPV-DNA in 90% (cervix uteri), 75% (anus), and 22% (oral cavity) of cases. HPV 16 was the most dominant (90%) subtype in these tumors. In contrast, none of the colorectal samples was characterized by HPV infection.

**Conclusions:** Currently, there is a great dissent in the literature regarding the prevalence of HPV infection in colorectal carcinoma with detection rates ranging from 0% to 90% which may to some extent reflect ethnical differences. According to our data, in German populations involvement of HPV in the development of colorectal cancer of immuno-competent patients represents at least a very rare event.

#### Fr-028

##### **Fourier transform infrared (FTIR) microscopy of intestinal mucosa and colonic neoplastic lesions**

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**Aims:** Our project aims at the identification of characteristic spectral biomarkers using spatial-resolved FTIR imaging to specify different neoplastic and preneoplastic colon lesions. This approach should provide a reliable, fast and perhaps earlier diagnosis of colorectal adenomas and carcinomas and will thus yield deeper insights into the development and effectiveness of new therapeutic approaches.

**Methods:** Frozen sections of intestinal mucosa of carcinomas, adenomas and normal mucosa were analysed by using FTIR-imaging. Furthermore, bioinformatical methods such as PCA and HCA were used in order to receive information for the different cell types.

**Results:** The first results show that we obtain characteristic spectra for the different cell types both of the healthy and the cancerous intestinal mucosa. These differentiations were performed by hierarchical cluster analysis and aligned with classical pathological approaches, thus providing helpful knowledge for the further training of artificial neuronal networks mainly aiming at an automated identification of the respective cell characteristics.

**Conclusions:** The spectral characteristics for different types of tissues were correlated with the corresponding pathology results. The intestinal mucosa was classified by FTIR-spectroscopy and bioinformatical methods like PCA and HCA. Complementary to the pathology results, we can show chemical changes at the molecular level.

#### Fr-029

##### **TTF-1 positive colon carcinoma diagnostic implications**

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**Aims:** We report on a 72 years old male with tumour in the left eye morphologically conform to a metastasis of a since 7 years known sigma carcinoma. Additionally lung cancer was diagnosed 1 year ago due to the fact of immunoreactivity for TTF-1, despite of morphological similarity to a colon carcinoma.

**Methods:** We examined tumour tissue from the eye, the locoregional relapse of the sigma carcinoma and from a bronchoscopy immunohistochemically with antibodies against CK7, CK18, CK20, CDX-2 and TTF-1 (clone 8G73G3/1).

**Results:** Tumour tissues of all locations showed an identical result with negativity for CK7 but clear positivity for CK18, CK20, CDX-2 and TTF-1 (clone 8G73G3/1). So an adenocarcinoma of the colon with aberrant positivity for TTF-1 and metastases to lung and eye was diagnosed.

**Conclusions:** Immunoreactivity for TTF-1 is generally considered suitable to prove lung cancer and to exclude colon carcinoma, at least by using clone 8G73G3/1. Our case demonstrates that this can be only a general rule. Even anti TTF-1 from clone 8G73G3/1 is not specific for thyroid or lung cancer and can be positive in a primary colon carcinoma and its metastases – as is already well known for clone SPT24. Despite of reactivity for TTF-1 additional immunohistochemistry (CK20, CDX-2) should be done in apparent primaries or probable metastases with morphological similarities to colon carcinoma.

#### Fr-030

##### **Correlation of molecular profiles and clinical outcome of stage UICC II colon cancer patients**

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**Background and Aims:** Published multi-gene classifiers suggested outcome prediction for patients with stage UICC II colon cancer based on several different gene expression signatures. However, there is currently no translation of these classifiers for application into routine diagnostic. Therefore, we validated own and published gene expression signatures and aimed to establish a protein expression classifier suitable for the application in routine diagnostic.

**Methods:** 68 stage UICC II colon cancer cases were investigated for the expression of five gene products (CDH17, LAT, CA2, EMR3, and TNFRSF11A) by immunohistochemistry. The cancer tissue of 53 of these 68 patients was profiled on Affymetrix GeneChips (HG-U133 Plus 2.0). Protein and RNA expression data were correlated with clinical data.

**Results:** For our immunohistochemical study we selected five genes which were previously reported to correlate with prognosis by gene expression profiling. However, by immunohistochemistry no correlation with clinical outcome could be observed. This result induced us to determine the expression profile of 53 of our cases by Affymetrix GeneChip analysis. Although we found genes which correlated with clinical outcome, no stable classification or prognosis signature could be established.

**Conclusions:** Our protein and gene expression analyses show that at present there is no robust molecular or immunohistochemical classifier available for the prediction of clinical outcome in patients with stage UICC II colon can-

cer. Further molecular studies are needed to develop robust prognosis signatures suitable for the application in routine diagnostic.

#### Fr-031

##### Detection of promotor hypermethylation of RASAL1 (RAS-activating-like-peptide1) in colorectal cancer

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**Aims:** GTPase activating proteins (GAPs) inhibit KRAS signaling by stimulating the intrinsic GTPase activity of KRAS. RAS-activating-like-peptide1 (RASAL1), a member of the GAP protein family, has been shown to inhibit KRAS activity in colorectal cancer. The aim of this study was to investigate if RASAL1 promotor hypermethylation occurs in colorectal cancer and leads to loss of RASAL1 expression and to correlate RASAL1 promoter hypermethylation with KRAS mutation status.

**Methods:** We examined RASAL1 promotor methylation in 205 colorectal cancer stage III patients using real-time PCR based quantitative promoter methylation analysis (MethyQESD). KRAS mutation status was determined by Sanger sequencing. RASAL1 expression was investigated both in RASAL1 hyper-methylated and unmethylated cancers and matched normal tissues by RT-qPCR, respectively. RASAL1 promoter methylation was also correlated with CIMP-phenotype.

**Results:** RASAL1 promotor hypermethylation was found in 6/205 cases (3%). RASAL1 methylation was significantly associated with wildtype-KRAS ( $p=0.043$ ) and was mutually exclusive to positive KRAS mutation status. Furthermore all cases with RASAL1 promotor hypermethylation showed a reduced expression of RASAL1 mRNA in cancer tissue compared to normal tissue ( $p=0.015$ ).

**Conclusions:** RASAL1 methylation is significantly associated with wildtype-KRAS and reduced expression of RASAL1 in colorectal cancer. Further studies are needed to clarify if RASAL1 promoter hypermethylation may be useful as an additional predictive marker for response to anti-EGFR targeted therapy.

## Unterer GI-Trakt II

#### Fr-032

##### Intratumoral budding (ITB) in colorectal cancer biopsy specimens is highly indicative of an advanced tumor stage in the corresponding resection specimens

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**Aims:** Peritumoral budding (PTB) is the histological hallmark of the epithelial-mesenchymal transition (EMT), associated with tumor progression and according to the AJCC/UICC an additional prognostic factor in colorectal cancer (CRC). Normally PTB is detected in CRC resection specimen, but the assessment of intratumoral budding (ITB) in preoperative biopsy specimens has not been systematically investigated yet. The aim of this study was to analyze the prognostic significance of ITB in preoperative biopsies and its implication on tumor progression.

**Methods:** 73 preoperative biopsy samples and the corresponding consecutive resection specimens were collected from the archive of the Institute of Pathology Aarau from 1990 to 2007. ITB and PTB were scored semiquantitatively as high (detectable at a low power magnification; 2.5x), intermediate (occasional budding at intermediate magnification 10x), low (difficult to find or absent) in all the biopsy samples and the consecutive resection specimens, respectively.

**Results:** In biopsy samples high ITB was observed in 17/73 patients (23.3%). Additionally, a high ITB score was associated with a high PTB in the resection

specimen (16/17 patients, 94.1%,  $p=0.014$ ), an infiltrating margin (13/17 patients, 76.5%,  $p=0.006$ ), lymph node metastasis (14/17 patients, 82.4%, specificity 50%, negative predictive value 90.3% and positive predictive value 33.3%,  $p=0.016$ ) and distant (10/14 patients, 71.4%,  $p=0.026$ ).

**Conclusions:** The assessment of ITB in preoperative biopsy predicts tumor aggressiveness and can be therefore implemented as an additional factor in the preoperative management of CRC patients.

#### Fr-033

##### Expression of FGF-2 is essential for the maintenance of cancer stem cell characteristics in human colorectal tumor cell lines

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**Aims:** Cancer stem cells (CSCs) are resistant to chemotherapeutics like 5-FU by an active export mechanism. Due to this trait CSCs enrich as side populations in FACS analyses. Colorectal CSCs (coCSCs) seem to be characterized by nuclear  $\beta$ -CATENIN as it leads to epithelial-mesenchymal transition (EMT), which was shown to induce stemness in formerly epithelial cells. Alternatively, coCSC can be isolated using the surface-markers CD44 and CD166 and kept in vitro strictly depending on the presence of FGF-2 in the culture medium. Since FGF-2 is a  $\beta$ -Catenin target gene and thus endogenously expressed in colorectal tumor cells, we hypothesized that this FGF-2 is essential for maintaining coCSCs.

**Methods:** 5-FU treatment, RNA interference (RNAi), real time PCR (qRT-PCR), Western Blot, transformation assay, mouse xenograft model, immunofluorescence, FACS.

**Results:** LoVo cells surviving 5-FU treatment showed 2–3 fold up-regulation of the stem cell markers CD44, CD166, an increase in EMT and expression of FGF-2. Vice versa, shRNA-mediated knockdown of FGF-2 in LoVo and Caco2 cells led to a strong reduction in anchorage independent growth as well as tumor formation after xenografting into nude mice. FGF-2 knockdown LoVo cells showed MET (mesenchymo-epithelial transition). Expectedly, these cells displayed an almost 90% reduction of the side population fraction.

**Conclusions:** We show that expression of FGF-2 is critical for maintaining the transformed phenotype as well as characteristics of stemness in CRC cells.

#### Fr-034

##### The subcellular localization of inhibitor of DNA binding (ID) proteins 1–4 changes differently in the carcinogenesis of colorectal cancer

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**Aims:** To investigate the expression of ID1, ID2, ID3 and ID4 proteins in the carcinogenesis of colorectal cancer (CRC).

**Methods:** Archival tissue specimens of normal epithelium (NE;  $n=126$ ), low- ( $n=37$ ) and high- ( $n=13$ ) grade intraepithelial neoplasia (IEN) and carcinomas (CA;  $n=126$ ) were stained for ID1–4 protein expression (immunohistochemistry) and evaluated for subcellular ID1–4 localization (cytoplasmic, nuclear). ID1–4 expression was correlated between NE, IEN, CA specimens and in CAs with clinico-pathological parameters and survival.

**Results:** Only few tissue specimens (<6%) were negative for ID1–4 expression. Significant nuclear to cytoplasmic shifts occurred for all IDs between NE, IEN and CA ( $p<0.0001$ ). Within carcinomas, no significant association was seen for ID1–4 localization with T and N categories or tumour differentiation. Nuclear ID1 ( $p=0.02$ ), ID2 ( $p=0.01$ ) and ID4 ( $p<0.001$ ) was linked to proliferation. Nuclear ID2 ( $p=0.002$ ) and ID4 ( $p<0.001$ ) localization correlated to Aurora-A expression in CAs. Cytoplasmic ID1, ID2, ID3 and ID4 was seen in 62%, 27%, 12% and 72% of CAs, respectively.

**Conclusions:** Nuclear ID localization is associated with proliferation, probably due to dimerization with bHLH and/or RB1. The distinct patterns of cytoplasm shifts of ID proteins within CAs suggests that ID1–4 may differentially change their function during CRC carcinogenesis and progression.

#### Fr-035

##### **ACSL5 function as modifier of intestinal pathways**

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**Aims:** The acyl-coA-synthetase 5 (ACSL5) is mitochondrial localized and activates long chain fatty acids. It is probably involved in several biochemical pathways like apoptosis and inflammation and development of colorectal cancer. The aim of the present study was to elucidate a putative crosstalk between ACSL5 function and established pathways in enterocytes.

**Methods:** Expression and activity of ACSL5 and genes involved in intestinal pathways were investigated in a cell culture model with molecular methods including qRT-PCR, Western blotting, reporter and activity assays. The data were proofed in an ACSL5 mouse model.

**Results:** Effects of ACSL5 on the signaling cascade were found in the canonical Wnt pathway which is involved in intestinal carcinogenesis. We also found ACSL5 dependent modification in lipid-activated transcription factors.

**Conclusions:** ACSL5 function is suggested as a putative modifying variable in established signaling cascades in the intestine. This association could be of relevance in the pathophysiological concept of several gastrointestinal disorders.

#### Fr-036

##### **hTERT (human telomerase RT-component) expression is regulated by $\beta$ -CATENIN in human colorectal cancer**

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**Aims:** Colorectal cancers (CRCs) are characterized by the activation of  $\beta$ -CATENIN due to mutations of components in the WNT-signaling pathway. When expressed in the nucleus it induces a program of proliferation, invasiveness, EMT (epithelio mesenchymal transition) and stemness. Lengthening of telomers is essential for tumor cells to gain the hallmark of eternal life. The function of the telomerase complex is regulated on the level of hTERT expression which is also found in adult stem cells. Thus, we hypothesized that hTERT is regulated by the activity of  $\beta$ -CATENIN in CRCs.

**Methods:** Immunohistochemistry, electrophoretic mobility shift assay (EMSA), chromatin immunoprecipitation (ChIP), luciferase-reporter assay, RNA interference (RNAi), quantitative PCR (qRT-PCR), Western Blot.

**Results:** Using immunohistochemistry, hTERT expression was found in tumor cells of CRCs at the invasive front which was accompanied by the nuclear localization of  $\beta$ -CATENIN. The hTERT promoter-enhancer contains four consensus TCF4 binding elements (TBEs) which interacted in vitro (EMSA) and in vivo (ChIP) with TCF4 or  $\beta$ -CATENIN, respectively. Finally, we demonstrate that hTERT-gene activity was directly regulated by  $\beta$ -CATENIN and TCF4.

**Conclusions:** In CRCs hTERT expression is mediated by  $\beta$ -CATENIN/TCF4 in a minority of mesenchymally organized tumor cells thus assigning another trait of stemness to these cells.

#### Fr-037

##### **Overexpression of DICER predicts poor survival in colorectal cancer**

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**Aims:** The RNASE III endonuclease Dicer is one of the key enzymes of microRNA biogenesis. The influence of DICER-expression in tumor cells on the prognosis of patients with several cancers has been studied with controversial

results among different cancer types. To date no one has examined this biomarker in colorectal carcinoma. Thus, we aimed to study the influence of Dicer expression on survival in colorectal cancer.

**Methods:** We performed immunohistochemical analyses on formalin-fixed paraffin embedded (FFPE) cancer tissue with an antibody against the DICER protein. Tumor material from 237 cases was available from patients with colorectal adenocarcinomas with moderate differentiation (G2) and without evidence of lymph-node (No) or distant metastasis (Mo). 64 cases were in T2 and 173 in T3 stage. A tissue microarray (TMA) was constructed with each tumor in triplicate. A Kaplan-Maier analysis was performed and the log-rank test was used for significance levels by using SPSS v.17 software.

**Results:** The expression of DICER in colorectal carcinoma shows a strong association with poor survival (cancer specific survival = CSS,  $p < 0,001$ ) as well as with reduced progression free survival (PFS,  $p < 0,001$ ).

**Conclusions:** Strong expression of the central microRNA biosynthesis enzyme DICER predicts poor prognosis in patients with colorectal cancer. This is in line with investigations on prostate cancer. Contradictory, in breast, lung and ovary cancer DICER has been shown to be a marker of good prognosis. Further studies on the cellular functions of DICER need to address these issues.

#### Fr-038

##### **CRM1 regulates EGFR expression in Colorectal Cancer in vitro and in vivo**

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**Aims:** Chromosomal region maintenance1 (CRM1) is a nuclear export factor for proteins relevant in tumor biology. Epidermal growth factor receptor (EGFR) regulates intracellular pathways that influence proliferation, cell migration, and survival. We aimed to investigate possible in vitro and in vivo interactions of CRM1 with EGFR in colorectal cancer.

**Methods:** CRM1 and EGFR expression were analysed in 355 human colorectal carcinomas by immunohistochemistry. Expression data was correlated with clinico-pathological factors and patient survival. Different colon cancer cell lines were investigated for protein levels of CRM1 and EGFR and incubated with Leptomycin B (LMB), a specific CRM1 inhibitor.

**Results:** Cytoplasmic and nuclear CRM1 expression was observed in 37.8% and 42.6% of colorectal carcinomas. Over-expression of cytoplasmic and membranous EGFR was observed in 26.2% and 22.3% of cases. High nuclear and cytoplasmic CRM1 expression was significantly associated with elevated EGFR protein levels. Cytoplasmic EGFR over-expression predicted a favourable patient prognosis ( $p = 0,024$ ). CRM1 as well as EGFR protein expression were observed in all investigated colon cancer cell lines. Inhibition of CRM1 resulted in a suppression of EGFR protein.

**Conclusions:** Our data suggest a role for CRM1 in the regulation of EGFR expression in colorectal cancer. Inhibition of CRM1 might influence the EGFR pathway and might modulate response to several novel EGFR inhibiting drugs.

#### Fr-039

##### **Modification of micro-RNAs miR-21, miR-143 and miR-145 expression in rectal cancer following neoadjuvant chemoradiotherapy**

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**Aims:** Colorectal cancer is associated with altered expression of micro-RNAs (miR). It has been shown that miR-21 is upregulated and that miR-143 and miR-145 are down-regulated in colorectal cancer. The aim of our study is to determine if these micro-RNAs change their expression levels in response to neoadjuvant chemoradiotherapy treatment.

**Methods:** 40 patients with advanced rectal cancer (clinical T3 or T4 or node-positive) were included. All patients underwent neoadjuvant chemoradiotherapy and surgical resection. Expression of miR-21, 143 and 145 was examined in macrodissected tumor tissue before and after chemoradiotherapy and normal rectal tissue (from the resection specimen). RNA was extracted from formalin-fixed and paraffin-embedded tissue by Trizol method, polyadenylated, reverse transcribed and subsequently analyzed by real time PCR.

**Results:** After treatment miR-21 showed higher expression levels in the tumor tissue than in the normal tissue. Compared to the tumor tissue before therapy the expression level decreased. For miR-143 and 145 treatment of the tumor resulted in an increase of expression for both micro-RNAs after treatment, while the tumor tissue had lower levels than the non-tumorous tissue.

**Conclusions:** The change of expression levels of microRNAs during the course of chemoradiotherapy in rectal cancer hints at their possible involvement in response to anticancer treatment.

#### Fr-040

##### Comparison of different methods for the analysis of codon G12 and G13 of the KRAS gene

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**Aims and Methods:** KRAS mutation screening has been achieved high importance in selecting the right therapy for patients with colorectal cancer and non-small-cell lung cancer especially in metastatic disease stage. Screening for KRAS mutations in these patients provide additional information on optimizing treatment options with targeted drugs. For comparative purposes, 72 mutated KRAS cases from 100 formalin fixed paraffin embedded colon or lung carcinoma tissue were selected as follows: G12A 5x, G12C 14x, G12D 14x, G12F 2x, G12R 1x, G12S 9x, G12 V 15x, G13D 8x, G13D +V14I 2x, G13E 1x, V14I 1x. These cases were investigated using 2 different methods: dideoxysequencing and pyrosequencing.

**Results:** By using the conventional laboratory protocol, we obtained a successful detection rate for dideoxy- and pyrosequencing of 94 and 92%, respectively. Three discrepancies out of 100 (3%) could be observed. In one case, we obtained a rare mutation (G13S) by dideoxysequencing while no mutation could be detected by pyrosequencing. The two other discrepancies were due to a double mutation in codon 12 (GGT → TTT; G12F) that is easy to read on sequence chromatograms but difficult to interpret (G12C) by pyrosequencing.

**Conclusion:** Discrepancies between the two methods were minimal. Both were accurate, whereas the major difference of these two methods in analyzing is the different approach with a numeric quantification using a cut off on the one hand and a visual approach on the other hand.

#### Fr-041

##### Pathological work-up after neoadjuvant treatment in rectal carcinoma: An interlaboratory test measuring interobserver agreement in assessing tumor regression grade

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**Aims:** Neoadjuvant therapy is considered standard practice for locally advanced rectal cancer. Several tumor regression grading systems have been proposed for use in histopathological evaluation of tumor response. In practice, their practicability varies, as we intend to demonstrate in this test.

**Methods:** Slides representing the tumor area of ten rectal carcinomas after neoadjuvant radiochemotherapy have been selected from the archives and submitted to seven Institutes of Pathology throughout Germany. Tumor regression grade for each tumor was assessed independently by seventeen participating pathologists according to six different grading systems. The degree of interobserver agreement was calculated using Kappa statistics.

**Results:** The overall  $\kappa$  scores range from 0.52 to 0.63 for the six grading systems corresponding to a moderate to good agreement. A newly created system came up with the best  $\kappa$  score. Regarding single categories, the extreme of

the spectrum in terms of complete regression delivers the highest  $\kappa$  score and causes least controversy.

**Conclusions:** So far, there is no standard system to assess tumor regression grade after neoadjuvant therapy of rectal carcinoma. However, this is essential, as pathological evaluation of tumor regression serves as a basis to further investigate the correlation between response and prognosis.

#### Fr-042

##### The prognostic role of histologically detected lymph node micrometastases in colorectal cancer

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**Aims:** The role of histologically detected lymph node micrometastases in colorectal cancer is still unclear. However, most studies did not differentiate between micrometastases (MM;  $\leq 2$  mm) and isolated tumor cells (ITC;  $< 0.2$  mm). Therefore, we performed a study investigating the clinical course of colorectal cancer patients with MM diagnosed during routine histopathological assessing.

**Methods:** A database research of our files was performed to identify cases with MM between 1995 and 2009 (N<sub>MM</sub>). Control groups with (No) and without macrometastases (N<sub>1</sub>) were built with otherwise equal characteristics in terms of date of diagnosis, age, gender, location, differentiation and pT-stage.

**Results:** 45 cases with MM have been identified. Including the control cases a total number of 135 patients were enrolled. The 5 year overall survival rates for N<sub>MM</sub>, N<sub>1</sub> and No were 62%, 60% and 78%, respectively. The Kaplan-Meier Curves were almost identical for N<sub>1</sub> and N<sub>MM</sub> whereas No patients showed a considerably favourable course. This difference was marginally significant ( $P=0.06$ ).

**Conclusions:** Histological detected lymph node micrometastases are associated with an adverse prognosis. The application of step serial section techniques to detect such metastases is therefore justified.

#### Fr-043

##### Lymph node number in locally advanced rectal cancer after preoperative 5-FU based radiochemotherapy (RCT): comparison of retrieval techniques

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**Aims:** For reliable postoperative staging after neoadjuvant RCT, adjuvant therapy decision and prediction of survival the number of examined lymph nodes (LN) is of utmost importance in rectal cancer. Recently, the median number of LN found has been described as 7.0 (Mekenkamp et al. AJSP 2009). We hypothesize that high quality of total mesorectal excision (TME) and of pathological work-up are key-determinants of sufficient LN retrieval.

**Methods:** LN in complete paraffin-embedded neoadjuvantly treated rectal cancer specimens (n=75) were compared with i.) gross LN dissection with and without perioperative arterial ink injection, and ii.) elution of whole perirectal fat by acetone with mechanical compression (n=10) (Basten et al. Pathologie, in press). Specimens are part of multicentric rectal cancer trials (e.g., CAO/ARO/AIO-04 and KFO 179, supported by DFG).

**Results:** The mean number of LN yielded by whole rectum embedding was 31 with 140–200 blocks prepared per case. After slicing and manual dissection the median LN count was 19 and 15 with and without ink injection. Process-

ing of fat acc. to ii. yielded 27 LN (median) per case and the number of tissue blocks ranged from 36 to 46 only.

**Conclusion:** High quality TME combined with elution and compression of whole perirectal fat is cost effective and yields a 3–4x increase of LN number compared to data of literature.

#### Fr-044

##### **A reliable immunohistochemical algorithm predicting distant metastasis in right-sided colon cancer**

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**Aims:** A test predicting distant metastases in colorectal cancer (CRC) would be relevant for the prognostication and the selection of patients for adjuvant chemotherapy. CRC with microsatellite instability (MSI) showed a low risk of distant metastases in previous studies. In addition, high expression of CD133 and  $\beta$ -catenin, which are related to cancer stem cells, might be predictive markers for distant metastasis according to recently published observations of our group. Based on these results we tried to establish a simple and reliable immunohistochemical algorithm to stratify the risk for distant metastasis in patients with right-sided CRC.

**Methods:** In a case-control study 57 cases of right-sided CRC specimens with synchronous distant metastasis were matched with 57 CRC without distant metastasis and a disease free survival of at least 5 years (stage I to III). Immunohistochemistry for hMLH1, as marker for sporadic MSI, and CD133 and nuclear  $\beta$ -catenin, as markers for cancer stem-cells, were applied to these cases and a diagnostic algorithm was defined.

**Results:** In the first step the tumors were stratified according to their hMLH1 expression. Loss of hMLH1 expression was significantly correlated with a very low risk of distant metastasis (1 of 19 patients; 5.3%;  $p=0.0003$ ). In hMLH1 positive cases combined high scores of CD133 and  $\beta$ -catenin exhibited a very high rate of distant metastasis (17 of 18 patients; 94.4%) whereas the risk of distant spread was intermediate for carcinomas with either low CD133 and/or low  $\beta$ -catenin expression ( $p=0.0007$ ).

**Conclusions:** By use of three well established immunohistochemical markers this algorithm allows to identify subgroups of CRC patients with extremely high and extremely low risk of distant metastasis, respectively.

#### Fr-045

##### **Endothelial VEGFR-3 expression in colorectal carcinomas is associated with hematogenous metastasis**

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**Aims:** Vascular endothelial growth factor receptor 3 (VEGFR-3) is a major inducer of lymphangiogenesis and probably angiogenesis. Since both processes are closely linked with tumor metastasis this study investigated the expression of VEGFR-3 in tumor-associated vessels in colorectal carcinomas (CRC) and evaluated its relevance for lymphogenous and hematogenous metastasis.

**Methods:** VEGFR-3 expression in normoxia and hypoxia was measured in tumoral (HCTEC) and corresponding normal (HCMEC) microvascular endothelial cells from 5 patients with CRC as well as macrovascular endothelial cells (HUVEC) using ELISA. The endothelial expression pattern of VEGFR-3 in 74 non-metastatic (No/Mo), lymphogenously-metastatic (N+) and hematogenously-metastatic (M+) CRC was immunohistochemically assessed.

**Results:** The VEGFR-3 expression pattern in HCTEC/HCMEC was individually different. The levels remained unchanged under hypoxia, in contrast to HUVEC where VEGFR-3 was downregulated. Positive VEGFR-3 expression was highly significantly associated with M+ tumors ( $p=0.0003$ ) but not with N+/- tumors. The majority of the detectable intratumoral VEGFR-3-positive vessels were of blood vascular origin. Whereas intratumoral lymphatic vessels were collapsed, VEGFR-3 positive peritumoral lymphatic vessels had mostly open lumina.

**Conclusions:** Colonic microvasculature seems to have a „hypoxia-resistant“ behaviour of VEGFR-3. VEGFR-3-positive tumor-associated blood vessels have a predominant significance in hematogenous metastasis of CRC. VEGFR-3-positive peritumoral but not intratumoral lymphatics could be the vascular type mediating lymphogenous metastasis.

#### Fr-046

##### **Down-regulation of CXCL1 Inhibits growth of colorectal liver metastases**

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**Aims:** To evaluate the possible role of CXCL cytokines for growth and invasion in colorectal liver metastases.

**Methods:** Gene expression profiling, shRNA knock down, in vitro proliferation and migration assays, Western blotting, qPCR, in vivo tumor growth in a xenograft model

**Results:** Examination of gene expression profiles between tumor cells of the invasion front and those from the inner parts of the tumor revealed an up-regulation of pro-angiogenic molecules at the invasion front which was further confirmed by qPCR. ShRNA mediated inhibition of CXCL1 showed inhibition of CXCL1 expression as judged by semiquantitative real-time PCR and Western blotting. In the proliferation assay, shCXCL1 cells in which CXCL1 expression has been ablated showed a much decreased proliferation rate compared with non-targeting controls (shNTC). ShRNA-mediated inhibition of CXCL1 resulted in 6-fold decrease in cell migration. ShNTC cells consistently showed levels of invasion similar to those observed for the parental LS174T cells. Transplantation of shCXCL1-, shNTC- or parental-LS174T cells to the livers of nude mice showed large tumors when using shNTC-cells similar to the parental LS174T cells whereas shCXCL1-cell clones seemed to have lost this tumorigenic potential.

**Conclusions:** Taken together, these data suggest that down regulation of CXCL1 results in reduced tumor growth and it may be a potential target to develop novel therapeutic strategies for some tumors.

#### Leber / Pankreas

#### Fr-047

##### **gp130-dependent pathways in host hepatocytes are important for liver repopulation in mice**

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**Aims:** Hepatocyte transplantation (HT) is an interesting tool for investigation of mechanisms involved in cellular engraftment, proliferation and in vivo selection to understand liver physiology and pathology. Here we aimed to generate an elegant model of liver repopulation and to evaluate the role of the IL-6/gp130 system.

**Methods:** Human alpha-1-antitrypsin (hAAT) transgenic mice were used to establish a highly efficient transplantation model. The established model was applied to mice carrying a conditional hepatocyte-specific deletion of the common IL-6 signaltransducer gp130 (gp130<sup>Δhepa</sup>).

**Results:** Bone-marrow-transplantation (BMT), partial hepatectomy (PH) and retrorsine treatment of recipient mice were used to optimise the in vivo selection of transplanted hepatocytes. BMT combined with PH was sufficient to induce a 30-fold increase in the number of transplanted donor hepatocytes, while additional retrorsine pre-treatment led to an up to 40-fold increase. WT-hepatocytes repopulated WT-recipients at the same rate as gp130<sup>Δhepa</sup> cells. In contrast, liver repopulation by transplanted cells was significantly enhanced in gp130<sup>Δhepa</sup> recipient mice. This was associated with higher proliferation of donor hepatocytes and more apoptosis in gp130<sup>Δhepa</sup> recipient livers.

Additionally, the acute-phase response was strongly induced after HT in WT recipients, but blunted in gp130<sup>Δhepa</sup> recipients. As a consequence significantly more liver remodelling, evidenced by stronger hepatic stellate cell activation and collagen accumulation, was found in gp130<sup>Δhepa</sup> mice after HT.

**Conclusion:** The here established HT-model can be efficiently applied to investigate cell specific mechanisms of liver repopulation and cell-cell interaction in the liver. Moreover we could show that gp130-dependent pathways in host hepatocytes are important to control liver repopulation.

#### Fr-048

##### Survivin is essential for in vivo regulation of apoptosis and proliferation in hepatocytes

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**Aims:** Survivin inhibits apoptosis and regulates mitosis and chromosome segregation. Liver regeneration is a complex process involving both proliferation and apoptosis. The role of survivin in liver regeneration is not well elucidated.

**Methods:** In a rat model 70% liver resection was used to study the role of survivin in liver regeneration. Survivin expression was quantified by RT-PCR, western blotting and immunohistochemistry. Apoptosis and proliferation were evaluated. Liver biopsies from 33 patients who underwent living donor liver-transplantation were studied. Due to the fact that global survivin KO mice die at 3.5 dpc we generated mice with a hepatocyte-restricted deletion of survivin (alb-survivin<sup>-/-</sup>). Cell restricted survivin deletion was conformed by laser microdissected nuclei followed by PCR. Proliferation, apoptosis and DNA-contents of hepatocyte nuclei were assessed.

**Results:** Survivin transcript and protein were significantly upregulated after 24–72 hours of partial hepatectomy and showed a significant correlation with proliferation but not with apoptosis. In humans survivin was nearly absent in donor and reperfused liver tissue but increased significantly after 5 to 7 days after transplantation and correlated with proliferation. Sections of the liver obtained from liver-specific survivin KO mice showed grossly enlarged nuclei with increased DNA contents. Basal proliferation and apoptosis of hepatocytes were enhanced compared to liver tissue obtained from wild-type mice.

**Conclusions:** Survivin is upregulated in human and in rodent liver regeneration and correlates with proliferation, thus indicating an association of survivin and cell division. In vivo deletion of survivin affects hepatocyte apoptosis and proliferation.

#### Fr-049

##### Crucial role of the AKT/mTOR signalling pathway in insulin-induced hepatocarcinogenesis

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**Aims:** To study the oncogenic effect of chronic and elevated secretion of insulin on hepatocytes in the presence of mild hyperglycemia, we developed a model of pancreatic islet transplantation into the liver via the portal vein. In this model, islets of a donor rat are transplanted into the liver of a recipient diabetic rat, with resulting local hyperinsulinism that leads to the development of preneoplastic lesions and hepatocellular carcinoma (HCC). Here, we assessed the functional relevance of AKT/mTOR cascade activation in hepatocarcinogenesis following islet transplantation into the liver.

**Methods:** Levels of AKT and its effectors were assessed via real-time RT-PCR, western blotting, and immunohistochemistry in preneoplastic and neoplastic lesions from transplanted rats. Human HCC cell lines were subjected to treatment with insulin and levels of proliferation and apoptosis were determined.

**Results:** AKT/mTOR pathway was progressively induced from preneoplastic lesions to HCC. In human HCC cell lines, insulin administration to the medium increased cell proliferation and reduced apoptosis via induction of the AKT/mTOR cascade. Insulin-induced growth was markedly reduced when

the same cells lines were concomitantly subjected to AKT silencing via siRNA.

**Conclusions:** Induction of AKT/mTOR pathway occurs early in the pancreatic islet transplantation model of liver cancer and might be an important candidate for innovative targeted therapies against human HCC associated with deregulated insulin levels.

#### Fr-050

##### Insulin deregulation promotes hepatocarcinogenesis by inducing a CpG methylator phenotype in a context of low genomic instability in a rat model

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**Aims:** People affected by diabetes mellitus and metabolic syndrome show an increased risk of hepatocellular carcinoma (HCC) occurrence, but the molecular mechanisms linking deregulated insulin and hepatocarcinogenesis are unknown. We generated a rat model in which chronic exposure of hepatocytes to elevated insulin levels (induced by pancreatic islet transplantation into the liver via the portal vein) leads to HCC development. Here, we determined whether insulin deregulation affects the levels of genomic instability and methylation in this model.

**Methods:** Genomic instability extent was assessed by random amplified polymorphic DNA (RAPD) and microsatellite analyses. Degree of methylation (genome-wide and at CpG islands) was determined via the cytosine extension assay. Levels of the main DNA methyltransferases (DNMTs) were assessed by western blotting.

**Results:** The rate of genomic instability was extremely low in preneoplastic lesions and HCC from transplanted rats. No changes in the levels of genome-wide DNA methylation were detected in preneoplastic lesions and HCC when compared with normal livers. In contrast, a rise in regional methylation at CpG islands occurred in rat preneoplastic lesions and HCC, and was paralleled by upregulation of the major DNMTs (DNMT1, DNMT3a, and DNMT3b).

**Conclusions:** The present data indicate that aberrant induction of insulin signalling might contribute to hepatocarcinogenesis by inducing an increase in methylation at CpG islands in a context of low genomic instability.

#### Fr-051

##### Hyperproliferative hepatocellular alterations after intraportal transplantation of thyroid follicles no carcinogenesis in a long-term trial

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**Aims:** Hyperproliferative hepatocellular lesions downstream of engrafted thyroid follicles emerge within three months after transplantation via the portal vein into the liver of thyroidectomized rats. The pre-neoplastic potential of these lesions was investigated in a long-term trial.

**Methods:** Lewis rats (male/female) (n=611) were divided into a main group (HG) and three control groups (KG1–3). Animals of HG (thyroidectomized and transplanted), KG1 (transplanted), KG2 (thyroidectomized) and KG3 (untreated) were killed after 6, 12, 18, 24, and 30 months. IGF-I, IGF-II, IGFBP1, IGFBP4, TGFα, EGF-receptor and the Bromodesoxyuridin-labeling-index were analyzed immunohistochemically and/or by mRNA-in-situ-hybridization.

**Results:** Hyperproliferative lesions were found downstream of the transplants throughout the entire duration of the experiment but only in the HG. In these lesions, IGF-I and TGFα were down-regulated, IGFBP1 was upregulated and EGF-receptor, IGFBP4 and IGF-II were unaffected. Interestingly, not a single

hepatocellular focus, induced by the follicle grafts, proceeded to a hepatocellular adenoma or carcinoma.

**Conclusions:** Triiodothyronine induces hyperproliferation of hepatocytes, but even stimulation for 30 months does not lead to the development of hepatocellular tumors. Therefore, we can assess the carcinogenic potential of this stimulation as being very low.

#### Fr-052

##### **Hepatocyte-specific deletion of the anti-apoptotic protein Mcl-1 triggers proliferation and hepatocarcinogenesis**

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**Aims:** To investigate the role of the anti-apoptotic Bcl-2 family member Myeloid cell leukemia-1 (Mcl-1) in liver homeostasis and hepatocarcinogenesis.

**Methods:** Conditional hepatocyte-specific Mcl-1 knockout mice (Mcl-1<sup>Δhep</sup>) were generated. Livers of Mcl-1<sup>Δhep</sup> mice were analyzed and compared to livers of control littermates (Mcl-1<sup>wt/wt</sup>) and heterozygous (Mcl-1<sup>fllox/wt</sup>) mice. Livers were studied morphologically (including immunohistochemistry), by expression analyses (qRT-PCR, immuno blotting), apoptosis assays, and comparative genomic hybridization (array CGH).

**Results:** Mcl-1<sup>Δhep</sup> mice revealed spontaneous apoptosis and severe liver damage. Chronically increased hepatocyte apoptosis coincided with strong hepatocyte proliferation and finally the development of hepatocellular carcinoma (HCC) in >50% of Mcl-1<sup>Δhep</sup> mice already at the age of 8 to 12 months, whereas Mcl-1<sup>wt/wt</sup> and Mcl-1<sup>fllox/wt</sup> mice lacked tumors. The tumors qualified as HCC which was confirmed by histology, expression of HCC markers and chromosomal aberrations.

**Conclusions:** The present study provides in vivo evidence that increased apoptosis of hepatocytes not only impairs liver homeostasis but also causes hepatocyte proliferation and hepatocarcinogenesis. Our findings have implications for the understanding of apoptosis-related human liver diseases.

#### Fr-053

##### **Endosialin expression is upregulated by interaction of hepatic stellate cells and hepatocellular carcinoma cell lines**

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**Aims:** Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide. We have recently shown that Endosialin, a marker of myofibroblastic differentiation, is overexpressed in fibrous septa of cirrhotic livers and along tumor vessels of human HCCs but not by the tumor cells themselves. Now, we aimed to analyze whether the interaction between HCC cells (HuH-7) and hepatic stellate cells (HSC) may alter Endosialin expression in vitro.

**Methods:** Immortalized HSCs and HuH-7 cells were cultured in DMEM Medium supplemented with 10% FCS and 4.5 g/dl glucose, either alone or in a 1:1 co-culture. After stimulation with Platelet Derived Growth Factor (PDGF, 50 ng/ml) or Transforming Growth Factor beta (TGF beta, 2 ng/ml) for 48 h, Endosialin mRNA expression was determined using realtime quantitative RT-PCR (qPCR) and Endosialin protein was detected by immunofluorescence for Endosialin. An Anti-alpha smooth muscle actin (ASMA) antibody was used for double fluorescence immunohistology to validate cellular localisation Endosialin expression.

**Results:** Unstimulated HSCs showed low levels of Endosialin mRNA and protein, while HCC cells were negative. Coculturing of untreated HuH-7 and HSCs resulted in a more than 2-fold upregulation (2.24-fold) of Endosialin mRNA. While stimulation of mono-cultured HSCs did not induce Endosialin mRNA (TGF beta 0.34-fold; PDGF 0.25-fold), stimulation of co-cultured

cells dramatically increased Endosialin expression (TGF beta 10.69-fold; PDGF 4.25-fold). Cytoplasmic Endosialin expression in HSCs was validated by co-staining with alpha smooth muscle actin.

**Conclusions:** Endosialin is expressed in (activated) HSCs but not in HCC cells and can be induced by profibrogenic factors. Additionally, the co-culture model suggests and interaction between HCC cells and HSCs for the upregulation of Endosialin expression, which either mediated by a yet undefined paracrine stimulation or a direct cell-cell interaction. Further functional analyses are needed to clarify the mode of Endosialin upregulation during liver fibrosis and hepatocarcinogenesis and its possible role as a therapeutic target.

#### Fr-054

##### **Acyl-CoA synthetase 5 promotes apoptosis in human hepatocytes by channelling acyl-CoAs into sphingolipid metabolism**

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**Aims:** Acyl-CoA synthetase 5 (ACSL5), a member of the ACSL gene family that activates fatty acids for utilization by numerous metabolic pathways, is the only ACSL isoform located on mitochondria and thought to be involved in apoptosis. Fatty acids up-regulate ACSL5 and increase apoptosis susceptibility in human hepatocytes, thus, we hypothesize that ACSL5 is a promoting factor in hepatocellular lipooptosis.

**Methods:** To investigate this mechanism, we used liquid chromatography, tandem mass spectrometry (LC-MS/MS), RNA interference and immunochromatological techniques.

**Results:** High ACSL5 activity decreased HepG2 cell viability and increased susceptibility to TRAIL and TNF $\alpha$ , whereas knock down of ACSL5 reduced apoptosis susceptibility in steatotic cells. Over-expression of ACSL5 in HepG2 cells increased synthesis of long-chain acyl-CoA by 50%. Ceramide and sphingomyelin levels were enhanced 2 to 3 fold.

**Conclusions:** Steatosis-induced up-regulation of ACSL5 promotes apoptosis in human hepatocytes which might be mediated by alterations in sphingolipid metabolism. We propose that ACSL5 could play a role in promoting fatty acid-induced lipooptosis as an important mechanism in fatty liver-related disorders.

#### Fr-055

##### **Expression of the extracellular matrix protein periostin in liver tumours and bile duct carcinomas**

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**Aims:** To study the relevance of Periostin, known to be involved in Epithelial-mesenchymal transition (EMT), in hepatocellular and bile duct cancer.

**Methods:** Immunohistochemical Periostin expression was semiquantitatively analyzed in normal liver tissue (n=20), hepatocellular carcinoma (HCC; n=91), liver-cell adenoma (n=9), focal nodular hyperplasia (n=13) and bile duct carcinomas (BDC; n=116) using tissue microarrays.

**Results:** Normal bile ducts, gall bladder epithelium and hepatocytes showed weak cytoplasmic periostin expression. There was strong epithelial periostin expression in 19/91 (20.9%) HCC and stromal periostin expression in 10/91 (11%). Epithelial expression in tumor cells was significantly associated with a higher tumor grade (p=0.048) and HBV infection (p=0.007). No periostin expression was found in benign liver tumors. Strong stromal periostin expression was detected in 78/116 (67.2%) BDC and strong epithelial expression in 39/116 (33.6%) BDC. Epithelial periostin expression was a prognostic factor for reduced overall-survival in univariate and multivariate analysis in patients with BDC.

**Conclusions:** The EMT protein Periostin is expressed in the stroma and epithelium of a subset of BDC and HCC. Epithelial Periostin expression is a

marker for malignant transformation of hepatocytes and a novel prognostic marker in BDC.

#### Fr-056

##### A lymphotoxin-driven pathway to hepatocellular carcinoma

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**Aims/Methods:** Hepatitis B and C viruses (HBV, HCV) cause chronic hepatitis and hepatocellular carcinoma (HCC) by poorly understood mechanisms. We aimed at understanding these mechanisms by analyzing HBV- and HCV-infected human liver tissue and generated transgenic mice mirroring HBV- or HCV-induced pathology.

**Results:** We show that the cytokines lymphotoxin (LT)  $\alpha$ ,  $\beta$  and their receptor (LT $\beta$ R) are increased in HBV- or HCV-induced hepatitis and HCC. Liver-specific LT $\alpha$  $\beta$  expression in mice causes liver inflammation and HCC linking sustained hepatic LT expression with hepatitis and HCC. HCC development depends on lymphocytes and I $\kappa$ B kinase  $\alpha$  expressed by hepatocytes but not on TNFR1. In vivo LT $\beta$ R stimulation implicates hepatocytes as the major LT-responsive liver cells. LT $\beta$ R inhibition in LT $\alpha$  $\beta$ -transgenic mice suppresses HCC formation.

**Conclusions:** Thus, sustained LT signaling represents a hitherto unknown pathway involved in hepatitis induced HCC.

#### Fr-057

##### Downregulation of AKAP12 in human hepatocellular carcinoma by epigenetic silencing

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**Aims:** We have previously shown a downregulation of the tumor suppressor A-kinase Anchoring Protein 12 (AKAP12) in human hepatocellular carcinoma (HCC) and in premalignant lesions. Here, we concentrated on epigenetic gene silencing that may cause the downregulation of AKAP12 in hepatocarcinogenesis.

**Methods:** BioCOBRA was used to achieve semi-quantitative DNA methylation data of the AKAP12 gene promoter in a panel of representative human samples (n=39), and in various cell lines. Consecutively, restoration experiments using 5-aza-2'-deoxycytidine (5-aza-dC) as demethylating agent were performed.

**Results:** BioCOBRA revealed a mean DNA methylation of 25.1% for HCC, 2.4% for cirrhotic liver (CL), and 6.6% for normal liver (NL), thus indicating a hypermethylation of the AKAP12 promoter in human HCC specimens. Mean DNA methylation levels of the cell lines: 91% (AKN-1), 34.9% (HepG2), 28.5% (Hep3B), 2.1% (HUH7), 1.5% (PLC) and 0% (HACL-1). Restoration experiments with two cell lines exhibiting highest DNA methylation levels, i.e. AKN-1 and HepG2, were performed and resulted in both cell lines in a decrease of promoter methylation paralleled by an increase in AKAP12 mRNA expression.

**Conclusions:** Our findings indicate that the previously described distinctive downregulation of AKAP12 in human hepatocarcinogenesis is due to epigenetic silencing of the AKAP12 promoter in HCC. Concerning CL, alternative epigenetic silencing mechanisms have to be taken into account.

#### Fr-058

##### Effects of induction and siRNA-mediated downregulation of lipid droplet-associated proteins in cell culture models of hepatocyte steatosis

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**Aims:** Hepatocellular lipid droplet (LD) accumulation is the most frequent liver pathology in western countries, and caused by a broad range of disorders such as alcohol abuse and metabolic syndrome. The LD-surface is governed by amphiphilic proteins of the PAT-family (perilipin, adipophilin, TIP47). Loss of adipophilin has been shown to inhibit fatty liver in mice. For human liver, we showed that other PAT-proteins, such as perilipin, are enrolled as well and differentially expressed.

**Methods:** The effects of induction of LD-accumulation with oleate and other adipogenic substances and of siRNA-mediated downregulation of PAT-proteins are evaluated in cell culture using immunofluorescence microscopy, immunoblot analysis and functional assays.

**Results:** In hepatocellular tumor-derived cells of the lines PLC, HuH7, Hep3B and HepG2, the PAT-proteins adipophilin and TIP47 surrounded small LDs. Upon lipid loading, Abhd5, caveolin-2 and CIDEA colocalized with growing sizes of LDs as well. Perilipin, however, which has been implicated in long term lipid storage, was not detected at LDs in normal or induced cultured cells. So far, in siRNA-mediated downregulation of adipophilin, TIP47 or both, no conspicuous upregulation of perilipin or other PAT-proteins was observed.

**Conclusions:** PAT-expression in common cell culture models show specific differences to human hepatocyte steatosis in vivo as in the case of perilipin. Cell culture models may be of value for the evaluation of LD-biogenesis, but not of chronic steatosis as it is most frequent in patients. Therefore, cell culture models are needed, which better reflect chronic hepatocyte steatosis.

#### Fr-059

##### Pancreatic-type acinar cell carcinoma of the liver: Clinicopathologic and immunohistochemical study of two cases of a rare unusual neoplasm

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**Aims:** Acinar cell carcinoma (ACC) is a rare neoplasm of the exocrine pancreas that is histologically distinct from salivary-type acinic cell adenocarcinoma. Anecdotal examples of this tumor have been reported in stomach, ampulla of Vater and jejunum, but their histogenesis remains unclear.

**Methods:** We describe two patients with hepatic neoplasms strikingly similar to pancreatic ACC, but with no evidence of a primary pancreatic tumour.

**Results:** The first patient was a 68-years-old woman with a 7 cm mass resected from segment 3 of the liver. No further treatment was recommended and the patient remained disease-free 38 months later. The second patient, a 68-years-old man, was diagnosed with multifocal bilobar liver lesions measuring up to 4.3 cm. Core needle biopsies were obtained. Both tumours showed a striking similarity to pancreatic ACC. Immunohistochemistry was consistent with pancreatic acinar differentiation. There was no evidence of liver cirrhosis or pancreatic tumour.

**Conclusions:** The findings in the two tumours are consistent with ACC. Absence of a primary pancreatic tumour favours a primary liver neoplasm. ACC of the liver probably arises by trans-differentiation from biliary stem cells or from ectopic pancreas tissue. Hepatic ACC might have been given different descriptive names in the past and might thus be under-recognized. Our two cases were seen prospectively during routine activity over a period of four years and both have been initially classified as unusual variants of adult hepatoblastoma.

#### Fr-060

##### **Immunohistochemical expression of interferon-stimulated genes in hepatitis C: a possible predictive role?**

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**Background:** Hepatitis C virus (HCV) is a major cause of chronic liver disease worldwide. The therapy is a combination of interferon (IFN) and Ribavirin, with a sustained viral response (SVR) in 50–60% of cases. IFN achieves its potent antiviral effects through the regulation of hundreds of IFN-stimulated genes (ISGs). IFN induces ISG transcription by activating the JAK-STAT pathway. Nonresponders show high mRNA levels of ISGs before the therapy. **Aims:** To correlate the immunohistochemical (IHC) expression of ISGs with the response to IFN-therapy.

**Methods:** 116 liver biopsies of 98 HCV-patients (44 F, 54 M) were taken at different times after the beginning of IFN-therapy (4, 16, 48, 96, 144 hours, 4, 8, 12 and 96 weeks). We performed IHC stainings for glypican 3 (GPC3), pSTAT1 and SOCS3. To characterize the inflammatory infiltrates we also performed stainings for CD3 (T-cells), CD20 (B-cells), CD56 (NK-cells) and CD68 (Macrophages). The number of positive cells for each marker was counted and afterwards correlated with the response to IFN at different times.

**Results:** There was a significant difference in the number of GPC3+ cells ( $p < 0.001$ ) between responders and not-responders at 4, 12 and 96 weeks. On the other hand, no significant difference ( $p = 0.619$ ) was found before and after the therapy. By a cut-off of 60 GPC3+ cells, the response to therapy could be predicted in 84.1% of the cases. An association between pSTAT1-positivity and activation of macrophages was seen ( $p < 0.001$ ).

**Conclusions:** The IHC analysis has shown a „preactivation“ of some ISGs (GPC3 and pSTAT1) in nonresponders. The mechanisms by which GPC3 influences the response are not clear. It could be involved in the control of the receptor-ligand interactions or play a role in macrophage recruiting, as suggested by the dramatic increase of CD68+ cells in biopsies with high pSTAT1 count. It is tempting to speculate that the determination of ISGs may help in select responders to IFN therapy.

#### Fr-061

##### **The role of tumor suppressor microRNAs in hepatocellular carcinoma**

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**Background and Aims:** MicroRNAs (miRNAs) are small noncoding, endogenous RNAs that function as post-transcriptional regulators, involved in many fundamental biological processes and in carcinogenesis. By previous miRNA profiling, we identified miR-125b and miR-143/145 as downregulated miRNA species in HCV-positive hepatocellular carcinoma (HCC). In the present approach we now address the function of miRNAs in hepatocellular carcinogenesis.

**Methods:** miRNA levels were analyzed in Huh7, Hep3B, HepG2, and Pop10 hepatoma cells and in different stages of the progression of HCC in HCC-developing mice by real-time PCR. AgoMiR-oligonucleotides, mimicking miR-143, miR-125b and miR-29c, respectively, were transfected into hepatoma cells and the agoMiR function was shown by reporter assays. Cell growth and apoptosis were determined.

**Results:** miR-125 and miR-143/145 expression level was repressed in all hepatoma cells tested. miRNA expression level in the knock-out mice, were progressively downregulated. In order to compensate this reduced miRNA levels in hepatoma cells, Pop10 and Huh7 cells were treated with the respective miRNA mimics. Overexpression of miR-143/145 and miR125b resulted in reduction of cell growth. Screening of databases identified various mitogenic pathways as putative targets of these miRNA. Reporter assays of selected 3'-UTR-regions identified the lin-28 transcript as miR-125b target.

**Conclusion:** miR-125b and miR-143/145 mediating reduced hepatocellular cell growth are suggested to be tumor suppressor miRNAs. The repression of miR-125b, resulting in upregulation of the lin28 axis as modulator of cell growth and differentiation, is considered to trigger hepatocellular carcinogenesis.

#### Fr-062

##### **Histomorphological and immunohistochemical characterisation of anaplastic carcinomas of the pancreas with or without osteoclast-like giant cells**

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**Aims:** Characterisation of a large series of anaplastic carcinomas of the pancreas with special emphasis on precursor lesions, growth pattern, and local spread, as well as characterisation of intratumoral osteoclast-like giant cells (OCGCs).

**Methods:** Histomorphological and immunohistochemical analysis of 15 anaplastic carcinomas of the pancreas.

**Results:** A significant number of tumors were found to contain PanIN lesions, some of them showing transformation into a carcinoma of ductal phenotype, followed by de-differentiation into an anaplastic pattern. The tumors displayed invasive growth patterns with infiltration of nerves, blood vessels and lymph vessels. 7 tumors contained OCGCs which, among others, expressed CD68, cathepsin K and TRAP, similar as found in intratumoral monocytes. Various cytokines and chemokines influencing the differentiation of monocytes and OCGCs were found to be produced in the tumor microenvironment by tumor infiltrating inflammatory cells and/ or by tumor cells.

**Conclusions:** In one of the largest series available to date we show that anaplastic carcinomas of the pancreas derive from pancreatic ducts. Furthermore we provide evidence that OCGCs derive from monocytes that differentiate into OCGCs in a multidimensional process which seems to be triggered by tumor-associated inflammation.

**Fr-063****Molecular marker for the prognosis of pancreatic carcinoma**

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**Aims:** Prognosis for patients with pancreatic carcinoma (PDAC) remains poor. Despite of increasing knowledge about the molecular basis of PDAC neither a specific marker for early diagnosis nor a target protein for a new therapeutic approach have been identified so far. However, reliable prediction of survival might help to improve the therapeutic outcome for patients with PDAC.

**Methods:** We analysed the gene expression profile of 29 PDAC using the Affymetrix GeneChip U133 2.0 array. We correlated gene expression values with postoperative survival time using a novel computational approach which incorporates background knowledge. Candidate genes were further validated using RT-PCR and immunohistochemistry of tissue microarrays containing cores from 458 patients with PDAC.

**Results:** We identified a signature of 8 genes, which were able to predict the survival of the patients with 70% accuracy. RT PCR and immunohistochemistry confirmed the findings of our gene expression experiments for most of the genes.

**Conclusions:** The combination of gene expression analysis, novel bioinformatic approaches and large-scale immunohistochemistry with TMAs is suited to identify novel marker genes for prognosis of PDAC patients.

**Fr-064****SIRT1 expression indicates poor prognosis in patients with pancreatic adenocarcinomas and correlates with tumor grade and the expression of conventional HDACs**

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**Aims:** Several lines of evidence indicate that the nuclear enzyme SIRT1 belonging to the class III histone deacetylases is implicated in the initiation and progression of various malignancies. SIRT1 exerts its function by regulating deacetylation and methylation of histone and non-histone substrates leading to transcriptional repression of tumor suppressor genes. Only recently, SIRT1 gained attraction as drugable target. Since data on SIRT1 expression in pancreatic cancer are lacking and treatment options are still very limited we aimed at investigating the expression profile and the putative role of SIRT1 as predictive and prognostic biomarker.

**Methods:** Employing immunohistochemistry SIRT1 expression was analyzed in a population-based cohort of 129 pancreatic ductal adenocarcinomas and subsequently correlated expression patterns with clinicopathological data and patient survival.

**Results:** We found nuclear SIRT1 expression in 36 (27.9%) pancreatic carcinomas. SIRT1 expression was significantly higher in poorly differentiated carcinomas ( $p=0.002$ ), other correlations were not observed. SIRT1 expression was positively correlated with the expression of conventional HDACs suggesting a shared regulation. Strong SIRT1 expression was a significant predictor of poor survival both in univariate ( $p=0.002$ ) and multivariate (HR 1.65,  $p=0.045$ ) analysis under inclusion of WHO stage and grade.

**Conclusions:** The data presented here 1) strongly argue for a prospective study to fully evaluate the predictive potential of SIRT1 and 2) provide a promising background for future studies to elucidate the specific function of SIRT1 in the origin and progression of pancreatic cancer.

**Fr-065****L1CAM expression in pancreatic ductal adenocarcinoma and in pancreatic intraepithelial neoplasia**

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**Aims:** The adhesion molecule L1CAM (CD171) has been found to account for chemoresistance and increased cell migration. Being expressed in various neoplasms, it has furthermore been postulated to represent a promising target for a specific tumor therapy. To determine the role of L1CAM in pancreatic ductal adenocarcinoma (PDAC), we investigated a comprehensive series of primary and metastatic PDAC, as well as pancreatic intraepithelial neoplasia (PanIN) lesions.

**Methods:** 110 primary PDAC, 15 lymph node metastases and 14 liver metastases, as well as 126 PanIN lesions of various grades were investigated immunohistochemically.

**Results:** L1CAM expression was detected in 92.7% of primary PDAC, 80% of lymph node metastases, and 100% of liver metastases. Tumor stage, grade, or survival did not significantly correlate with L1CAM expression. L1CAM was expressed in PanIN, where the frequency of expression significantly increased with the grade.

**Conclusions:** L1CAM is likely to play a role in the initiation and progression of PDAC, probably contributing to the aggressive phenotype and the chemoresistance of these tumors. Furthermore, L1CAM represents a promising novel target for a specific therapy of PDAC.

**Poster: Hämatopathologie****Thymus****Fr-066****Evaluation of efficient targeted therapies for the treatment of thymic malignancies**

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**Aims:** Primary thymic malignancies are rare and no efficient targeted therapeutic approaches are currently available. We evaluated novel therapeutic strategies using two recently established thymic tumor cell lines.

**Methods:** Different inhibitors were employed to investigate the anti-tumorigenic effects and molecular mechanisms of Hsp90 inhibition. A pharmacological approach was performed to evaluate the efficacy of targeting select Hsp90-clients independently. In vitro effects on cell viability, cell cycle, apoptosis induction and signaling pathways were analyzed using MTT-assays, FACS analyses, and Western immunoblotting. IGF-1R expression was investigated in human thymic tumor samples using immunohistochemistry and tissue micro-arrays.

**Results:** Hsp90 inhibition induces multimodal antitumorigenic effects, i.e. induction of cell cycle arrest and apoptosis, and abrogation of invasiveness, associated with the degradation of EGFR, IGF-1R, CDK4, Akt, and RAF-1. Independent inhibition of these clients revealed that targeting IGF-1R also elicits strong anti-tumorigenic effects. IGF-1R is overexpressed in thymic tu-

mors. IGF-1R inhibition activated compensatory signaling pathways. Hsp90 inhibition circumvents the activation of compensatory signaling pathways.

**Conclusions:** We have identified novel targeted therapeutic strategies for the treatment of thymic tumors. Our data suggest that Hsp90 inhibition represents a highly promising therapeutic option in thymic malignancies.

## Lymphozyten / Lymphome allgemein

### Fr-067

#### Increased phosphorylation of ribosomal S6 protein in mitotic human lymphoma cells

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**Aims:** The localization and the upstream regulators of phosphorylated ribosomal S6 protein (p-rpS6) suggest a role for this protein in the regulation of protein synthesis and cell proliferation. Our goal was to investigate the expression of p-rpS6 in cycling non-neoplastic lymphoid and lymphoma cells.

**Methods:** The expression of p-rpS6 and pHH3 (a known mitotic marker) were studied by double immunocytochemistry labelling, fluorescent microscopy, flow cytometry and Western blot in different lymphoma cell lines after different treatments. Upstream regulators of rpS6 were analysed by p-mTOR and p-S6 K immunocytochemistry. p-rpS6 and pHH3 immunohistochemical stainings were carried out on tissue microarray (TMA) of several malignant and normal tissues.

**Results:** High expression levels of pHH3, p-rpS6 and its upstream regulators were found in different mitotic lymphoma cells by immunocytochemistry, flow cytometry and Western blot analysis. However, mitotic cells were consistently p-rpS6 negative in all examined non-lymphoid tumor types (TMA). Rapamycin, a known inhibitor of mTOR kinase significantly decreased the mitotic p-rpS6 levels in lymphoma cells.

**Conclusions:** Our findings suggest the ribosomal S6 protein to be a promising target in future therapeutical approaches of hematologic malignancies.

## B-Zellen

### Fr-068

#### Atypical mantle cell lymphoma or CLL/B-PLL with aberrant immunoreactivity for cyclin D1 ?

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**Aims:** We report on a 70 years old female with clinical symptoms of B-CLL since 3/2006 (Binet C) and initially 12,500 leukocytes/nl. The clinical course was indolent with 18,950 leukocytes/nl (79 % lymphocytes), Hb 13.5 g/dl and 50,000 thrombocytes/nl in 9/2009. Because of decreasing thrombocytes (with splenomegaly) the patient was re-evaluated at our hospital.

**Methods:** Cytological, FACS and FISH analyses of blood and bone marrow were done as well as histological and immunohistological examinations of a bone trephine.

**Results:** Most nuclei of blood lymphocytes (>55%) showed prominent solitary nucleoli and grooves. Smudge cells were visible. FISH bone marrow: trisomy 12, del17p, t(11;14) were detectable; FACS: positivity for CD19, CD20, CD43, FMC7, kappa; negativity for CD5, CD38, CD103, CD11c. Bone trephine (70% infiltration): Immunoreactivity for CD20, cyclin D1, CD79a and BCL2; negativity for CD5 and CD23.

**Conclusions:** This lymphoma shows transitions of CLL, B-cell prolymphocytic leukaemia and mantle cell lymphoma concerning morphology, cytogenetic findings and immunophenotype. Such cases are usually subsumed as atypical mantle cell lymphomas because of immunoreactivity for cyclin D1 or t(11;14). But this is only an agreement. It should be considered whether such

lymphomas are better diagnosed according to morphology with the additional indication of aberrant immunoreactivity for cyclin D1.

### Fr-069

#### Rare BCL2 gene rearrangement in follicular lymphomas of Iran – a country with very low incidence rate of this lymphoma type

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**Background and Aims:** BCL2 gene rearrangement has been considered as a fundamental genetic aberration in pathogenesis of follicular lymphoma (FL) with reported incidence rate from 96% to 31%. The detection rate depends highly on the detection technique and geographical region. Wide geographical difference in incidence rate of FL raises the question of variable molecular pathophysiology for this lymphoma in different geographical areas. In our current study we investigated FL cases from Iran a country with very low incidence rate for this lymphoma - for BCL2 gene rearrangement with FISH assay which seems to be the most sensitive and specific method for the detection of BCL2 breaks.

**Methods:** We identified 11 follicular lymphomas by reviewing 160 NHL lymphoma cases from the archives of Faghihi Hospital and Khalili Hospital in Shiraz and applied Vysis LSI BCL2 Dual Color Break Apart Probe with interphase FISH technique on paraffin-embedded tissue sections.

**Results:** BCL2 break was identified only in one of our 11 FL cases (9%). This is significantly less than the rate of this rearrangement in western.

**Conclusions:** Our results suggest that the molecular pathogenesis of FL differs in Iran from that observed in Western countries. This finding highlights the significant geographical variation in biology of FL.

### Fr-070

#### M protein deposition in the skin a rare manifestation of Waldenström macroglobulinemia

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**Aims:** Waldenström macroglobulinemia (WM) is a low-grade B-cell lymphoproliferative disorder accounting for approximately 2% of hematologic neoplasms. Although immunoelectrophoresis may present characteristic elevation of M gradient, diagnosis is often hampered by uncharacteristic symptoms and by little significance of randomly taken trephine biopsies. However, in these cases IgM deposited in tissue may lead to the diagnosis. Here, we present the case of a patient with imposing dermal IgM deposition.

**Methods:** Dermal lesions were documented by the dermatologists and histomorphologic evaluation of bone marrow and skin specimens was performed at the Institute of Pathology.

**Results:** In the bone marrow biopsies, a diffuse and nodular lymphoplasmocytic infiltrate with partial monotypic expression of the  $\kappa$ -light chain and IgM heavy chain was seen. Dermal lesions were characterized by clustered glassy papules on both knees and buttocks. Histomorphologically, these papules consisted of nodular IgM deposits.

**Conclusions:** WM is a rare lymphoproliferative disorder. According to the literature, dermal IgM deposition has only been described in single cases. Though, as seen in this report, accurate examination of the skin may lead to initial diagnosis.

**Fr-071****Two unusual EBV- and HHV-8 associated lymphoproliferative disorders occurring after solid organ transplantation.**I. Anagnostopoulos<sup>1</sup>, R. Trappe<sup>2</sup>, K. Jöhrens<sup>1</sup><sup>1</sup>Institut für Pathologie, Charité, Campus Mitte, Berlin<sup>2</sup>Medizinische Klinik mit Schwerpunkt Hämatologie und Onkologie, Charité, Campus Virchow, Berlin

**Aims:** Epstein-Barr virus (EBV) and human herpesvirus type 8 (HHV-8) share the ability of infecting lymphocytes and inducing a spectrum of lymphoproliferations. Here we describe 2 patients with solid organ transplantation who developed peculiar lymphoproliferations associated with an infection of both viruses.

**Methods:** Lymph node specimens were analyzed by conventional histology, immunohistology and in-situ hybridisation for EBER detection (EBER-ISH).

**Results:** Case 1: The lymph node showed a focal Kaposi's sarcoma manifestation and in addition morphological features of the plasma cell type of Castleman disease. The plasmablasts and plasma cells within the mantle zones showed a polytypic expression of the Ig light chains with a predominance of the lambda-positive cells. A low number of plasma cells expressed the LANA-1 of HHV-8. EBER-ISH revealed several positive cells morphologically corresponding to plasmablasts and occasional plasma cells. Case 2: The lymph node showed features of a germinotropic lymphoproliferative disorder. All plasmablasts expressed LANA-1 and EBER transcripts.

**Conclusions:** The presence of a coinfection with EBV and HHV-8 in patients after solid organ transplantation seems to be a rare phenomenon. However a careful investigation of such specimens for the presence of both viruses is indicated.

**Fr-072****T-bet expression by the tumor cells of hairy cell leukemia correlates with Interferon gamma production**K. Jöhrens<sup>1</sup>, V. Moos<sup>2</sup>, T. Schneider<sup>2</sup>, I. Anagnostopoulos<sup>1</sup><sup>1</sup>Institut für Pathologie, Charité, Campus Mitte, Berlin<sup>2</sup>Abteilung für Gastroenterologie, Rheumatologie und Infektiologie, Charité, Campus Benjamin Franklin, Berlin

**Aims:** Hairy cell leukemia (HCL) is an uncommon B-cell malignancy with unknown pathogenesis. In an earlier study we demonstrated that HCL cells highly express the transcription factor T-bet, the master regulator of the Th1 cell response regulating IFN- $\gamma$  production. Here we aimed to investigate whether the neoplastic hairy B cells also possess the ability to produce IFN- $\gamma$ .

**Methods:** IFN- $\gamma$  concentrations in the sera of 5 HCL patients and 55 healthy subjects were quantified by a cytometric bead array. Cytokine secreting T and B cells were determined through short-term stimulation and subsequent analysis by four-color FACS. CD3<sup>+</sup>CD19<sup>+</sup> or CD3<sup>+</sup>CD103<sup>+</sup> B cells, respectively, and CD3<sup>+</sup>T cells were analyzed separately. At least 50000 T cells and B cells were analyzed using following antibodies: CD3, CD19, IFN $\gamma$ , TNF $\alpha$ , IL-2, IL-4 and IL-10, CD103, and T-bet.

**Results:** Neoplastic cells from the peripheral blood of five patients with HCL showed an enhanced expression of IFN- $\gamma$  after stimulation. Additionally, a comparison with 55 healthy individuals revealed a significant elevation of IFN- $\gamma$  in the sera of HCL patients.

**Conclusions:** Based on our recent findings that a non-neoplastic B cell subset, the monocytoid B cells, are T-bet positive and produce IFN- $\gamma$  we propose that monocytoid and hairy B cells have a similar function and that the T-bet-IFN- $\gamma$  axis is involved in the pathogenesis of HCL.

**Fr-073****Diagnostic and prognostic utility of PD-1 in B cell lymphomas**

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**Aims:** Programmed death-1 (PD-1), a member of the CD28 co-stimulatory receptor superfamily, is physiologically expressed by germinal center-associated helper T-cells and acts as a negative regulator of the immune system. PD-1 is specifically expressed by tumor cells in angioimmunoblastic T-cell lymphoma and is a postulated diagnostic marker in chronic lymphocytic leukemia (CLL/SLL). Furthermore, a recent study suggests prognostic importance of PD-1 in follicular lymphoma (FL). We assessed the diagnostic potential as well as the possible prognostic importance of PD-1 in B-cell lymphomas.

**Methods:** Distribution of PD-1+ lymphocytes in various B-cell lymphomas was studied on tissue microarray (TMA) platforms encompassing 671 cases. Correlation with known biologic and clinical key data was performed. Prognostic cut-off scores were determined by receiver operating curve analysis.

**Results:** PD-1+ tumor-infiltrating lymphocytes were numerous in marginal zone lymphomas and FL. Their amount decreased from FL grade 1 to grade 3 and to FL with transformation to diffuse large B-cell lymphoma. Contrary to previously published results, we could not confirm PD-1 positivity of neoplastic cells in CLL/SLL. PD-1 tumor-infiltrating lymphocytes above the prognostic cut-off score (158/mm<sup>2</sup>) was a positive prognostic factor of disease-specific survival (DSS) in FL-patients (p=0.020), independent of increased FOXP3+ cells and age.

**Conclusions:** Our results show that PD-1 has no diagnostic value in CLL/SLL. Increased numbers of PD-1+ tumor-infiltrating lymphocytes above the prognostic cut-off score are associated with significantly improved DSS in FL and may thus be useful to predict the heterogeneous clinical behavior of FL.

**Fr-074****c-Myc expression in mature aggressive B-cell lymphoma in correlation with MYC chromosomal breaks and molecular diagnosis**M. Szczepanowski<sup>1</sup>, W. Klapper<sup>1</sup>, S. Bentink<sup>2</sup> for the German Cancer Aid project "Molecular Mechanisms In Malignant Lymphoma"<sup>1</sup>Department of Pathology, Hematopathology Section and Lymph Node Registry, University of Kiel, Kiel, Germany<sup>2</sup>Institute of Functional Genomics, University of Regensburg, Regensburg, Germany

**Aims:** The goal was to correlate c-Myc mRNA and protein expression with MYC translocation status and molecular diagnosis in Burkitt (mBL) and Diffuse Large B-cell lymphoma (non-mBL and intermediate lymphomas) and to evaluate Myc expression as an alternative diagnostic method to screen for diagnostically and prognostically important MYC-translocations.

**Methods:** Myc expression was measured by immunohistochemistry, quantitative RT-PCR and gene expression profiling (GEP) in 21 molecularly characterised B-NHL (Hummel et al., 2006) without MYC-break (MYC-negative, n=5), MYC-Immunoglobulin translocations (IG-MYC, n=11) or MYC-translocations with non-Immunoglobulin genes (non-IG-MYC, n=5).

**Results:** Lymphomas with IG-MYC translocations displayed higher Myc expression compared to MYC-negative and non-IG-MYC lymphomas by qRT-PCR (p=0.0194, ANOVA), GEP (p=0.012, ANOVA) and immunohistochemistry, with the latter yielding no statistically significant results. Restricting the analysis to non-mBL/intermediate lymphomas, Myc expression did not show statistically relevant differences between MYC-negative lymphomas and lymphomas with any kind of MYC-translocation. Although average values of Myc expression were generally higher in MYC-translocation positive lymphomas, a subset of MYC-negative non-mBL expressed Myc at similar levels as lymphomas with MYC-translocations.

**Conclusions:** Detection of MYC-translocations and determination of the MYC translocation partner are of great importance for the diagnosis of BL and as a prognostic marker in non-mBL mature aggressive B-NHL. Herein, we demonstrate, that all three tested methods (immunohistochemistry, qRT-PCR and GEP) do not allow reliably to discriminate between mBL and non-

mBL, non-mBL with or without MYC-translocations or MYC-translocation type on the single case level. Thus, detection of MYC translocations and translocation partners by cytogenetics or FISH remains the gold standard for diagnosis and prognostication in aggressive B-NHL.

#### Fr-075

##### **Cyclin D1 transcripts in mantle cell lymphoma and CyD1+ myeloma cell lines**

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**Aims:** The Cyclin D1 (CyD1) protein is overexpressed in mantle cell lymphoma (MCL) and a subset of myeloma (MM). The identification of differential expression of CyD1 mRNA variants raises questions regarding the role it plays in pathogenesis. We analysed CyD1 mRNA variant expression levels, genomic CyD1 locus and A870G genotype in pre-existing lymphoma cell lines with CyD1 overexpression.

**Methods:** 8 MCL cell lines (Granta 519, HBL-2, UPN-1, Jeko-1, Rec-1, JVM-2, NCEB-1, Mino) and 2 MM cell lines (KMS 12, U266) were analysed by real-time RT-PCR for expression levels of total CyD1 (relative to TBP as reference gene),  $\Delta 3'$ UTR CyD1, CyD1 $\alpha$  and CyD1 $\beta$  variants. CyD1 locus and A870G genotype was analysed by genomic PCR and RFLP.

**Results:** MM cell lines (KMS 12 and U266) and three MCL cell lines (Granta 519, JVM-2, NCEB-1) expressed both CyD1 $\alpha$  and  $\Delta 3'$ UTR CyD1 transcripts. In three MCL cell lines (Jeko-1, Rec-1 and UPN-1)  $\Delta 3'$ UTR CyD1 mRNA was exclusively found, accompanied by deletion of  $3'$ UTR locus in Jeko-1 and UPN-1. HBL-2 and Mino did not express  $\Delta 3'$ UTR CyD1. CyD1 $\beta$  splice variant was expressed at low levels both in MCL and MM cell lines and did not correlate with A870G genotype.

**Conclusions:** A group of MCL cell lines (Jeko-1, Rec-1 and UPN-1) exclusively express  $\Delta 3'$ UTR CyD1 mRNA. MCL models will be useful in helping to understand the role of differential CyD1 mRNA isoforms in pathogenesis and make progress in delineating the steps to these diseases.

#### Fr-076

##### **Primary testicular lymphoma: a strictly homogenous hematological disease?**

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**Aims:** Primary testicular lymphomas (PTL) display for the most aggressive diffuse large cell B-cell lymphoma (DLBCL) which could be further subclassified into germinal center B-cell-like and activated B-cell phenotype by immunohistochemistry.

**Methods:** A retrospective analysis of primary testicular lymphomas diagnosed at the Institute of Pathology, SALK between January 1997 and December 2008 was done. Immunohistochemical staining and complete clinical data evaluation was carried out and linked to overall survival time.

**Results:** 18 cases of PTL were diagnosed in elderly patients of aggressive clinical type. Most of the lymphomas were classified as DLBCL (15/18 (83.3%)) showing a non significant prevalence of activated B cell phenotype (9/15 (60%)) compared to the germinal centre phenotype (6/15 (40%)). Additionally, we diagnosed two cases of mantle cell lymphomas (pleomorphic subtype) and one case could not further be characterized (due to sparse biopsy material). The survival analysis revealed no significant difference for any of the investigated antigens.

**Conclusions:** Primary testicular lymphomas are for the most DLBCL, but subtype classification reveal molecular heterogeneity inside this lymphoma

entity, therefore differentiation could be important for future approaches of treatment.

#### Fr-077

##### **FOXP1 protein overexpression is associated with inferior outcome in diffuse large B cell lymphomas with non-germinal center phenotypes, independent of gains and structural aberrations of its gene**

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**Aims:** We investigated the molecular epidemiology of structural and numeric FOXP1 gene aberrations in correlation to those of the BCL-6 gene and the prognostic importance of FOXP1 protein expression in a large cohort of DLBCL.

**Methods and Results:** By interphase fluorescence in situ hybridization with color-labeled bacterial artificial chromosome clones, 12% (27/223) analyzable cases showed FOXP1 gains and 1% (2/210) FOXP1 breaks. Eight of 159 cases with known BCL-6 and FOXP1 gene status showed an isolated FOXP1 gain, 30 cases an isolated BCL-6 gain and 29 cases a trisomy 3. FOXP1 gains (isolated and due to trisomy 3) were more frequent in nodal than extranodal DLBCL and in non-germinal center B-cell-like (non-GCB) DLBCL than in GCB DLBCL. By immunohistochemistry, FOXP1 protein was more often overexpressed in non-GCB than in GCB cases. FOXP1 overexpression was associated with a poor disease-specific survival in all DLBCL, particularly in nodal- and non-GCB cases. There was no correlation between FOXP1 gene aberrations and both, FOXP1 protein expression and survival.

**Conclusions:** FOXP1 is recurrently targeted by genetic aberrations in DLBCL. Only the presence of FOXP1 protein, irrespective of its gene status, is decisive for prognosis in DLBCL particularly in nodal- and non-GCB cases.

#### Fr-078

##### **Tumor subpopulations and intratumoral heterogeneity in diffuse large B-cell lymphomas (DLBCL) – coexpression of the transcription factors involved in B-cell differentiation**

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**Aims:** Tightly controlled expression of transcription factors (TF) in B-cells regulates germinal center (GC) formation (BCL6) as well as GC exit and plasmacellular differentiation (IRF4 and BLIMP1). DLBCL, the most common type of GC derived lymphoma display multiple aberrations of these TF including aberrant somatic hypermutation, translocations and overexpression. Herein, we studied the coexpression of TF in DLBCL cells which are not coexpressed under physiological conditions. Our aim was to quantify tumor subpopulations as a feature reflecting the intratumoral heterogeneity in order to gain insight into lymphomagenesis.

**Methods:** 115 DLBCL (38 molecular Burkitts (mBL), 77 non-mBL) characterized by molecular profiling were stained by immunofluorescence-triple-staining with two antibody-combinations: PAX5/BCL6/MUM1 (the latter detecting IRF4 protein) and PAX5/BCL6/BLIMP1 followed by quantitative digital image analysis.

**Results:** As expected, DLBCL display a non-physiological coexpression pattern of TF. Within DLBCL multiple populations of tumor cells with different phenotypes were detectable and quantified. Under physiological conditions, BCL6 and MUM1 expression is virtually exclusive in GC and regulated in opposite directions. Indeed, MUM-1/IRF4 has been reported to downregulate BCL6. Surprisingly, in the large subgroup of DLBCL with BCL6 and MUM1 coexpression, our initial analysis showed a correlation between the percentage of BCL6 and the percentage of BCL6/MUM1 co-expressing cells. These data indicate that the downregulation of BCL6 by MUM-1 is not functional in this subset of DLBCL. Moreover, the positive correlation of BCL6 and MUM1 expression in these DLBCL might suggest the presence of an ab-

errant regulative factor in DLBCL that upregulates both proteins or overcomes MUM1 function in downregulating BCL6. Further correlations with molecular and clinical findings will be presented.

**Conclusions:** Our results indicate that the tumor cells in DLBCL are heterogeneous and display different phenotypes in respect to expression of TF. The presence of „non-physiological“ TF coexpression pattern in DLBCL might explain their oncogenic potential and will help to gain further insight into lymphoma pathogenesis.

## T-Zellen

### Fr-079

#### A cytotoxic gamma/delta T-cell lymphoma in the large intestine

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**Aims:** Enteropathy-associated T-cell lymphomas (EATLs) account for less than 5% of all gastrointestinal lymphomas. Most arise in the small intestine and are strongly associated with celiac disease or EBV-infection. We report a case of a 62 year old man with a primary T-cell lymphoma in the large intestine, without a history of celiac disease.

**Methods:** Recurrent colonoscopies for hematochezia were performed over a period of 4 months in the otherwise healthy patient. Stool and blood tests for parasitic or microbiological infections were prepared. Colon biopsies were analyzed by histology, immunohistochemistry, polymerase chain reaction and in-situ hybridization with EBER probes detecting EBV-infection.

**Results:** Colonoscopies showed lumen-constricting ulcerations in the large intestine. All screening tests for an infection were negative. Blood leukocyte counts were normal. Histology showed numerous monomorphous, medium-sized intraepithelial lymphocytes (IELs). The immunophenotype of these IELs was CD3+, CD8+, Perforin+, TIA+, while CD4, CD5, CD56 and CD30 were all negative. Staining for TCRbeta was also negative, suggesting gamma/delta T-cell lymphoma.

**Conclusions:** Most T-cell lymphomas in the gastrointestinal tract are alpha/beta T-cell receptor expressing EATLs, which are located in the small intestine and are associated with poor prognosis. The case presented here is unusual since the cyto-toxic T-cells express presumably gamma/delta T-cell receptors but is not associated with celiac disease or EBV-infection. The few published cases, including our own, suggest that lymphomas of the large intestine seem to have a better prognosis.

## Morbus Hodgkin

### Fr-080

#### Clonal relationship in relapsing Hodgkin lymphoma

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**Aims:** Though the majority of Hodgkin lymphomas (HL) can be currently cured by multimodal therapy, a small number of patients will experience a relapse. Early relapses seem to be associated with a worse prognosis than late ones. We aimed to investigate if the first and all further manifestations of HL are morphologically, virologically and clonally related to each other.

**Methods:** Thirteen patients with relapsing HL were identified. A total number of 31 formalin-fixed, paraffin embedded tissue samples of these patients [primary tumours and (multiple) recurrences] were analyzable. Hodgkin and Reed-Sternberg cells were microdissected after immunohistochemical staining for CD30 using the laser-capture technique. Immunoglobulin heavy chain (IgH) fragment lengths were analyzed after DNA pre-amplification and polymerase chain reaction, applying FR<sub>3</sub> and J primers by ABI 310 Genetic Analyzer.

**Results:** All 2 early relapses after first HL diagnosis were clonally related to the initial tumor, while 3 of 4 early relapses after a second or third relapse were

not. Two out of 10 late relapses were clonally unrelated, which was accompanied by phenotypic and EBV-association switch in one case. One patient had two simultaneous clonally unrelated early relapses after a second recurrence at different anatomical sites with divergent EBV-association. Another 2 cases, initially presenting as HL and relapsing as diffuse large B-cell lymphoma or vice versa, showed clonal relationships between both entities.

**Conclusions:** Recurrent HL especially after a second or third relapse or those accompanied by phenotypic and/or EBV-association switch may represent clonally unrelated second neoplasms. This might be important in clinical decision making.

## Methoden und Varia

### Fr-081

#### A technique to coverslip a histological slide stained with Leder procedure with a xylene based mounting medium

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**Aims:** Leder procedure, also known as naphthol AS-D chloroacetate esterase procedure, is an enzyme histochemical staining method which is a substantial stain in bone marrow diagnostics. Until now, all slides were coverslipped with an aqueous mounting medium in our laboratory as recommended by several books on histochemistry.

**Methods:** However, inspired by the publication of Nitta et al. (Diagn Pathol 2008, 3:41) I dried the slides after the last step of the Leder procedure at room temperature. Alternatively a hot plate or a hair dryer was used. By omitting the dehydrating alcohol series, the slides were directly coverslipped with a xylene based mounting medium (pertex, Medite GmbH, Burgdorf, Germany).

**Results:** By omitting the alcohol series it was possible to coverslip the slides stained with a Leder procedure with a xylene based mounting medium. The stain appeared to be alcohol sensitive and xylene stable.

**Conclusions:** No longer a slide stained with a Leder procedure must be coverslipped with an aqueous mounting medium. The use of a xylene based mounting medium results in a better quality of the slide and simplifies the laboratory procedure.

### Fr-082

#### Formation of gouty tophi is initiated by extranuclear DNA

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**Aims:** Analysis of gouty tophi initiated by injection of MSU (monosodium urate) crystals intraperitoneally in mice and patients suffering from gout.

**Methods:** Human PMN (polymorphonuclear cells) were incubated with MSU crystals and analysed by fluorescence microscopy (DAPI). Moreover, mice were treated intraperitoneally with MSU crystals resulting in generation of gouty tophi. Paraffin sections of the tophi were analysed by nuclear staining and immunohistology. Furthermore, synovial fluids and tophi of patients with acute gouty attacks were analysed.

**Results:** In the in vitro assays we found the formation of extracellular DNA induced by MSU crystals. Next we analysed the gouty tophi generated intraperitoneally in mice and again detected extranuclear DNA and histones. Last we investigated material of gouty patients and observed the formation of extranuclear DNA in the synovial fluid and in tophous material.

**Conclusions:** We conclude that the extranuclear DNA has been ejected by the neutrophils in order to trap the inflammation inducing crystals. A similar mechanism is employed by neutrophils in the defense against massive bacterial or fungal infection, where the extranuclear DNA helps for immobiliza-

tion and inactivation of pathogenic microorganisms. Further investigations are required to elucidate whether the extranuclear DNA is ameliorating or precipitating inflammation and pathology in gout.

#### Fr-083

##### **ED-B fibronectin: A marker of neoangiogenesis as an immunotherapeutic target**

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**Aims:** Current treatment of malignancies often involves large doses of rather unspecific drugs, frequently resulting in severe adverse events. Thus, modern clinical cancer research focuses on compounds able to discriminate between malignant and normal tissues.

**Methods:** The extra-domain B of fibronectin (ED-B FN) is extracellularly expressed around newly formed blood vessels in neoplasms but not in normal mature tissues. We developed a modified immunohistologic technique for the detection of this epitope and performed a first phase 1 study with 2 patients with relapsed classical Hodgkin lymphoma using an anti-ED-B-<sup>39</sup>I.

**Results:** We show high amounts of perivascular ED-B FN in biopsies of more than 300 patients with Hodgkin, Non-Hodgkin lymphoma, and myeloproliferative neoplasms. ED-B FN expression was nearly absent in normal lymph nodes and bone marrow biopsies. The extent of vascular ED-B FN expression in neoplasias was positively correlated with grade of malignancy. Both patients showed a sustained partial response after application of a anti-ED-B FN-radioimmunotherapy. The reagent revealed no toxicity.

**Conclusions:** ED-B FN is a promising target structure for the immunotherapy of haematologic malignancies.

## Poster: Orthopädische Pathologie

#### Fr-084

##### **Overexpression of the drug resistance-associated protein metallothionein does not correlate with isolated limb perfusion treatment of sarcomas**

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**Aims:** Hyperthermic isolated limb perfusion with tumor necrosis factor alpha (TNF- $\alpha$ ) and melphalan (HILP-TM) achieves high response rates in non-resectable sarcomas. Melphalan resistance was previously reported to be strongly associated with overexpression of metallothioneins (MTs). MTs are ubiquitous proteins with a high affinity for heavy metal ions. The objective of this patient-based study was to investigate the influence of MT expression on tumor responses in a larger series of HILP-TM-treated soft tissue (STS) and bone sarcomas (BS).

**Methods:** In primary biopsies of 41 HILP-TM-treated sarcomas (37 STS and 4 BS), MT expression was assessed by an immunoreactive score (MT-IRS). We studied the association of MT-IRS between histological regression (responder >90%, or non-responder  $\leq$ 90% regression), tumor proliferation, and other clinico-pathological parameters.

**Results:** MT expression was found in 70.7% (N=29) of tumors (high 12.2%, moderate 19.5%, and low 39.0%). After HILP-TM, 20 cases (48.8%) were categorized as "responders" and 21 (51.2%) as "non-responders". Six "responders" (14.6%) presented with complete regression. MT expression positively correlated with tumor proliferation but not with HILP-TM.

**Conclusions:** HILP-TM showed a histologically favorable response in a high rate of advanced limb sarcomas. Although MT overexpression was observed in this cohort of sarcomas, the immunohistochemical MT status was not predictive of the tumor response after HILP-TM treatment.

#### Fr-085

##### **DNA ploidy and core classification of Angiosarcoma**

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**Aims:** Angiosarcoma is a highly malignant neoplasm originating from blood or lymphatic vessels. As a relatively rare tumor representing about 1% of soft tissue tumors it is not well characterized. We wanted to explore the DNA ploidy in correlation to morphological parameters as well as chromosomal alterations.

**Methods:** Angiosarcoma cases were retrieved from the files of the Jena soft tissue tumor reference center. The cases were reviewed morphologically according to core classification analyzing in particular the size of tumor nuclei and mitoses. A subset of cases were analyzed by static DNA cytometry. In addition database searches (PubMed, Mitelman database) were performed to identify chromosomal aberrations.

**Results:** In total, more than 200 angiosarcomas were identified. Of these, 50 cases were analysed by DNA cytometry and the core classification. In addition, a tissue microarray of 138 cases was generated. The morphological analysis and the DNA measurements indicated that angiosarcoma can be divided into two subgroups. One is harbouring a near diploid DNA content while the other is aneuploid, meaning hypodiploid or clearly hyperdiploid. This correlated with differences in the nuclear sizes showing tumors with predominantly small nuclei versus tumors with medium and/or large nuclei. There is a considerable variability in DNA content and nuclear size correlating with the fact that angiosarcomas harbour complex karyotypes.

**Conclusions:** Our data indicate that angiosarcomas can be further subdivided by morphological and DNA parameters. The impact for patient survival still needs to be determined. In addition, we will search for specific biomarker with potential relevance for tumor diagnosis and therapy.

#### Fr-086

##### **Characterisation of epitheloid haemangioendothelioma with a new Interphase-FISH-Test**

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**Aims:** Epitheloid haemangioendothelioma (EHE) are rare vascular tumours whose dignity, clinical course and histological appearance lies between benign vascular tumours (haemangioma) and malignant angiosarcoma. By cytogenetic analysis two cases of EHE were found to harbour a balanced translocation t(1;3)(p36.3;q25) suggesting a characteristic chromosomal rearrangement as cause for the development of EHE. In this study 15 cases of EHE were investigated by interphase-FISH directed against this translocation.

**Methods:** FISH was done on extracted nuclei and on thin tissue sections. Nuclei were extracted from formalin-fixed and in paraffin-embedded tumour material. Suitable FISH probes, located at the 1p and 3q breakpoint region, were identified based on database search, ordered from the CHORI BAC repository and processed by plasmid-isolation and DOP-PCR. Probe labelling was performed by direct nick translation. Subsequently, FISH with 3 minutes of microwave treatment was done. Slides were evaluated by fluorescence microscopy.

**Results:** 5 cases could be successfully analyzed which harboured a chromosomal break in the 1p36.6 region supporting the observation that the t(1;3)(p36.3;q25) translocation is present in primary EHE. The break apart signals were present in diploid nuclei; less frequently also in tetraploid nuclei. In the latter the translocation was present twice.

**Conclusions:** We could confirm that EHE is characterized by a recurrent chromosomal translocation. With further optimisation, our interphase-FISH

approach should be suitable for pathological routine diagnosis. In addition for the simplification of the protocol and an easier detection of signals a better characterisation of the breakpoint is needed. Additional studies need to be performed to identify the genes involved in this translocation and the corresponding molecular mechanisms leading to the EHE tumor development.

**Fr-087****Pigmented villonodular synovitis (PVNS) - like changes in periprosthetic membranes**

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**Aims:** PVNS is a tumour-like mesenchymal lesion of uncertain histogenesis that may occur at both intra-articular and extra-articular sites. In this study, we describe the occurrence of PVNS-like lesions in the neosynovia following total endoprosthesis (TEP).

**Methods:** Periprosthetic membrane specimens from patients undergoing replacement of their knee TEP were paraffin embedded, sectioned and hematoxylin-eosin stained. For evaluation of iron deposits the classic Prussian blue reaction was used.

**Results:** The neosynovia displayed a broad spectrum of alterations. In several periprosthetic membranes we found histological lesions showing some features of PVNS. In some cases the histology closely resembled PVNS with marked villous hyperplasia, fibrosis, iron deposits and a mixed cellular infiltrate composed of mononuclear cells, fibroblasts, foam histiocytes and multinucleated giant cells with varying hemosiderin deposits.

**Conclusions:** PVNS-like lesion found in the neosynovia of periprosthetic membranes represent an exuberant fibrohistiocytic reaction. Their development might be governed by the same pathological principle responsible for the classical PVNS in synovial membranes, probably involving chronic inflammatory/irritation-induced synovial hyperplasia. These findings might provide an additional clue for the understanding of the pathological mechanism of classical PVNS.

**Fr-088****Detection of minimal infection in synovial fluid of patients with arthritis and prosthesis loosening**

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**Aims:** Minimal infections in synovial membranes can be diagnosed using standardized morphological criteria. On one hand culturing synovial fluid provides a high rate of false negative results, on the other hand the disadvantage of molecular analysis was until now a high amount of false positive results.

**Methods:** We have adapted a commercial sepsis test, validated for whole blood, for the use in synovial fluid specimens. The test system detects 40 bacterial and fungal species as well as 5 types of resistances against antibiotics. A special method of enrichment of bacterial and fungal DNA, which eliminates the human background DNA, provides high specificity and sensitivity. Synovial fluids of 29 patients with degenerative joint disease and total joint replacement were collected and stored at 4°C and was analysed using the commercial test system (VYOO<sup>®</sup>, Jena, Germany).

**Results:** Four of the 29 collected specimens showed bacterial infection. Histological analysis of synovial membranes confirmed joint infection in those cases which were positive with the molecular pathological test system. There were no false positive results based on morphological analysis.

**Conclusions:** Analysis of synovial fluid samples by multiplex-PCR for bacterial and fungal DNA is a reliable, highly sensitive tool for detecting „low level“ infections. The major diagnostic problem of false positive results could be eliminated by a special adsorption technique, using specific binding of non-methylated CpG, which improved the sensitivity/specificity of the test.

**Poster: Urothologie****Nephrologie****Fr-089****Reduced expression of ADAMTS13 in vascular smooth muscle cells in benign nephrosclerosis**

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**Aims:** Deficiency of the antithrombotic von Willebrand factor cleaving protease ADAMTS13 is involved in the pathogenesis of thrombotic microangiopathies (TMAs). To examine whether ADAMTS13 may be involved in the pathogenesis of hypertensive renal injury its expression was examined in human kidney samples with and without benign nephrosclerosis.

**Methods:** All specimens were examined by immunohistochemistry for the presence of ADAMTS13 in preglomerular VSMCs. Intrarenal arterioles of normal kidneys (n=5) and patients with benign nephrosclerosis (n=62) were scored for hyalinosis, fibrosis and the sum of both defined as benign nephrosclerosis score.

**Results:** Arterial and arteriolar VSMCs revealed a significant inverse correlation between benign nephrosclerosis score and ADAMTS13 staining of VSMCs. The fibrosis score and ADAMTS13 staining was also significantly inverse correlated. There was no significant correlation between hyalinosis score and ADAMTS13 staining in VSMCs.

**Conclusions:** Our study demonstrates a decreased ADAMTS13 staining in preglomerular VSMCs in benign nephrosclerosis. ADAMTS13 seems to be a marker of contractile differentiation with unclear physiological role of preglomerular smooth muscle cells.

**Fr-090****Ultrastructural findings in C4d-positive endothelial cells from ABO-incompatible renal allografts**

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**Aims:** ABO-incompatible renal allografts (ABO-i) are often C4d positive. The significance of this finding is still indetermined. While the ultrastructural features of C4d-positive ABO-compatible grafts (ABO-c) have been described, the ultrastructural appearance of the peritubular capillary endothelium (PTCE) and glomerular endothelium (GE) in ABO-i has not been reported.

**Methods:** PTCE, GE and underlying basement membranes of 11 biopsies of C4d+ ABO-i and 19 biopsies of C4d+ ABO-c with histological signs of humoral rejection (glomerulitis, capillaritis) were investigated by electron microscopy.

**Results:** In all analyzed parameters (widening of glomerular subendothelial space, endothelial swelling in glomerular and peritubular capillaries (ptc), basement membrane lamellation and double contours, loss of glomerular endothelial fenestration) ultrastructural changes were more frequently seen in ABO-c. This was statistically significant concerning widening of subendothelial space (53 versus 0%, p<0,05), loss of endothelial fenestration (53 versus 9%, p<0,05), ptc basement membrane lamellation (47 versus 9%, p<0,05) and swelling of ptc endothelial cells (53 versus 0%, p<0,05).

**Conclusions:** Although complement fixation takes place in both, ultrastructural alteration of glomerular and capillary endothelium is less severe in C4d+ ABO-i compared to ABO-c. This is in accordance with favorable outcome after ABO-i transplantation and indicates, that C4d positivity alone does not implicate endothelial damage.

**Fr-091****Electron microscopic studies of the role of gadolinium in nephrogenic systemic fibrosis (NSF) and encapsulating peritoneal sclerosis (EPS)**J.A. Schröder, E. Goffin<sup>1</sup>, Ch. Weingart<sup>2</sup>, T. Vogt<sup>3</sup>, B.K. Krämer<sup>4</sup>

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**Aims:** Since 2000 growing clinical data refer to a very disabling fibrosing disease, NSF, in a fraction of patients with renal insufficiency exposed to gadolinium-based (Gd) MR-imaging contrast agents. The pathogenesis of NSF and the mechanism by which Gd acts as a trigger remain elusive. EPS is also a rare fibrotic process observed in long-term peritoneal dialysis patients. Its cause is still unknown; we tested the hypothesis of a Gd implication.

**Methods:** We performed electron microscopic examinations of skin of NSF and of peritoneal membrane samples of EPS patients to search for tissular Gd using the very sensitive Electron Spectroscopic Imaging and Electron Energy Loss Spectroscopic analysis.

**Results:** Multiple Gd-aggregates were found in the skin adhering to cell profiles and collagen fibers in the dermis forming a perivascular „Gd-deposit zone“ of approx. 5 µm width. Iron signal was also present in singular Gd-positive deposits as well as in adjacent connective tissue in the skin supporting the postulated Gd transmetallation hypothesis. Iron was also evidenced in the peritoneal samples of the EPS-patients but no Gd signal was found this later finding arguing against Gd implication in EPS development.

**Conclusions:** Our data point to the specific role of gadolinium in NSF but not in EPS patients.

**Fr-092****Podocyte expression of ADAMTS13 in renal biopsies with thrombotic microangiopathy (TMA)**F. Modde<sup>1</sup>, C.L. Bockmeyer<sup>1</sup>, V. Forstmeier<sup>1</sup>, P.A. Agustian<sup>1</sup>, V. Bröcker<sup>1</sup>,J. Traeder<sup>1</sup>, U. Lehmann<sup>1</sup>, K. Theophile<sup>1</sup>, H.H. Kreipe<sup>1</sup>, M. Schiffer<sup>2</sup>,J.U. Becker<sup>1</sup><sup>1</sup>Institut für Pathologie, Medizinische Hochschule Hannover<sup>2</sup>Klinik für Nephrologie, Medizinische Hochschule Hannover

**Aims:** ADAMTS13 is regarded to function as a critical mediator for development of thrombotic microangiopathy (TMA). ADAMTS13 is synthesised in renal podocytes and glomerular endothelial cells. Therefore we hypothesised that renal TMA correlates with reduced glomerular expression of ADAMTS13.

**Methods:** Formalin fixated paraffin embedded renal biopsies of 22 patients with TMA were analyzed and compared to normal renal tissue. Immunohistochemistry for ADAMTS13 was performed to stain podocytes. For mRNA analysis glomeruli were lasermicrodissected using MMI laser technique and software. Quantitative mRNA analysis was done by TaqMan real time PCR after cDNA synthesis and preamplification.

**Results:** ADAMTS13 immunostains of TMA patients showed no reduced visceral epithelial and glomerular endothelial signal when compared with tissue from healthy patients. ADAMTS13 mRNA transcript was not diminished compared to normal renal tissue. Podocyte staining patterns and mRNA expression were independent on thrombus localization or chronic glomerular injury with glomerular basement membrane duplication.

**Conclusions:** Renal TMAs are not associated with locally decreased glomerular expression of ADAMTS13. Further investigations, ideally in podocyte-specific ADAMTS13-knockout mice, should address the role of ADAMTS13 in podocytes.

**Fr-093****Silver enhanced in situ hybridisation detects of BK virus DNA in renal biopsies from renal transplant patients**F.R. Fritzsche<sup>1</sup>, A. Gaspert<sup>1</sup>, S. Pianca<sup>2</sup>, T. Fehr<sup>2</sup>, R. Tubbs<sup>3</sup>, H. Moch<sup>1</sup><sup>1</sup>Institut für Klinische Pathologie, Universitätsspital Zürich<sup>2</sup>Klinik für Nephrologie, Universitätsspital Zürich<sup>3</sup>Institut für Klinische Pathologie, Cleveland Clinic Ohio

**Aims:** Polyomavirus type BK (BK virus) nephropathy is a common complication of renal transplantations associated with high rates of graft loss. We evaluated a new silver-enhanced in situ hybridisation (SISH) technique for the detection of BK virus in renal transplant kidney specimen.

**Methods:** The SISH analysis was applied on a Ventana Benchmark platform to detect BK virus infections. As a reference test the SV40 immunohistochemistry was used.

**Results:** The BK virus SISH is highly concordant with conventional chromogen in situ hybridisation. The SISH was positive in all 26 immunohistochemically confirmed cases of BK virus nephropathy.

**Conclusions:** BK virus SISH is a sensitive tool for the detection of BK virus infections in renal transplant patients. From the diagnostic point of view, no advantage in comparison to existing tests (SV40/PCR) could be demonstrated.

**Fr-094****The inflammatory infiltrate: A potential clue to differentiate BK nephropathy from T-cell mediated rejection in kidney transplant?**Hui Xu<sup>1</sup>, M. Buettner<sup>1</sup>, R. Boehme<sup>1</sup>, K. Amann<sup>1</sup><sup>1</sup>Institut für Pathologie, Universitätsklinikum Erlangen

**Aims:** It is notoriously difficult to differentiate T-cell mediated rejection from polyoma virus infection in the context of kidney transplantation. Although stainings for HLA-DR, C4d and Polyoma virus can sometimes be of great assistance, the definite decision may be problematic. Therefore, it was investigated whether the composition of the inflammatory infiltrate, with special regard to Treg, can give a hint towards the derivation of the inflammation.

**Methods:** 18 renal biopsy specimens with immunohisto-chemically proven polyoma virus infection were compared with 22 specimens with interstitial T-cell mediated rejection. Immunohistochemical stainings with antibodies against CD3, CD8, c-maf, FoxP3, CD20 and CD21 were performed to characterize the infiltrate, which was subsequently quantified. Statistical analysis was performed.

**Results:** Comparing the subtypes of lymphoid cells, there was in general a predominance of T cells (CD3) over B cells (CD20). Th2 (c-maf) cells usually outnumbered cytotoxic T cell (CD8). Treg (FoxP3) were the smallest subgroup of T cells with only few scattered cells in the infiltrate. No differences were observed in the composition of the infiltrates in BK nephropathy compared to T-cell mediated rejection.

**Conclusions:** The characterization of the inflammatory infiltrate does not serve as a discrimination aid to differentiate BK nephropathy from T-cell mediated rejection. Independent of the antigen-stimulus the infiltrate is dominated by T cells. The number of Treg is very small and only few scattered cells are found.

## Nierenzellkarzinom

## Fr-095

**Expression and functional characterization of the BNIP3 protein in renal cell carcinomas**

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**Aims:** BNIP3 (Bcl-2/adenovirus E1B 19 kDa-interacting protein 3) is a BH3-only protein that regulates apoptosis and autophagy. BNIP3 plays also an important role in hypoxia-induced cell response and is regulated by HIF1. Here, we studied a possible association of BNIP3 expression and the prognosis of patients with renal cell carcinomas (RCCs) and examined the functional relevance of BNIP3 in the regulation of cell survival and apoptosis of renal carcinoma cells.

**Methods:** BNIP3 expression was examined by immunohistochemistry using a tissue microarray with RCC tumor tissue samples of 838 patients. The functional effects of BNIP3 in renal carcinoma cells were investigated by Western blot, apoptosis and cytotoxicity assays, immunofluorescence, transfections and siRNA-mediated gene knockdown.

**Results:** The expression levels and subcellular localization of BNIP3 showed a great variability in RCCs. High cytoplasmatic BNIP3 expression was associated with high grade RCCs ( $p < 0.001$ ) and regional lymph node metastasis ( $p = 0.02$ ). BNIP3 expression correlated negatively with disease specific survival ( $p < 0.001$ ). Multivariate Cox regression analysis retained BNIP3 expression as an independent prognostic factor in patients without distant metastasis ( $p = 0.004$ ).

**Conclusions:** BNIP3 regulates cell survival in RCCs and its expression is a powerful independent prognostic marker in patients with RCCs.

## Fr-096

**Aberrations of DNA-copy number and expression of PARK2 and PACRG in cc-RCC: correlation with clinico-pathological parameters and prognosis**

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**Aims:** The present study correlates PARK2 and PACRG expression with copy number abnormalities, clinico-pathological parameters and survival in cc-RCC.

**Methods:** RNA and DNA were isolated from fresh-frozen primary ccRCC samples and corresponding non-malignant tissues from 100 patients (59 male, 41 female, median age 63 years). Follow-up data were available for 80 patients. mRNA expression and copy number analyses were performed by quantitative PCR on the LightCycler 480 (Roche) using gene-specific Taq-Man gene expression and copy number assays. Relative quantification was performed by the  $\Delta\Delta CT$  method using non-malignant tissue (mRNA expression) or lymphocyte DNA of healthy donors (copy number;  $n = 20$ ) as reference. Statistical analysis was done by SPSS 17 for Windows.

**Results:** Eighty patients had an organ-confined, 20 a non-organ-confined tumor. Five patients showed a G1, 76 a G2 and 19 a G3 tumor. PACRG was significantly down-regulated in ccRCC compared to corresponding normal kidney tissue ( $p < 0.001$ ). A lower expression was found in 92 tumours, whereby 66 cases showed a complete loss of PACRG expression. PACRG expression was reduced in higher tumor stages (I/II vs. III/IV,  $p = 0.046$ ), and in metastasized tumors ( $p = 0.012$ ). A complete loss of PACRG expression was associated with a significantly shorter disease-free survival ( $p = 0.046$ ). A down-regulation of PARK2 was demonstrated in 57% of cc-RCC ( $p < 0.001$ ), in 13 cases with a complete loss of PARK2 expression. A reduced PARK2 expression was

associated with higher tumor stage ( $p = 0.034$ ), grade ( $p = 0.004$ ) and metastatic spread ( $p = 0.02$ ). DNA copy number analysis showed losses of PACRG in 47% of cases, but the down-regulation of PACRG is not always associated with low PACRG DNA copy number.

**Conclusions:** Loss of PACRG and PARK2 are common events in cc-RCC and are associated with aggressive disease. PACRG and PARK2 might represent tumor suppressors in ccRCC and their loss is involved in tumor progression as it has been reported for a few other tumor entities.

## Fr-097

**Expression of the forkhead transcription factor FOXP1 is associated with tumor grade and Ki67 expression in clear cell renal cell carcinoma**

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**Aims:** The aim of this study was to prove the expression of FOXP1 in clear cell renal cell carcinoma and to correlate the FOXP1 expression with clinical and pathological features.

**Methods:** 129 cases of clear cell renal cell carcinomas treated by radical nephrectomy between 1993 and 2000 with known follow-up were included in the study. Tissue microarrays from malignant and corresponding non malignant formalin fixed, paraffin-embedded archive material were constructed. Immunohistochemical FOXP1 expression was assessed semiquantitatively and correlated with disease specific-, overall -, and recurrence free survival and Ki67 expression.

**Results:** Disease specific survival of the patients with clear cell renal cell carcinomas correlated with tumor grading ( $p < 0.05$ ). Cases with lymph node metastasis showed a significant shorter overall and disease specific survival than cases without lymph node metastasis ( $p < 0.05$ ). Short recurrence free survival correlated with increasing tumor stage and grade and with lymph node metastasis ( $p < 0.05$ ). Tumor tissues showed loss of FOXP1 expression or low FOXP1 expression in 69 cases (53.4%). Expression of FOXP1 correlated negatively with tumor grading ( $p = 0.02$ ), but not with tumor stage or lymph node metastasis. Significant positive correlation was shown for Ki67 expression and tumor grade, stage and lymph node metastasis ( $p < 0.05$ ). The overall survival as well as disease specific survival correlated with Ki67 status ( $p < 0.05$ ). The Spearman-Rho correlation showed that FOXP1 expression negatively correlates with Ki67 expression in clear cell renal cell carcinomas ( $p = 0.036$ ).

**Conclusions:** Survival of patients with clear cell renal cell carcinomas is influenced by tumor grade and lymph node metastasis, as well as by tumor proliferation. FOXP1 transcription factor seems to play an important role in loss of differentiation of clear cell renal cell carcinomas and its expression correlates with proliferation of the tumor cells.

## Fr-098

**Mining tissue microarray data to unhide combinations of biomarker expression patterns that improve intermediate staging and grading of clear cell renal cell cancer (ccRCC)**

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**Aims:** The progression risk of ccRCC is difficult to predict particularly for tumors with organ-confined stage and intermediate differentiation grade. Applying appropriate mathematical models for comprehensive analyses of molecular pathways deregulated in ccRCC may improve prognosis.

**Results:** Patients with pT2 and pT3 tumors that were p27 and CAIX positive had a better outcome than those with all remaining marker combinations. A prolonged survival among patients with intermediate grade correlated with

p27 and PTEN expression, as well as with inactive ribosomal protein S6. Only a weak conditional dependence existed between the expression of p27, PTEN, CAIX and p-S6 suggesting that the dysregulation of several independent pathways are crucial for tumor progression

**Methods:** Using tissue microarrays, expression patterns of 15 different proteins were evaluated in over 800 ccRCC patients to analyze pathways reported to be physiologically controlled by the tumor suppressors pVHL and PTEN. recursive bootstrap elimination and graphical log-linear modeling were applied to analyze the TMA data.

**Conclusions:** The use of recursive bootstrap elimination and graphical log-linear modeling for comprehensive TMA data analysis allows the unraveling of complex molecular contexts.

#### Fr-099

##### Comparison of P-AKT EXPRESSION in renal cell carcinomas and their metastasis

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**Aims:** In advanced and metastatic renal cell carcinoma a significant resistance to chemo- and radiotherapy was previously shown. For these patients novel and partly still experimental therapeutic concepts focus on the use of various inhibitors of the PI3 K/ p-AKT/ mTOR. Associations between overexpression of p-AKT and sensitivity to inhibitors of mTOR (e.g. temsirolimus) were previously found in different tumours. The present study evaluated expression of p-AKT in primary renal cell carcinomas and their metastasis.

**Methods:** P-AKT expression was immunohistochemically analyzed in a tissue microarray from renal cell carcinoma (n=45), their metastasis (first onset n=45, second onset n=5), and adjacent tumour-negative renal tissue (n=45).

**Results:** P-AKT was overexpressed in 53.3% (n=24) and 71.1% (n=32) of primary tumours and metastasis compared with normal renal parenchyma. In 60% of patients concordant p-AKT expression was shown between primary tumour and first onset metastasis. Second onset of metastasis showed p-AKT overexpression in 60% compared with matched first onset metastasis, showing overexpression of p-AKT in 40%.

**Conclusion:** Overexpression of p-AKT was more often demonstrated in metastasis than in primary RCC. Comparison of p-AKT expression in primary RCC and first onset metastasis showed discordance in 40%. Hypothesizing that not only the p-AKT overexpression of the primary tumour but also the p-AKT status of metastasis would be a predictive marker for tumour response to mTOR inhibitors, evaluation of metastatic tissue would be of great interest.

#### Fr-100

##### p53 isoforms in clear cell renal cell carcinomas

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**Aims:** Renal cell carcinomas (RCCs) exhibit a marked resistance towards conventional chemotherapy, which is – at least in part – due to functional suppression of p53. The responsible inhibitory mechanisms, however, have not been completely understood yet and differential expression of p53 isoforms, which can influence the transcriptional activity of p53, might participate in this inhibition. Therefore, the aim of our study was to elucidate the relevance of differential p53 isoform expression for carcinogenesis, progression and therapy-resistance of RCCs.

**Methods:** Semiquantitative „Realtime PCR“, Western Blot and caspase-assays.

**Results:** RCCs revealed a shift towards a more p53 activating isoform expression pattern during tumour initiation and progression, in vivo. In vitro, two cell lines exhibiting a similar sensitivity towards Topotecan-induced cell death revealed a similar induction of p53 target genes by Topotecan but differed in the extend of Topotecan-induced apoptosis. Furthermore, they strongly differed in their basal expression patterns of the p53 isoforms as well as in the differential regulation of p53 isoform expression upon Topotecan-treatment.

**Conclusions:** p53 isoform expression and regulation seems to be neither responsible for induction and progression of RCCs in vivo, nor for regulation of p53 gene expression, sensitivity towards chemotherapy and induction of apoptosis in vitro.

#### Peniskarzinom

#### Fr-101

##### c-met and HGF- $\alpha$ possible therapeutic targets in squamous cell carcinoma of the penis ?

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**Aims:** Der hepatocyte growth factor (HGF; scatter factor) und sein rezeptor c-met sind bei malignen Tumoren eingebunden in die Zellproliferation, deren Motilität, die Angiogenese und das peritumorale Stromaremodellierung. Rezente Untersuchungen ergaben zudem die Möglichkeit der gezielten Inhibition des c-met/HGF-Systems (Tosche & Jänne 2008). Ziel dieser Untersuchung war die Evaluierung der HGF/c-met Expression beim Peniskarzinom.

**Methods:** 34 Peniskarzinom-Fälle der Tumor-Stadien I und III wurden immunhistochemisch mit einem polyklonalen anti-c-met und anti-HGF Antikörper untersucht. Die zytoplasmatische Färbereaktion wurde semiquantitativ evaluiert.

**Results:** Unter Verwendung eines in der Literatur etablierten cut-off Wertes von 33% (Furukawa et al. 1995) zeigten 73,5% (25/34) aller Penis-Ca eine c-met- und 72,7% (24/33) eine HGF-Expression.

**Conclusions:** Möglicherweise kann anhand dieses Expressionsmusters wie es in ähnlicher Weise beim Cervix-Ca zu finden ist, auch eine Therapieempfehlung gestaltet werden. Wie bereits im Cervix-Ca gezeigt, korreliert eine c-met-Überexpression mit einer erhöhten Rezidivwahrscheinlichkeit (Baykal et 2003). Somit könnte eine gezielte therapeutische Intervention in Form einer Inhibition der c-met-Aktivität (z.B. durch AMG102 oder L2G7) bzw. seines Liganden HGF- $\alpha$  bzw. eine Inhibition der MET kinase Aktivität (z.B. durch PHA-665752 und SU11274) die Progression des Penis-Ca verhindern und damit eine radikale chirurgische Intervention dem Patienten erspart bleiben.

#### Fr-102

##### Leiomyosarcoma of the penis

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**Aims:** Less than 5% of penile malignancies are sarcomas. Primary leiomyosarcoma is the second most common sarcoma of the penis following Kaposi sarcoma. Deep-seated, large (>5 cm) leiomyosarcomas have a poor prognosis because of their tendency for widespread metastases.

**Methods and Results:** A 44 year old male patient presented with pain, dysuria and swelling of the glans penis within two months. Intraoperative frozen section examination revealed a malignant soft tissue tumor, so total penectomy was performed. The resection specimen represented a grey-white, pale tumor of 6.4 cm, located in corpus spongiosum et cavernosum with involvement of the glans penis. The resection margins were free of tumor. Immunohistochemistry showed positivity of the tumor cells for vimentin, desmin and

smooth muscle actin and negativity for keratin. One week after diagnosis a CT-scan showed multiple pulmonary and hepatic metastatic deposits. Chemotherapy was performed. The patient died 4 months after surgical treatment.  
**Conclusions:** The most common differential diagnosis of leiomyosarcomas of the penis includes sarcomatoid carcinoma and Kaposi sarcoma. Primary leiomyosarcomas arising in the penis are very rare with about 40 reported cases. Surgery remains the mainstay of treatment. The best morphologic predictors for outcome are tumor depth and size of the lesion.

## Harnblasenkarzinom

### Fr-103

#### Epithelial mesenchymal transition (EMT) in urothelial carcinoma of the urinary bladder (UBC) in vitro relation to oncofetal matrix remodelling

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**Aims:** Invasion requires changes in carcinoma cell phenotype and matrix microenvironment enabling migration with EMT as a key step. The study was aimed at elucidating the role of different growth factors for EMT of UBC cells in vitro and its influence on oncofetal matrix synthesis and adhesion.

**Methods:** RT112 cells were used for in vitro stimulation with TGFβ<sub>1</sub>, EGF, FGF acidic and HGF. mRNA expression of E-cadherin, Vimentin, N-Cadherin, snail, and oncofetal fibronectin (oncFn) and tenascin-C (oncTn) variants was assessed by rtRT-PCR. Adhesion to matrix proteins was analysed using multi substrate arrays. OncFn and oncTn proteins were analysed by immunofluorescence in RT112 and T24 xenografts.

**Results:** Among the growth factors tested only TGFβ<sub>1</sub> induces EMT in RT112 indicated by an increase in snail, Vimentin and N-Cadherin and a general decrease in adhesiveness to several matrix proteins. This is accompanied by an increase in oncFn and oncTn-C mRNA. T24 xenografts (mesenchymal phenotype) show an enhanced deposition of oncFn and oncTn-C in comparison to RT112 xenografts (epithelial phenotype).

**Conclusions:** In UBC, TGFβ<sub>1</sub> can induce EMT implicating a crucial role of tumor-stroma cross talk in this process. EMT leads to an upregulation of embryonal matrix proteins which are important for migration, angiogenesis, and myofibroblast development as key events in carcinoma progression.

### Fr-104

#### Decreased RECK and increased EMMPRIN expression in urothelial carcinoma of the bladder are associated with tumour aggressiveness

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**Aims:** Decreased expression of the reversion-inducing cysteine-rich protein with Kazal motifs (RECK) a downregulator of the matrix metalloproteinases MMP-2, MMP-9 and MMP-14 was recently shown for several tumours. Besides prostate cancer, however, there is only little known about RECK in urological cancers. Hence, this study aimed at investigating the expression of RECK and its opponent the MMP activator EMMPRIN in the urothelial carcinoma of the bladder.

**Methods:** Expression analysis of 70 human urothelial carcinomas from TUR-B and cystectomy specimens was performed using immunohistochemistry

against RECK, EMMPRIN, MMP-2, MMP-9 and MMP-14. Correlations with pT stage and tumour grade were analyzed.

**Results:** Consistent with findings in other cancer types, RECK expression is decreased in invasive and high grade urothelial carcinomas whereas EMMPRIN is shown to be upregulated in those tumours. No correlation could be established regarding the MMPs investigated.

**Conclusions:** Decreased RECK and increased EMMPRIN expression is associated with more aggressive urothelial carcinomas, therefore further studies are warranted to shed light on the role of RECK and EMMPRIN in the origin and progression of urothelial carcinomas.

### Fr-105

#### No FGFR3 mutations in normal urothelium of bladder cancer patients with urothelial carcinoma with activating FGFR3 mutations

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**Aims:** Frequent activating FGFR3 mutations have been shown to be frequent in papillary non-invasive bladder tumors and flat urothelial hyperplasia and are suggested to be one of the earliest molecular changes in bladder tumorigenesis. These mutations are associated with a favourable clinical outcome. The aim of the present study was to analyze the incidence of FGFR3 mutations in normal urothelium in patients with bladder cancer.

**Methods:** FGFR3-mutation analysis was performed on 64 samples of unsuspected urothelium from 53 patients (38 with FGFR3-mutated bladder tumors, 15 without any urothelial malignancy) by SNaPshot analysis.

**Results:** All samples showed a wildtype sequence for FGFR3. No mutations were detected, neither in the control group nor in the bladder cancer group.

**Conclusions:** FGFR3 mutations cannot be proved as the earliest events in bladder carcinogenesis and are associated with hyperproliferative lesions like flat urothelial hyperplasia and urothelial papillomas. To date chromosome 9 deletions and possibly epigenetic changes with promoter hypermethylation of several genes remain the earliest detectable alterations in bladder cancer development.

### Fr-106

#### M-FISH (UroVysion) in the urologic outpatient practice: a prospective follow-up-study of non-muscle-invasive bladder cancer (NMIBC)

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**Aims:** The diagnostic benefit of UroVysion (UV) test (M-FISH) in the follow-up of patients with NMIBC is unclear. The aim was to assess the value of UV on follow-up of patients with high-grade urothelial bladder cancer (UCB) in an outpatient setting.

**Methods:** 25 unselected patients with a history of high-grade UCB were prospectively followed in 185 events by cystoscopy, cytology and UV by a single centre. TUR-B was performed if cystoscopy was suspect. Sensitivities and specificities, positive and negative predictive values for recurrence were calculated.

**Results:** Sensitivities and specificities were 84% and 88% for cytology and 96% and 86% for UV. Taking into account the anticipatory positive test results sensitivity and specificity for standard follow-up scheme consisting of combined cystoscopy and cytology showed 78.4% and 83.1%. UV yielded 94.6% and 92.6%. 92.3% of patients with a history of previous CIS, negative cystoscopy and a positive UV finding developed CIS recurrence within 5 months.

**Conclusions:** The value of UV seems to be limited in NMIBC, but it may be a worthwhile approach in patients with previous CIS, high risk for CIS or previous unequivocal cytology suspicious for CIS, especially during or shortly after instillation therapy. Tighter follow-up or photodynamic assessment should be considered in positive UV and concomitant negative cystoscopy and cytology results in surveillance of patients with a history of high-grade NMIBC.

#### Fr-107

##### **c-met and HGF- $\alpha$ in muscle invasive and local advanced bladder cancer a therapeutic target ?**

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**Fragestellung:** Der hepatocyte growth factor (HGF; scatter factor) und sein Rezeptor c-met sind bei malignen Tumoren eingebunden in die Zellproliferation, deren Motilität, die Angiogenese und das peritumorale Stromaremodellierung. Rezente Untersuchungen ergaben zudem die Möglichkeit der gezielten Inhibition des c-met/HGF-Systems (Tosche & Jänne 2008). Ziel dieser Untersuchung war die Evaluierung der HGF/c-met Expression beim muskelinvasiven, papillären Urothelkarzinom der Harnblase (HCA).

**Methodik:** 103 HCA der Stadien pT pT2a bis pT4b wurden immunhistochemisch mit einem polyklonalen anti-c-met und anti-HGF Antikörper untersucht. Die zyttoplasmatische Färbereaktion wurde semiquantitativ evaluiert.

**Ergebnisse:** Unter Verwendung eines in der Literatur etablierten cut-off Wertes von 33% (Furukawa et al. 1995) zeigten 78,6% aller HCA eine c-met- und 99% eine HGF-Expression.

**Schlussfolgerung:** Möglicherweise spielt das HGF/c-met System eine Rolle bei der malignen Progression des HCA. Die Majorität der untersuchten HCA zeigt eine c-met-Überexpression. Somit könnten muskelinvasive bzw. ö lokal fortgeschrittene HCA möglicherweise von einer Inhibition von c-met (z.B. durch AMG102 oder L2G7) bzw. eine Inhibition der MET-Kinase Aktivität (z.B. durch PHA-665752 und SU11274) profitieren.

#### Fr-108

##### **Imaging mass spectrometry of bladder tissue microarrays**

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**Aims:** The detection of disease specific marker proteins for the diagnosis or prediction of progression in bladder cancer is a current and ongoing research focus. The emerging technique of imaging mass spectrometry (IMS) applied to bladder tissue microarrays (TMA) enables visualization of the spatial distribution of specific protein/peptide expression profiles in correlation with histological features of cancerous and non-cancerous tissues.

**Methods:** TMAs with matched pairs (n=23) of normal urothelium and bladder cancer were subjected to on-tissue tryptic digestion. Briefly, sections underwent paraffin removal and antigen retrieval (tris-EDTA buffer). On-tissue digestion was achieved by spotting trypsin onto the tissue using a Portrait 630 reagent multi-spotter. Following digestion, matrix (CHCA) was spotted directly onto the array of tryptic spots. Samples were analyzed utilizing the UltrafleXtreme MALDI-TOF/TOF mass spectrometer. Data analysis was performed by using the ClinProTools 2.2 and FlexImaging 2.1 software.

**Results:** On-tissue tryptic digestion of bladder tissue revealed 127 differentially expressed peptides in the mass range from m/z 600–4000. Combining five peptides in a genetic algorithm based model to discriminate bladder cancer from normal urothelium resulted in a sensitivity and specificity of 89.7% and 96.1%, respectively.

**Conclusions:** The identification of these differentially expressed peptides might elucidate important changes in the proteome of the urothelium during the development of cancer.

#### Fr-109

##### **Immunohistochemical expression of EGFR, HB-EGF, CD9 and MAPK in superficial bladder cancer could be marker for recurrence and progression**

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**Aims:** This study explores the expression of EGFR, HB-EGF, MAPK and CD9 in superficial and invasive bladder cancer (BCa) and its correlation with recurrence and disease progression.

**Methods:** Tissue microarrays from archival material from 117 patients undergoing transurethral resection or cystoprostatectomy for superficial BCa were included in the study. 55 patients showed no recurrence or progression after transurethral resection, 45 patients showed recurrence after first therapy and 17 patients showed disease progression. The immunohistochemistry was done by incubation with mono- and polyclonal antibodies (EGFR from Fa Dako, HB-EGF from Dr. R. M. Adam, Harvard Medical School, Boston, MAPK from Cell Signaling and CD9 from Fa. Serotec), followed by incubation with Envision Plus (Dako, Denmark). Cytoplasmic expression was evaluated and the cases were separated in negative, weak, moderate or strong staining for EGFR. For CD9 and MAPK intensity of staining and percent of stained cells were noticed. HB-EGF was evaluated as staining intensity of the cytoplasm and percent of stained nuclei. Statistical analysis was done by SPSS 17 for Windows.

**Results:** Significant correlations were noticed in EGFR, MAPK and CD9 expression patterns in recurrent or progressive BCa comparing with tumors without recurrence or progress. Statistic significant differences were also observed between normal tissue and pTa and pT1 BCa for EGFR expression and between pTa and pT1 BCa for the cytoplasmic HB-EGF expression. Expression of EGFR and nuclear expression of MAPK correlated with tumor grade. Tumor specific, but not overall survival, is shorter in CD9 negative bladder cancer cases than in CD9 positive cases (log rank test, p=0.015) as well as in tumor with positive cytoplasmic expression for HB-EGF (log rank test, p=0.037). The tumor specific survival is correlated with cytoplasmic as well as with nuclear MAPK expression in bladder cancer (log rank test; p=0.009, p=0.007, respectively). The Spearman correlation showed an association between immunohistochemical expression of CD9, MAPK and HB-EGF.

**Conclusions:** Expression of EGFR, HB-EGF, CD9 and MAPK are associated with tumor stage and grade in superficial BCa and could predict progression and recurrence, and may play an important role for tumor specific survival in BCa.

#### Fr-110

##### **DBC1 and FGFR3 expression as indicators of tumor progression and recurrence in superficial bladder cancer**

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**Aims:** This study explores the expression of DBC1 (deleted in bladder cancer locus 1, a candidate tumor suppressor gene on 9p32–33) and FGFR3 in superficial and invasive bladder cancer (BCa) and the correlation to recurrence and progress.

**Methods:** Tissue microarrays (TMA) from archival material from 117 patients undergoing transurethral resection (TUR) or cystoprostatectomy for superficial BCa were included in the study. 55 patients showed no recurrence or progression after TUR, 45 patients showed recurrence after first therapy and 17 patients showed disease progression. The immunohistochemistry for DBC1 was done with a polyclonal antibody (Protein Tech Group). For FGFR3 we used a mouse monoclonal antibody (Santa Cruz Biotech). Primary antibody incubation was followed by detection with EnVision Plus (Dako, Denmark). For DBC1, cytoplasmic expression was evaluated and the cases were separated in positive and negative; for FGFR3 cytoplasmic and nuclear expression

was studied and separated in negative, weak, moderate or strong. Statistical analysis was done by SPSS 17 for Windows.

**Results:** Normal urothelium is always positive for DBC1 and FGFR3. A statistically significant loss of DBC1 expression was observed in progressive and recurrent BCa compared with cases without recurrence ( $p < 0.05$ ). G2 tumors showed statistically significant loss of DBC1 compared with G1 and G3 tumors ( $p < 0.001$ ). Also significant differences in DBC1 expression were noticed in pTa tumors compared with pT1 tumors ( $p = 0.002$ ). Significant differences in FGFR3 expression was noticed also in cases with progression and recurrence compared with cases without progression or recurrence ( $p < 0.05$ ). The FGFR3 expression is different in normal urothelium, pTa and pT1 cases, respectively. Also significant differences were found in BCa depending on differentiation ( $p < 0.02$ ). No correlation to overall or tumor specific survival was noticed for DBC1 or FGFR3 expression.

**Conclusions:** Loss of DBC1 is correlated with increased tumor stage in BCa. The FGFR3 expression is significantly correlated with tumor stage and grade in BCa. FGFR3 expression and loss of DBC1 could be indicators for tumor progression or recurrence in superficial BCa.

## Prostatakarzinom

### Fr-111

#### ADAM12 is overexpressed in prostate cancer and is associated with disease progression

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**Aims:** In a mouse model of prostate cancer the disintegrin and metalloprotease ADAM12 is upregulated in carcinoma-associated stroma and is involved in tumour progression. In this study, we analyzed the expression of ADAM12 in a large cohort of human prostate cancers.

**Methods:** 502 clinically characterized prostate tissues were immunohistochemically analysed for ADAM12 expression using a well characterized rabbit polyclonal antibody. This data was correlated to clinico-pathological parameters including relapse-free survival.

**Results:** ADAM12 is overexpressed in prostate tumors in epithelia (92% vs. 46%) and adjacent stroma (54% vs. 6%) in comparison to normal tissues. Epithelial ADAM12 expression was correlated with higher pT stage, larger tumour size, higher pre-operative PSA levels and high stromal ADAM12 expression. In univariate survival analysis, strong ADAM12 was associated with shortened relapse-free survival ( $p < 0.05$ ).

**Conclusions:** ADAM12 is overexpressed in prostate cancer, correlates to conventional parameters of tumor progression and shorter survival times. We conclude that ADAM12 might play a role in progression of prostate cancer.

### Fr-112

#### ERG rearrangement as a marker to differentiate between small cell lung cancer and small cell prostate cancer

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**Aims:** Small cell prostate cancer is a rare but aggressive disease. Currently, its histogenetic origin is unclear and its distinction from metastatic small cell

lung cancer is challenging. The aim of our study was to determine whether the ERG rearrangement commonly observed in acinar prostate cancer can distinguish small cell prostate cancer from small cell lung cancer samples.

**Methods:** We assessed 15 small cell prostate cancers and 22 small cell lung cancers for ERG rearrangement using FISH. Commonly used and novel immunohistochemical markers (i.e. AR, CANT1, GOLPH2, PSA, PSMA, CD56, EMA, TTF1, Chromogranin A, Synaptophysin and Ki-67) were further studied.

**Results:** ERG rearrangement occurs in 86% small cell prostate cancers but in none of the small cell lung cancers. The prostate targeting markers AR, CANT1, PSA, and PSMA were positive in a minority of small cell prostate cancer samples but none of the small cell lung cancer samples whereas GOLPH2 was positive in the majority of both entities. CD56, EMA, and TTF1 were negative in most small cell prostate cancer samples and positive in the majority of small cell lung cancer samples. Thus, ERG rearrangement is the best marker to differentiate between both tumours ( $p < 0.0001$ ).

**Conclusions:** The ERG rearrangement is commonly observed in small cell prostate cancer supporting the hypothesis that ERG rearrangement occurs in aggressive prostate cancers. Furthermore, the ERG rearrangement is the most significant marker to differentiate between small cell prostate cancer and small cell lung cancer. Moreover, our data suggest that small cell prostate cancer is not a tumour entity on its own but a dedifferentiated variant of common acinar prostate cancer.

### Fr-113

#### p16/ARF promoter methylation in prostate carcinoma

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**Aims:** p16INK4a and p14ARF are gene products of the CDKN2A locus and play an important role in the regulation of cell proliferation and apoptosis, via p53, and the RB pathway. We investigated on the methylation of these promoters in prostate cancer and their influence on p53.

**Methods:** Methylation of the p16 and ARF promoters in 91 cases of prostate carcinoma was checked by methylation sensitive PCR (MSP). Immunohistochemistry on paraffin sections was performed to reveal the expression of p53.

**Results:** Methylation of the p16 promoter increased with increasing T-category ( $p < 0.0001$ ), and in Gleason grade 4+3 tumours, as compared to Gleason grade 3+3 and 3+4 ( $p < 0.05$ ). It was independent of tumour size. ARF-methylation was highest in Gleason 3+3 tumours ( $p < 0.05$ ), and decreased with the T-category ( $p < 0.0001$ ). In immunohistochemistry, p53 positive cells were on average less in cases with methylated p16 (19.9% +/- 21.8% (sd)) than with unmethylated p16 (39.2% +/- 35.2%). For ARF methylation no correlation with p53 expression was observed.

**Conclusions:** Methylation of the p16 promoter in prostate cancer correlates with T-stage and Gleason grading. It influences cell cycle regulation via the expression of p53. ARF promoter methylation showed indirect correlation with T-stage, but no correlation with p53 expression. In summary, increased p16 promoter methylation in prostate cancer is associated with higher T-stage and Gleason grading and might be a prognostic marker for prostate cancer.

### Fr-114

#### Margin status of the vas deferens in radical prostatectomy specimens. Relevance or waste of time?

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**Aims:** Examination of the margin status of radical prostatectomy specimens is of interest since a positive margin has implications in the risk of a relapse and possible adjuvant therapies. It is surprising that the examination of the vas

deferens margin is not generally recommended and not general practice. To test the relevance we examined the vas deferens margin status in radical prostatectomies.

**Methods:** A total of 2701 consecutive prostatectomy specimens from 1995/2009 (Mannheim, n=1932) and 2007/2009 (Homburg, n=769) were reviewed for a tumor infiltration of the vas deferens margin and these data were correlated with clinico-pathological data.

**Results:** In 41/2701 cases (1.5%) a positive margin of the vas deferens was detected. In 12 cases even a bilateral infiltration was present. All tumors were locally advanced [pT3a (n=1), pT3b (n=34), pT4 (n=6)], 15 (37%) had lymph node metastases. The Gleason scores ranged from 7 to 9. Preoperative PSA was between 3.1 and 127 ng/ml (mean 28.02 ng/ml). In all cases with seminal vesicle infiltration (40/41) the positive margin of the vas deferens was on the same side. In 11/15 patients (73%) with pN1 status lymph node metastases, seminal vesicle infiltration and positive margin of vas deferens were seen on the same side. In 16 cases (39%) infiltration of the vas deferens was the only positive margin of the whole specimen.

**Conclusions:** A positive margin of the vas deferens is an infrequent finding but in 39% of cases the vas deferens represented the only positive margin. The question whether and what kind of adjuvant therapy is indicated remains to be discussed. A histological evaluation of the vas deferens margin seems therefore reasonable.

#### Fr-115

##### TMA study to enlight the influence of LOXL-2 and SNAIL on the E/N-Cadherin switch in prostate cancer

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**Aims:** Loss of cell adhesion plays a major role in cancer progression. Especially E- and N-Cadherine expression can be used to keep track for loss of cell adhesion and invasion in prostate cancer. These matrix associated molecules are some from a broader set of other stromal associated genes which are dysregulated in prostate cancer like e.g. collagens and are triggers or tracers of tumour growth and progression.

**Methods:** The study is based 963 prostate cancer cases without preoperative treatment. The samples collected on tissue microarrays were investigated immunohistochemically. A marker panel (LOXL-2, E-, N-Cadherin, SNAIL) was analyzed to decode the interaction network in-between these factors. Special attention was given to the E- and N-Cadherin switch.

**Results:** LOXL-2 and SNAIL were positively correlated to the Expression of E-Cadherin ( $P < 0,001$ ), whereas significant association of LOXL-2 and elevated N-Cadherin expression to a high Gleason score ( $P = 0,026$ ) could be seen. No correlation could be found between N- or E-Cadherin and preoperative PSA or stage.

**Conclusions:** High expression levels of SNAIL are corresponding with E-Cadherin expression, whereas the role of LOXL-2 remains indistinct. N-Cadherin expression and loss of E-Cadherin seems to trace or support aggressive tumour behaviour like the metastatic potential.

#### Fr-116

##### Correlation between prostate cancer volume by computer aided three dimensional reconstruction of radical prostate ctomy and preoperative PCA3-score

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**Aims:** Serum PSA is a widely used diagnostic marker for prostate cancer, but is significantly prone to bias. Recently, a test for prostate cancer gene 3 (PCA3) has been developed using voided urine samples. In this study, we correlated PCA3-score, preoperative PSA and overall Gleason score with the tumor volume, tumor location and prostate volume.

**Methods:** 55 patients with biopsy-proven cancer were included in this study. PCA3 score and serum PSA were quantified pre-operatively. Radical prostatectomy was performed. Prostatectomy specimens were whole-mounted and sectioned. Cancer areas were marked on the slides and computer-aided 3D-reconstruction along the urethra was performed.

**Results:** Mean total serum PSA was 7.6 ng/ml (range 3.5–17 ng/ml), mean PCA3-score was 48 (range 5–233), mean tumor volume was 1.4ccm (range 0.3–9.9 ccm), and mean prostate specimen volume 28ccm (range 13–95 ccm). Using the colliculus spermaticus as topographic reference, tumors were divided in groups according to their distance from the prostatic part of the urethra, using 8, 16 and 32 mm radius as cutoff points. No significant correlation was observed for PCA3-score and location, tumor volume and overall Gleason score but slight tendency of association ( $p < 0,7$ ) with periurethral location.

**Conclusions:** PCA3-Score is a helpful tool in diagnosis of prostate cancer independent of features like organ volume, tumor volume at time of diagnosis or Gleason grading in contrast to serum PSA.

#### Fr-117

##### P53 Codon 72 SNP (R72P) and prostate cancer risk

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**Aims:** The tumor suppressor p53 plays a major role in stress responses of the cell and modulates cell cycle arrest and apoptosis. The p53 R72P SNP was found to be associated with increased risk of various malignancies. However, for prostate cancer only limited data are available, especially in Caucasian population. We therefore analysed the distribution of the R72P SNP in male Caucasian prostate cancer patients and a healthy control group.

**Methods:** Peripheral blood or normal prostate tissue from formalin-fixed, paraffin-embedded tissue sections were used for DNA isolation. Allelic variants of p53 Codon 72 SNP were determined using RFLP analysis. Overall, 194 male patients without any malignancy and 118 male consecutive prostate cancer patients were investigated.

**Results:** The distribution of the p53 R72P SNP did not differ significantly between prostate cancer patients and the control group. There was also no association between the allelic variants and Gleason score. Significant difference in the distribution of the R72P SNP regarding onset of the disease was found ( $p = 0,035$ ), showing increased frequency of the proline variant in younger patients ( $\leq 60$  years). Regarding recurrence, a trend towards increase of the G-allele was found in patients with early recurrence.

**Conclusions:** The overall risk of prostate cancer is not associated with R72P SNP. However, disease onset might be modulated by the p53 variant allele suggesting an important role of apoptosis regulation in prostate carcinogenesis.

## Fr-118

**Diagnostic and prognostic value of T-cell receptor gamma alternative reading frame protein (TARP) expression in prostate cancer**

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**Aims:** T-cell receptor gamma chain alternative reading frame protein (TARP) has recently been proposed as being up-regulated in prostate cancer (PCA). Additionally, TARP has been proposed as a potential therapeutic target for cancer therapy.

**Methods:** We analysed the protein expression of TARP in a large well characterised prostate cancer cohort to assess its diagnostic and prognostic value. TARP protein expression was carefully analysed and associated with clinicopathological parameters.

**Results:** TARP is significantly over-expressed in the vast majority (~85%) of PCA in comparison to non neoplastic prostate tissue. Its expression was associated with conventional markers of unfavourable tumour behaviour. However, a prognostic value of TARP was not found.

**Conclusions:** The diagnostic value of TARP is limited in comparison to AM-ACR, p63 or GOLPH2. Since TARP specific immunologic therapy regimen are currently being tested, the high frequency of TARP over-expression in PCA conveys a high potential for a predictive and potentially therapeutic use of this biomarker.

## Fr-119

**FGFR3 mutation analysis in prostate cancer**

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**Aims:** In prostate cancer, the FGF system has already been well studied, but the role of FGFR3 is still unknown. To date, only limited data of FGFR3 mutations in prostate cancer are available. Most recently, activating FGFR3 mutations were described to be associated with low-grade prostate tumors. Therefore the aim of this study was to investigate the FGFR3 mutation status in a comprehensive series of prostate tumors.

**Methods:** Analyses were done on human FFPE specimen. Overall, 102 archival prostate tumors achieved by radical prostatectomy and 29 incidental prostate tumors obtained by TUR-P (low grade tumors (Gleason score ≤6): n=22) were investigated. After microdissection and DNA isolation, all FGFR3 mutation hotspots known from human malignancies were analyzed using SNaP-shot or RFLP assays.

**Results:** All cases could successfully be analyzed by SNaP-shot, 80 cases were investigated using RFLP. No mutation in FGFR3 could be detected in any of the analyzed cases. There were also no mutations in patients with concomitant bladder tumors as reported previously.

**Conclusions:** Our results indicate that mutational activation of FGFR3 plays no important role in prostate carcinogenesis. The most recently reported FGFR3 mutations in low-grade prostate tumors could not be verified in our series.

## Vorträge: Beste Forschungsbeiträge

## Fr-120

**fAChR/CD3 bispecific T cell engaged antibody for immunotherapy of rhabdomyosarcomas**

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**Aims:** Rhabdomyosarcomas (RMS) are the most frequent malignant soft tissue tumors in childhood with a 5-year survival rate of 5–10% in advanced disease stages (~40% of patients). Based on our preliminary work with identification of the fetal acetylcholine receptor (fAChR) as a tumor-specific antigen in RMS, we have generated a specific human antibody fragment (scFv) directed against the fAChR. This shows as an immune toxin (Pseudomonas exotoxin coupled with A (ETA)) as well as a chimeric T cell receptor a very strong tumor-specific toxicity in vitro and in animal models respectively.

**Methods:** Western Blot, immunofluorescence, bacterial protein expression, cell transfection, FPLC purification, XTT-cytotoxicity assay

**Results:** In order to increase functionality and decrease undesirable toxicity of the existing scFv based anti-fAChR constructs we generated a CD3/fAChR scFv based BiTE (bispecific T cell engaged antibody) that showed strong RMS specific cell lysis in vitro and in vivo after coincubation with peripheral blood lymphocytes (PBL).

**Conclusions:** Since established CD3/CD19 BiTEs are highly specific and functional against B cell lymphomas/leukemias with low unspecific toxicity, the CD3/fAChR BiTE will be the basis for subsequent clinical phase I trials for the immunotherapy of aggressive/advanced RMS.

## Fr-121

**Morphological and molecular characterization of a novel mouse mutant for the peripheral myelin protein 22 (Pmp22 gene) - associated with peripheral neuropathy**

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**Aims:** Mutations in the human peripheral myelin protein 22 (Pmp22) gene cause peripheral neuropathies such as the Charcot-Marie-Tooth (CMT) disease. Here we describe a novel mutant mouse line (TRE002) created using N-ethyl-N-nitrosourea mutagenesis that shows a new point mutation in the Pmp22 gene.

**Methods:** A total of 33 mice were analyzed (15 mutant, 18 control littermates). After X-ray analysis, all organs underwent gross and histopathological analysis. The sciatic nerve and the spinal cord were studied by electronic microscopy. Single nucleotide polymorphism and sequence analysis were performed for the identification of the mutation.

**Results:** The TRE002 mutant mice exhibited a marked resting tremor, were hypoactive and showed abnormal gaits with marked muscle atrophy. In electron microscopy, hypomyelination of the spinal cord and peripheral nerves as well as a decrease in the axon number was observed. In addition, X-ray analysis accompanied by high plasma levels of alkaline phosphatase revealed bone alterations in the mutant mice. A T→A transversion in the third coding exon of the Pmp22 gene caused an aminoacids substitution such as in patients affected by CMT.

**Conclusions:** This new pmp22 mouse mutant line is a powerful tool to elucidate new pathophysiological events of the hereditary peripheral neuropathies.

**Fr-122****Differential matrix remodelling of adult bone marrow and perinatal umbilical cord mesenchymal stem cells on 3D- collagen scaffolds for bone tissue engineering**

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Adult human mesenchymal stem cells from bone marrow (BM-MS) represent a promising source for skeletal regeneration. Perinatal MSC from Wharton's Jelly of the umbilical cord (UC-MS) are expected to possess enhanced differentiation capacities due to partial expression of pluripotency markers. This study compares the cell-mediated remodelling of three-dimensional collagen I/III gels during osteogenic differentiation of both cell types regarding migration, matrix remodelling and differentiation. When activated through collagen contact and subjected to osteogenic differentiation, UC-MS differ from BM-MS in expression and synthesis of extracellular matrix (ECM) proteins as shown by histology, immunohistochemistry, Western Blot analysis and realtime-RT-PCR. The biosynthetic activity was accompanied in both cell types by the ultrastructural appearance of hydroxyapatite/calcium crystals and osteogenic gene induction. Following secretion of matrixmetalloproteinases (MMP), both MSC types migrated into and colonised the collagenous matrix causing matrix strengthening and contraction. These results indicate that UC-MS and BM-MS display all features needed for effective bone fracture healing. The expression of ECM differs in both cell types considerably, suggesting different mechanisms for bone formation and significant impact for bone tissue engineering (Biomaterials, in press, 2009).

**Fr-123****ABC1/MDR1 mediates anticancer drug-efflux in IPH-926 chemoresistant human lobular breast cancer cells**

T. Krech, E. Scheuerer, M. Noskowitz, H. Christgen, U. Lehmann, H. Kreipe, M. Christgen

Institut für Pathologie, Medizinische Hochschule Hannover

**Fr-124****Intermediate miRNA expression profile of patients with Graves' disease compared to normal thyroid and papillary thyroid carcinoma**

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**Aims:** Several studies reported on a higher risk of developing papillary thyroid carcinomas (PTC) in patients with Graves' disease (GD) with cancer rates ranging from 1% to 9%. MicroRNAs (miRNAs) are endogenous, non-coding, small RNAs that regulate gene expression. Selected miRNAs are known to be deregulated in PTC. The aim of the study was to determine the miRNA profile in patients with GD and PTCs.

**Methods:** MiRNA expression of five selected miRNA types (146b, 181b, 21, 211, 222) was analysed in 158 consecutive patients who underwent surgery for GD (age 14–83 yr, mean 45 yr, male:female 1:4.1) by RT-PCR TaqMan miRNA assay and compared with 50 PTCs and 4 normal thyroid tissues.

**Results:** The prevalence of PTC in this series with GD was 7% (11/158). Calculating relative changes in gene expression all five miRNAs were significantly upregulated in GD compared with normal tissue with fold changes up to 31 for miRNA 146b ( $p=0,001$ ). No significant fold changes could be demonstrated between PTC (without GD) and PTC associated with GD ( $n=11$ ) whereas all PTC (with or without GD) differ significantly from (adjacent), non-neoplastic thyroid tissue in at least 4/5 miRNAs ( $p=0,047$ ).

**Conclusions:** The miRNA expression profile of patients with GD is intermediate between normal thyroid tissue and PTC suggesting that Graves' disease is a risk factor for developing PTC.

**Fr-125****Epigenetic reprogramming of hematopoietic stem cells by fusion with embryonic stem cells**

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All mature blood cells develop from hematopoietic stem cells (HSC), which due to their multilineage differentiation potential and self-renewal capacity maintain hematopoiesis throughout life. Here we show that after fusion of HSC with embryonic stem cells (ESC), HSC acquired a pluripotent, ESC-like state by epigenetic reprogramming. These HSC/ESC hybrids gave rise to non-hematopoietic derivatives as demonstrated in vitro by embryoid body (EB) assay and in vivo by teratoma formation. Gene array analysis on a global scale confirmed the pluripotent state of HSC/ESC hybrids by clustering with ESC and induced pluripotent stem cells (iPS cells). Further, the demethylated status of CpG sites of Oct4 promotor/enhancer regions demonstrated efficient epigenetic reprogramming of the hybrids. The HSC/ESC hybrids are stable tetraploid as shown by flow cytometry and karyotype analysis. Interestingly, microarray and real-time RT-PCR analyses revealed that the reprogrammed HSC/ESC hybrids express gene clusters from both parental population but also clusters that are not expressed in either parental cell population. The HSC/ESC hybrids can be efficiently differentiated into hematopoietic progenitor cells and functional, mature cardiomyocytes suggesting a somatic memory by an increased and accelerated propensity towards mesodermal cell differentiation. Furthermore, HSC/ESC hybrids were differentiated into bipolar neuronal stem cells underlining their developmental flexibility.

## Vorträge: Molekulare gastrointestinale Tumorpathologie

### Fr-126

#### Molekulare Pathologie des Kolonkarzinoms – Stand des Wissens

T. Kirchner  
München

### Fr-127

#### Pankreaskarzinom – Molekulare und Chirurgische Pathologie

A. Tannapfel  
Bochum

### Fr-128

#### HER2 testing in gastric cancer: Guideline validation and development

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**Aims:** Trastuzumab therapy showed overall survival benefit in Her2-pos. advanced gastric cancer (GC) within the ToGA trail. It was the aim of this study to validate Her2 testing procedure in GC in terms of inter-laboratory and inter-observer variation.

**Methods:** Based on TMA samples with 30 cores immunohistochemical Her2 scoring issues specific for GC among 8 French and German laboratories were identified. In a 2<sup>nd</sup> step a 547 GC core TMA was used to determine scoring concordance between 6 German pathologists.

**Results:** A HER2 score deviation  $\leq 1$  was found in 14/29 cores (48.3%) when HercepTest was used as compared to 22/29 cores (75.9%,  $p=0.002$ ) when 4B5 was used. After discussion of these results inter-observer variation reached 95.6% concordance for intensity and 91.8% for area scoring using 4B5 by 6 pathologists. Inter-observer agreement was especially low if less than 5 tumor cells were stained.

**Conclusions:** Her2 testing by IHC is quite different from breast as intensity scoring is of major importance and the area cut-off (>10%) is omitted in biopsies. Reproducible Her2 immuno-scoring in GC needs guidelines and training. Thus, trials for proficiency testing are planned to be established parallel to the approval of the drug for stomach cancer treatment.

### Fr-129

#### Lack of CCR7 expression is rate limiting for lymphatic spread of pancreatic ductal adenocarcinoma

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**Aims:** We studied the role of the chemokine receptor CCR7, which has been described to be a key regulator in the homing of immune cells from peripheral tissues to lymph nodes, in the lymphatic spread of pancreatic ductal adenocarcinomas (PDACs).

**Methods and Results:** CCR7 was expressed in the majority (10/12) of PDAC cell lines at mRNA level. While FACS analysis revealed only low constitutive CCR7 protein expression in most cell lines in monolayer cultures, CCR7 expression was significantly elevated in 7 of 11 cell lines upon spheroid culture. In transwell migration assays CCR7 expressing PDAC cells showed enhanced migration towards CCL21. In an orthotopic nude mouse model, CCR7 transfected Pt45P1 cells gave rise to significantly larger tumors and showed a higher frequency of lymph vessel invasion and lymph node metastases than mock transfected cells. Likewise, of 121 human PDACs 46% exhibited moderate-to-strong CCR7 expression by immunohistochemistry, which correlated with high rates of lymph vessel invasion and the presence of lymph node metastases. Moreover, in PDAC completely lacking CCR7 expression, high rates of lymph vessel invasion and >3 lymph node metastases were not detected. The evaluation of CCL21 expression by immunofluorescent staining revealed a significant upregulation of CCL21 in peritumoral and intratumoral lymph vessels compared with lymph vessels in disease-free pancreata.

**Conclusion:** CCR7 expression significantly promotes lymphatic spread in PDAC. Lymph vessel invasion of PDAC cells may be additionally enhanced by upregulation of CCL21 in tumor-associated lymph vessels, representing a previously unknown factor of lymphatic spread.

### Fr-130

#### MiRNome for microdissected pancreatic ductal adenocarcinoma

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**Aims:** Pancreatic ductal adenocarcinoma (PDAC) is known for its very poor overall prognosis. Recently, misregulated micro RNAs have been identified as a novel class of therapeutic and diagnostic targets for cancer. In this study we aimed at the identification of differently expressed miRNAs in microdissected PDAC.

**Methods:** A protocol for microdissection and RNA isolation from FFPE PDAC and normal ductal tissues to enable miRNA array analyses using the Agilent MiRNA Array technology was established. MiRNA expression profiles were also generated from chronic pancreatitis specimen. Differentially regulated miRNAs were validated with qRT-PCR.

**Results:** MiRNA profiles from FFPE tissues were successfully established. Following statistical analyses we identified to this end 57 differentially expressed miRNAs. Upon validation of selected miRNAs we identified a miRNA which has not been described to be overexpressed in PDAC, previously. A qRT-PCR index using only miR-24 and the newly identified miRNA was found to discriminate normal pancreas, chronic pancreatitis and cancerous tissues, establishing a potential utility for miRNAs in diagnostic procedures.

**Conclusions:** MicroRNA profiles from microdissected pancreatic tissues were able to identify previously not described misregulated miRNAs. The newly identified miRNAs serve as novel candidates to establish diagnostic and also therapeutic strategies for PDAC.

#### Fr-131

##### **Aurora-A inhibition induces PLK-1 up-regulation in chromosomal- instable colorectal carcinoma cell lines**

C. Herz, C.D. Fichter, C. Münch, A. May, A. Schöpflin, J. Köller, M. Werner, S. Lassmann

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**Aims:** Due to its over-expression in several human carcinomas, the centrosomal kinase Aurora-A is discussed as therapeutic target. Here, we investigated the effect of Aurora-A inhibition in chromosomal- (CIN) and microsatellite- (MIN) instable CRC cell lines, focussing on the Aurora-A interaction partner Polo kinase 1 (PLK-1).

**Methods:** Aneuploid CIN- (HT29, CaCo-2) and diploid MIN- (HCT116, DLD1) -type CRC cell lines were analyzed for PLK-1 mRNA (Q-RT-PCR) and protein (Western blot, immunofluorescence) expression. Cells were either treated with Aurora-A-specific or unspecific siRNA or were incubated with medium only. Aurora-A and PLK-1 mRNA/protein expression and localization were then analyzed in treated versus untreated cells.

**Results:** In untreated HT29 cells, PLK-1 mRNA and protein was highly expressed and correlated to strong Aurora-A expression. Also in untreated HCT116 cells, PLK-1 protein was highly expressed, but independent of Aurora-A expression. Inhibition of Aurora-A resulted in significant up-regulation of PLK-1 mRNA and protein expression in HT29 ( $p < 0.001$ ) and CaCo-2 ( $p = 0.013$ ) cells.

**Conclusions:** High PLK-1 expression may occur in diploid and aneuploid CRC cells. However, significant PLK-1 up-regulation due to Aurora-A inhibition is only seen in aneuploid CRC cells. This suggests that CRC cells have distinct functional levels of Aurora-A/PLK-1 interactions, which may influence the efficiency of Aurora-A and/or PLK-1 targeted therapies.

#### Keynote Lecture:

#### Das Humane Krebssequenzierungsprojekt

#### Fr-132

P. Lichter  
Heidelberg

#### Vorträge: Molekulare Tumorpathologie I

#### Fr-133

##### **Morphologische und molekulare Pathologie des Lungenkarzinoms**

I. Petersen  
Jena

#### Fr-134

##### **Molekularpathologie des Prostatakarzinoms 2010**

A. Hartmann  
Erlangen

#### Fr-135

##### **Endothelial factors in the prostate: Loss of Caveolin-1 in prostate cancer correlates with tumour aggressiveness**

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**Aims:** Understanding the mechanisms of angiogenesis in prostate cancer (PCa) could be helpful for the development of prognostic markers and therapeutic options. Therefore, we investigated angiogenic and endothelial factors (VEGFA, VEGFR2, Cav-1, CD31, CD34, CD105, CD144, CD146) on the mRNA and protein level in well defined cohorts of prostate cancers and correlated the data with microvessel density (MVD) and clinicopathological parameters.

**Methods:** Tissue Microarrays (TMA) of 3.261 prostatectomy specimens from patients with PCa and a validating cohort of 64 specimens were analysed for MVD by immunohistochemistry (CD31, CD34). The smaller cohort was additionally used to assess endothelial Cav-1 protein expression and qPCR-based mRNA levels of normal and tumour tissue.

**Results:** In both cohorts, MVD correlated significantly with tumour grade and pT status. In comparison to normal tissue, Cav-1 mRNA was significantly down-regulated in tumour samples and correlated inversely with pT status ( $P = 0.006$ ) and Gleason score ( $P = 0.012$ ). Higher MVD tended to result in lower Cav-1 mRNA expression ( $r_s = -0.277$ ,  $P = 0.056$ ). In a univariate Kaplan Meier survival analysis, lower Cav-1 mRNA expression was associated with biochemical recurrence.

**Conclusions:** The results suggest that loss of Caveolin-1 expression plays a role in the development and progression of prostate cancer.

#### Fr-136

##### **MYC and TPD52 (PrLZ) are „8q amplification targets“ with strong prognostic relevance in prostate cancer**

S. Minner<sup>1</sup>, Julia Rohwer<sup>1</sup>, E. Burandt<sup>1</sup>, P. Tennstedt<sup>1</sup>, S. Kurtz<sup>2</sup>, M. Mader<sup>1</sup>, L. Burkhardt<sup>1</sup>, T. Schlomm<sup>2</sup>, M. Graefen<sup>2</sup>, H. Huland<sup>2</sup>, G. Sauter<sup>1</sup>, R. Simon<sup>1</sup>

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**Aims:** Overrepresentation (gain) of 8q is one of the most common genomic alterations in prostate cancer. MYC is the most frequently reviewed „8q amplification target“ but other 8q genes may also be involved. Increased expression of TPD52 (PrLZ), localized on 8q21.1, has been suggested as a marker for prostate cancer progression and metastasis. We thus aimed to determine the relative importance of genomic TPD52 and MYC alterations.

**Methods:** A total of 1018 prostate cancers treated by radical prostatectomy with long-term follow-up data were successfully analyzed by fluorescence in situ hybridization (FISH) for both MYC and TPD52 in a tissue microarray (TMA) format.

**Results:** Increased gene copy number of MYC were found in 127 (12.5%) and of TPD52 in 209 (20.5%) of these cases. Both genes were simultaneously affected in 76 cases while gains of MYC alone occurred in 51 and of TPD52 alone in 133 cases. Gains of both genes were strongly related to Gleason grade ( $p < 0.0001$  for MYC and TPD52) and tumor stage ( $p < 0.0001$  each). TPD52 and MYC gains were also associated with an increased risk for PSA recurrence ( $p < 0.0001$  for TPD52;  $p = 0.006$  for MYC). Remarkably, the combined analysis of both genomic areas revealed that this association was largely driven by TPD52 while tumors with overrepresentation of MYC alone had a similar clinical outcome as cancers without any 8q gains.

**Conclusions:** Although the number of cases with TPD52 alterations was not very large in this study, these data suggest a much stronger impact of TPD52 gene copy numbers than seen for MYC. This is interesting in the light of recent reports suggesting TPD52 protein as a target for vaccination in tumors.

**Fr-137****Global analysis of CTCF regulated genes in early stages of prostate cancer**

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**Aims:** Age is the most important factor in the development of prostate cancer (PCA) and epigenetic silencing of genes is one of the earliest molecular alterations commonly found in prostate neoplasia. It was recently shown that loss of imprinting (LOI) of IGF2 occurs in the normal ageing prostate. The resulting increased IGF2 levels are believed to contribute to prostate carcinogenesis and may also account for the strong association with older age. LOI of IGF2 is due to altered regulation by the enhancer-blocking element CCCTC-binding factor (CTCF). CTCF is a chromatin insulator that is required for repression of the maternal imprinting at the imprint control region (ICR) and influences the activation and inhibition of thousands of genes in the genome. Using IGF2 as an indicator of altered epigenetic CTCF control, we identified potential CTCF target genes by specific CTCF knock down in PCA cell lines. The expression of these genes was further analyzed in non-neoplastic and neoplastic prostate tissues with and without LOI of IGF2.

**Methods:** Gene expression profiles were performed on prostate cancer cells (PC3) with a specific CTCF knock down 48 h after transfection. Custom arrays containing potential CTCF target genes were performed on 7 normal morphologically healthy prostate tissues tested for IGF2 LOI and ROI status and on 12 PCA tissues of radical prostatectomy. The most significant genes were tested in 24 normal prostate tissues with LOI and ROI.

**Results:** 390 genes were differentially expressed in histologically non-neoplastic prostate tissue with and without LOI (taken from radical prostatectomy specimens resected for PCA). Of these, 111 genes were also significantly regulated in PCA compared to non-neoplastic prostate and 104 were significant when comparing different Gleason grades (Gleason 6 vs 8). 50 genes were commonly deregulated in all comparisons.

**Conclusions:** Our data suggest an important role of the insulator and transcription factor CTCF in very early stages of PCA, potentially by global relaxation of the imprinting of a large array of CTCF target genes. In vitro and in vivo analyses using CTCF knock out animals are underway to further define the exact mechanisms of this process.

**Fr-138****K-RAS-Mutations and expression of p53 and p16: A comprehensive study of 27 borderline tumors (LMP) of the ovary and associated peritoneal implants**

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**Aims:** Mutation of K-RAS and coincidental missing of p53-mutation have been described in borderline tumors of the ovary. Data concerning different peritoneal implants of the same patient are scarce. Therefore, we assessed KRAS-mutation as well as p53- and p16-overexpression in a series of 27 LMPs, 25 of them with peritoneal implants.

**Methods:** Paraffin embedded material of 27 patients, including 26 serous and one serous-mucinous subtype of ovarian LMPs, 25 of them with between 3 to 10 implants was available (total number=140). Expression of p53 and p16 was revealed by immunohistochemistry (IHC). DNA analysis was performed by PCR and direct sequencing. All tumors/lesions underwent microdissection prior to PCR. K-RAS codon 12/13 in exon 2 was analysed.

**Results:** 11 (42%) of LMPs contained a KRAS-mutation. But only in 5 (19.2%) of these cases the same mutation could also be demonstrated in the associated implants; whereas in no case the KRAS-mutation was seen in all implants of the same patient. Strong p53-positivity was seen in only 6.6%, p16-positivity in 31.8%.

**Conclusions:** Mutation of K-RAS is not essential for the development of borderline tumours and implants. These results are a sign of other unknown initial genetic events. The detection of the same mutation in 45% in a part of the implants at least point to a partial metastatic-like nature.

**Vorträge: Aktuelle Methoden der Molekularpathologie I****Fr-139****Whole genome and targeted next generation sequencing of formalin fixed and paraffin embedded tumor tissues**

M.R. Schweiger  
Berlin

**Fr-140****“Non-coding RNA – A new world beyond proteins of Tumor Markers and Regulators?”**

S. Diederichs  
Heidelberg

**Fr-141****Neues zur Proteinanalytik von archivierten Gewebeproben**

K.F. Becker  
München

**Fr-142****“Knut, Rosa and the rest of the gang – little helpers in establishing genetically modified mice”**

H. Schorle  
Bonn

**Vorträge: Plasmazytom und B-Zellneoplasien****Fr-143****Molekulare Pathologie von Plasmazellneoplasien: Stand des Wissens**

F. Fend  
Tübingen

**Fr-144****Klassisches Hodgkin-Lymphom und mediastinales B-Zell-Lymphom – zwei Endpunkte einer gemeinsamen Onkogenese**

P. Möller  
Ulm

**Fr-145****MYC-break-positive B-cell lymphomas with a favourable and adverse prognosis differ dramatically in their MYC target gene expression**

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**Background and Aims:** Aggressive B-cell lymphoma can be separated by gene expression profiling into molecular Burkitt lymphoma (mBL) and cases lacking the mBL signature (non-mBL and intermediate cases). MYC-breaks are present in almost all cases of mBL and are associated with excellent clinical outcome. In contrast, MYC-break-positive non-mBL/intermediate cases display a very short overall survival. In this study we investigated the role of MYC in these lymphomas.

**Methods:** To elucidate the genome-wide MYC binding pattern we performed chromatin immunoprecipitation followed by deep sequencing (ChIP-Seq) employing 3 MYC break positive cell lines. The resulting MYC binding sites were correlated with the gene expression profile of 220 published mature aggressive B-cell lymphoma cases.

**Results:** More than 3000 Myc binding sites were identified by ChIP-Seq. Linkage of these binding sites to gene expression revealed an almost inverse expression pattern in MYC-break-positive mBL as compared to non-mBL/intermediate cases.

**Conclusions:** Our data demonstrate that the expression of MYC target genes dramatically differ in mBL and MYC-break-positive non-mBL/intermediate cases. This might contribute to reciprocal clinical outcome in these lymphomas.

**Fr-146****Functional and therapeutic relevance of CD56(NCAM) expression in multiple myeloma**

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**Aims:** The neural cell adhesion molecule and signal transducer CD56 (NCAM) is an essential marker for abnormal plasma cells and is expressed in ~70% of multiple myelomas (MM) with impact on tumor progression and osteolysis, but neither the expression profile nor the functional relevance of CD56 isoforms in MM has been investigated.

**Methods:** RNase Protection Assay, radioactive qRT-PCR, Western Blot, immunofluorescence, cell transfection and generation of stable transfectants, siRNA knock down, cDNA microarrays.

**Results:** Using new CD56 specific qRT-PCR strategies and antibodies, we could show that CD56<sup>40kD</sup> is the exclusively expressed CD56 isoform in MM and is associated with the progression of MGUS to manifest MM. Moreover myeloma cell lines stably overexpressing the recently by us identified CD56 mutation/polymorphism Gln/Gly599Arg present in 100% of multiple myelomas showed 6-fold decrease of apoptosis and activation of CD56 dependent kinase signalling cascades such as erk, AKT and CamKII.

**Conclusions:** We conclude that CD56 expression in MM has impact on tumor progression and activation of anti-apoptotic/pro-proliferative pathways. Therefore CD56 dependent signalling pathways but also CD56 itself regarding the Gln/Gly599Arg mutation/polymorphism might be potential targets for novel immunotherapeutical strategies in MM.

**Fr-147****Micro RNA expression profiling of Burkitt lymphoma variants**

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**Background and Aims:** Although Burkitt lymphoma (BL) is homogeneous in most of its morphological, immunophenotypical and genetic features except incidence of EBV infection it is divided into three clinical/epidemiological variants (endemic BL, sporadic BL and immunodeficiency-associated BL). This subclassification is based on differences in the geographical appearance and the association with immunosuppression. To clarify whether the BL variants harbour differences at the molecular level they were subjected to micro RNA expression profiling.

**Methods:** Micro RNA expression profiles of 71 BL cases (24 endemic BL, 31 sporadic BL and 16 immunodeficiency-associated BL) were established by microarray analysis. Differential expression of micro RNAs between subgroups was validated by real-time PCR.

**Results:** The micro RNA profiling revealed merely minor expression differences among the BL variants, which was confirmed by real-time PCR. No micro RNA signature could be generated which is suitable for the identification of the BL variants.

**Conclusions:** The results of the micro RNA profiling strengthen the concept that all three BL variants represent one homogeneous disease entity. The clinical/epidemiological differences of the three variants appear to be mainly due to environmental factors.

**Fr-148****Characterization of 32 gastrointestinal (GI-) B cell lymphomas by FISH and SNP-analysis**

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**Aims:** 32 cryopreserved B-cell lymphomas of the GI tract (8 MALT lymphomas, 14 DLBCL, 10 composite lymphomas) were screened by FISH and SNP-analysis.

**Methods:** We performed FISH analysis with commercially available break apart probes for c-Myc, Bcl2, IgH, Bcl6, CyclinD1, Malt1 and Bcl10, and fusion probes for the IgH/Bcl2, c-Myc/IgH, Api2/Malt1 and Malt1/IgH -translocations and a BAC-probes set for the FoxP1-split and compared these data with a FISH data base of 250 lymphomas. SNP-analysis (Affymetrix HGW SNP array 6.0) was performed.

**Results:** All 32 cases were negative for Bcl2, C-Myc, CyclinD1 and FoxP1-splits, as well as for the Bcl2/IgH and Malt1/IgH-translocations. 2/32 cases had a Malt1-split; one of those showed a fusion of the Api2 with the Malt1-gene. One case had an amplification of the Malt1-gene and one case had a Bcl10-split. 9/32 cases showed a Bcl6-split and 12/32 an IgH-rearrangement with unknown translocation partner. Compared with the FISH database of B cell NHL only 16% have such an aberration while the Bcl6-split is twice as high in the GI lymphomas. Of 4 lymphomas with BCL6 split 4 showed no aberrations in the SNP profile pointing to a putative balanced translocation with an unknown partner. No difference in BCL6 protein expression was found in the +/- BCL6 rearranged groups.

**Conclusions:** Apart from morphology, immunohistochemistry and clinics, GI B cell lymphomas have a characteristic pattern of chromosomal breakpoints and translocations.

## Vorträge: Hämatopathologie I

## Fr-149

**Molekulare Pathologie des Hodgkin-Lymphoms**

M.L. Hansmann  
Frankfurt

## Fr-150

**Molekulare Klassifikation maligner Lymphome**

A. Rosenwald  
Würzburg

## Fr-151

**Assessment of clonality in lymphoproliferations – state of the art and caveats**

H.J.M. van Krieken  
Nijmegen

## Fr-152

**Apoptotic lymphoma cells evoke a pro-senescent stromal signal that limits Myc-driven lymphomagenesis**

C. Loddenkemper<sup>1</sup>, M. Reimann<sup>2</sup>, S. Lee<sup>2,3\*</sup>, J. Dörr<sup>2</sup>, V. Tabor<sup>3</sup>, H. Stein<sup>1</sup>, B. Dörken<sup>2,3</sup>, C. Schmitt<sup>2,3</sup>

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**Aims:** To investigate whether cellular senescence plays a role as an additional safeguard program in aggressive B-cell lymphoma with constitutive Myc expression who typically exhibit high amounts of apoptotic cell death.

**Methods:** Transgenic mouse models, immunohistochemistry, immunoblotting, flow cytometry, gene expression analysis.

**Results:** Using the Burkitt like E $\mu$ -myc transgenic mouse lymphoma model, we show that cellular senescence serves as another crucial anti-neoplastic barrier during Myc-driven tumorigenesis in addition to apoptosis. E $\mu$ -myc lymphomas harbor a substantial fraction of senescent tumor cells and lymphomas lacking the methyltransferase Suv39h1 - essential for senescence - develop significantly faster. In addition, a panel of 30 human diffuse large B-cell lymphoma (DLBCL) samples was sub-divided based on Ki-67 immunoreactivity. The group with the lower Ki-67 index presented with a higher fraction of apoptotic cells, more lymphoma-infiltrating macrophages, and stronger reactivity for the TGF- $\beta$  signaling mediator Smad3-P highly reminiscent of a macrophage-derived mechanism of senescence induction in the mouse model.

**Conclusions:** Our study expands the relevance of oncogene-induced senescence (OIS) to Myc-driven cancers and demonstrates that different tumor suppressor programs such as apoptosis and senescence- are enforced in an interdependent fashion between tumor- and non-malignant stroma cells during lymphomagenesis.

## Vorträge: Hämatopathologie II

## Fr-153

**Stand des Wissens zur molekularen Pathologie der myeloproliferativen Neoplasien**

O. Bock  
Hannover

## Fr-154

**Neue Erkenntnisse zur Thrombozytogenese**

H. Schulze  
Berlin

## Fr-155

**Aberrant proplatelet formation in chronic myeloproliferative neoplasms**

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**Aims:** Proplatelets represent cytoplasmic pseudopodia of megakaryocytes which extend into bone marrow sinuses to release platelets. Proplatelets are not visible in conventional bone marrow histology and have not been studied in chronic myeloproliferative neoplasms (MPN) so far.

**Methods:** In this study proplatelets were visualized in situ by immunohistochemical labelling of platelet glycoprotein Ib and confocal laser scanning microscopy.

**Results:** In bone marrow trephines from essential thrombocythaemia, fibrotic and prefibrotic primary myelofibrosis (PMF) there was a significant increase of proplatelet density when compared with normal bone marrow samples ( $p < 0.001$ ). Manifest fibrosis in PMF exhibited the highest density and volume ratio of proplatelets with significant differences to non-fibrotic PMF ( $p < 0.001$ ) and ET ( $p < 0.001$ ).

**Conclusions:** We demonstrate that besides megakaryocytic proliferation extensive pseudopodial proplatelet formation provides a hallmark of MPN. Fibrosing differ from non-fibrosing MPN by density and size of aberrant proplatelets.

## Fr-156

**Essential role for Stat5 in myeloproliferative neoplasms induced by BCR-ABL1 and Jak2V617F**

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**Aims:** The STAT5 proteins are activated in malignant cells from patients with myeloproliferative neoplasms (MPNs) such as chronic myeloid leukemia (CML) and polycythemia vera (PV), but the role of STAT5 in the pathogenesis of these diseases has not been fully elucidated. Here, we sought to determine the requirement for Stat5 in MPNs induced by BCR-ABL1 and Jak2V617F.

**Methods:** Mice with a conditional null mutation in the Stat5 gene were used in retroviral bone marrow (BM) transduction / transplantation models of CML and PV, respectively.

**Results:** In recipients of BCR-ABL1-transplanted BM, loss of one Stat5 allele resulted in attenuation of CML and the appearance of acute B-lymphoblastic leukemia. Complete deletion of the Stat5 gene resulted in the absence of any myeloid or lymphoid leukemia. Recipients of Jak2V617F-transplanted BM failed to develop polycythemia but had evidence of subclinical MPN and substantial myelofibrosis in the BM.

**Conclusions:** We show that Stat5 is necessary for the pathogenesis of CML-like disease induced by BCR-ABL1 and of polycythemia by Jak2V617F, and validate the Stat5 pathway as a target for therapy in MPNs associated with dysregulated tyrosine kinases. However, targeting Stat5 alone may not prevent Jak2V617F-induced myelofibrosis.

**Fr-157****Asymmetric chromosome segregation in dysplastic megakaryocytes in myelodysplastic syndromes**C. Münch<sup>1</sup>, A.M. May<sup>1</sup>, D. Hauschke<sup>2</sup>, J. Roth<sup>1</sup>, S. Lassmann<sup>1</sup>, M. Werner<sup>1</sup>Institut für Pathologie, Universitätsklinikum Freiburg<sup>2</sup>Institut für med. Biometrie u. med. Informatik, Universitätsklinikum Freiburg

**Aims:** Myelodysplastic syndromes (MDS) are clonal disorders of pluripotent hematopoietic stem cells. Distinct morphologically recognisable nuclear features (e.g. polynucleation) occur in dysplastic megakaryocytes (MKs) in MDS and may be due to imperfect endomitotic cell cycles. The aim of our study was to elucidate the chromosome distribution within dysplastic polynuclear MKs.

**Methods:** Bone marrow smears of 7 MDS patients and 4 healthy controls were analysed by May-Grünwald-Giemsa (MGG) staining. The positions of selected MKs were recorded for subsequent FISH (Fluorescence in situ hybridization) imaging. Sequential multilocus FISH was performed with probes specific for chromosomes 1 (RP11-79E5), 7 (pZ7.5), 8 (pZ8.4) and 18 (2xbA). FISH signal distribution in the nuclei of the previously recorded MKs was examined by 3D imaging. The number of nuclei of polynuclear MKs was evaluated by MGG and DAPI staining.

**Results:** MKs of healthy controls predominantly showed a single multilobulated nucleus. In contrast, MKs of MDS cases exhibited a high degree of polynucleation with up to nine separate nuclei. In binuclear MKs with a combined DNA content of 4 N both nuclei were diploid. Interestingly, varying chromosome numbers were detected within the separate nuclei of polynuclear MKs with a combined DNA content of 8 N and 16 N.

**Conclusions:** Our study indicates an asymmetrical segregation of chromosomes in polynuclear MKs resulting in aneuploidy of the separate nuclei. Our observations provide the basis for further examination of cell cycle associated proteins responsible for dysregulated endomitosis and hence potential causes of polynucleation in MDS.

**Fr-158****GATA1 mutations are essential in transient myeloproliferative disorder (TMD) but not in acute myeloid leukemias (AML) with t(8;21)**S. Hoeller<sup>1</sup>, M.P. Bihl<sup>1</sup>, C. Arber<sup>2</sup>, S. Dirnhofer<sup>1</sup>, A. Tzankov<sup>1</sup><sup>1</sup>Institut für Pathologie, Universitätsspital Basel, Schweiz<sup>2</sup>Klinik für Hämatologie, Universitätsspital Basel, Schweiz

**Aims:** Recently, we detected the earliest known GATA1 mutation in the literature, leading to a premature stop at codon 2 (E2Term). This patient harbored a trisomy 21, a concomitant event in the pathogenesis of TMD. Since GATA1 interacts with RUNX1, which is located on chromosome 21 as well and is rearranged in a subset of AML, i.e. AML with t(8;21) leading to RUNX1-RUNX1T1 fusion, we aimed to analyze the frequency and type of GATA1 mutations in such instances.

**Methods:** Sixteen formalin-fixed and paraffin-embedded bone marrow trephines of AML with RUNX1-RUNX1T1 fusion were analyzed for GATA1 mutations by sequencing the whole exon 2.

**Results:** In this series only one AML case (6%) with GATA1 mutation could be detected. This mutation replaces the serine at position 26 by a phenylalanine, which leads to abrogation of the serine phosphorylation site. Based on the findings of Yu et al. (J Biol Chem 2005, 280:29533), an alteration of this GATA1 serine phosphorylation site leads to an apoptotic stimulus due to decreased GATA1-mediated transcription of the E4BP4 and BCL-X<sub>L</sub> genes.

**Conclusions:** Taken together, exon 2 mutations of GATA1 are infrequent in AML with RUNX1-RUNX1T1 fusion. Particularly the GATA1 S26F mutation doesn't seem to have a potential tumorigenic effect. Contrary to TMD, GATA1 mutations are not essential in the pathogenesis of AML with t(8;21); RUNX1-RUNX1T1 fusion.

**Vorträge: Hämatopathologie III****Fr-159****Gene deregulation and spatial genome reorganization near breakpoints prior to formation of translocations in ALCL**Stephan Mathas<sup>1,2</sup>, Stephan Kreher<sup>1,2</sup>, Karen J. Meaburn<sup>3</sup>, Korinna Jöhrens<sup>4</sup>, Björn Lamprecht<sup>1,2</sup>, Chalid Assaf<sup>5</sup>, Wolfram Sterry<sup>5</sup>, Marshall E. Kadin<sup>6</sup>, Masanori Daibata<sup>7</sup>, Stefan Joos<sup>8</sup>, Michael Hummel<sup>4</sup>, Harald Stein<sup>4</sup>, Martin Janz<sup>1,2</sup>, Ioannis Anagnostopoulos<sup>4</sup>, Evelin Schrock<sup>9</sup>, Tom Misteli<sup>3</sup>, Bernd Dörken<sup>1,2</sup><sup>1</sup>Max-Delbrück-Center for Molecular Medicine, Berlin, Germany<sup>2</sup>Hematology, Oncology and Tumorimmunology, Charité Medical University, Berlin, Germany<sup>3</sup>Cell Biology of Genomes, National Cancer Institute, NIH, Bethesda, USA<sup>4</sup>Institute of Pathology, Charité Campus Benjamin Franklin, Berlin, Germany<sup>5</sup>Department of Dermatology, Allergy and Venerology, Skin Cancer Center Charité, Medical University Berlin, Germany<sup>6</sup>Department of Pathology, Harvard Medical School, Boston, USA<sup>7</sup>Department of Hematology, Kochi Medical School, Japan<sup>8</sup>German Cancer Research Center, Heidelberg, Germany<sup>9</sup>Institute for Clinical Genetics, University of Technology, Dresden, Germany

**Aims:** Whereas the identification and characterization of translocations rapidly increases, little is known about the mechanisms of how translocations occur in vivo. Anaplastic large cell lymphoma (ALCL) is a subgroup of peripheral T cell lymphomas. In approximately 40% of ALCL the characteristic t(2;5)(p23;q35) translocation is found. Here, we used ALCL cell lines and primary tumor samples with and without the characteristic t(2;5)(p23;q35) translocation to study the mechanisms of formation of translocations and of ALCL transformation.

**Methods:** Northern and Western Blot analyses; EMSA; co-immunoprecipitations; 3D interphase and conventional 2-color FISH analyses; RT-PCR analyses; immunohistology.

**Results:** We report deregulation of several genes located near the ALCL translocation breakpoint, regardless of whether the tumor contains the t(2;5). The affected genes include the oncogenic transcription factor Fraz (a member of the AP-1 transcription factor complex; located on 2p23), the helix-loop-helix (HLH) protein Id2 (2p25) and the oncogenic tyrosine kinase CSF1-receptor (5q33.1). For all these genes we identified an t(2;5)-independent ALCL-specific up-regulation. This up-regulation promotes cell survival and repression of T cell-specific gene expression programs that are characteristic for ALCL. Furthermore, the analysis of the spatial localisation of these genes in the nuclear space by 3D interphase FISH analysis revealed that they are in spatial proximity within the nuclear space of t(2;5)-negative ALCL cells. This proximity facilitated formation of t(2;5) translocations upon experimental induction of double-strand breaks in t(2;5)-negative ALCL cell lines.

**Conclusions:** These data suggest that deregulation of breakpoint proximal genes occurs prior to the formation of translocations and that aberrant transcriptional activity of genomic regions is linked to their propensity to undergo chromosomal translocations. In addition, our data demonstrate that deregulation of breakpoint proximal genes plays a key role in ALCL.

**Fr-160****cFLIP diminishes CD95-induced apoptosis of CD30-stimulated cutaneous anaplastic large cell lymphoma (cALCL) cells**B. Hirsch<sup>1</sup>, F.K. Braun<sup>2</sup>, N. Al-Yacoub<sup>2</sup>, C. Assaf<sup>2</sup>, M.E. Kadin<sup>3</sup>, W. Sterry<sup>2</sup>, J. Eberle<sup>2</sup>, H. Dürkop<sup>1</sup><sup>1</sup>Institut für Pathologie, Charité Universitätsmedizin Berlin, Campus Benjamin Franklin Berlin<sup>2</sup>Charité - Dept. of Dermatology and Allergy, HTCC -Skin Cancer Center, Berlin<sup>3</sup>Dept. of Dermatology and Skin Surgery, R.W. Medical Center, Providence RI, USA

**Background/Aims:** Stimulation of the TNF receptors CD30 and CD95 exerts opposite effects in cALCL cells. Crosstalk of both receptors is unknown. We

aimed to reveal regulatory mechanisms of CD30-induced effects on CD95 signaling of cALCL cell lines.

**Methods:** cALCL cell lines were cultured in vitro and "CD30/CD95 crosstalk analysis" following CD30-, CD95-, and CD30/CD95-costimulation was performed. Cell surface expression of CD30 and CD95 and induction of apoptosis was investigated by FACS. RNA of treated cells was analyzed by RT-RQ-PCR. A lentiviral-based shRNA-mediated approach was used to inhibit cFLIP expression.

**Results:** CD30/CD95 crosstalk experiments revealed that CD30 ligation leads to NFκB-mediated cFLIP up-regulation in cALCL cells, which in turn enhanced resistance to CD95-mediated apoptosis. Knockdown of cFLIP increased basic apoptosis rates and diminished CD30-mediated suppression of CD95-induced apoptosis of cALCL cells.

**Conclusions:** CD95-mediated apoptosis is diminished following prestimulation of CD30 in cALCL cells. This effect is based on the CD30-induced up-regulation of cFLIP. These results may contribute to improved therapy concepts for CD30-positive cutaneous lymphomas.

#### Fr-161

##### The role of epigenetic modifications in the extinction of the B-cell gene expression program in classical Hodgkin lymphoma

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**Background:** Epigenetic changes were found to be involved in the extinction of the B-cell gene expression program of classical Hodgkin lymphoma (cHL). However, it is unclear whether global or specific epigenetic changes are responsible for the loss of the B-cell identity of cHL.

**Aims and Methods:** To identify the epigenetic differences between Hodgkin and B-cell lines we analyzed their global acetylation pattern by chromatin-immunoprecipitation (ChIP) and subsequent Affymetrix promoter tiling arrays (chip) and compared these results with gene expression data.

**Results:** Only a limited number of genes appear to be significantly differentially acetylated in Hodgkin and B-cell lines including genes of the B-cell pathway. Interestingly, many of the differentially acetylated genes (e.g. CD19, ID2, RYBP) were found to be also affected by epigenetic treatment (AZA/TSA) of B-cells as shown by gene expression profiling.

**Conclusion:** Our data demonstrate that the spectrum of the epigenetic difference between Hodgkin and B-cell lines is less wide than previously expected and suggests that only a limited number of epigenetically regulated genes are involved in the extinction of the B-cell expression program of cHL. Some of the epigenetically regulated genes may also be involved in the pathogenesis of cHL.

#### Fr-162

##### In situ localization of follicular lymphoma a single center survey on 1294 lymph nodes

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**Aims:** Follicular lymphoma (FL) is one of the most common lymphomas of the adult in Europe and the USA and characterized by a chromosomal translocation t(14;18), resulting in an overexpression of the antiapoptotic BCL-2 protein. Recent investigations documented a prevalence of t(14;18) positive cells in the peripheral blood in up to 66% of healthy individuals older than 50 years. Further, single reports on BCL-2 positive B-cells in physiologic germinal centres are on record, so called "in situ-localization of follicular lymphoma (ISLFL)". However, frequency of occurrence and clinical impact of ISLFL remain unclear.

**Methods:** Immunostaining for BCL-2 was performed in a study group of 1,294 unselected lymph nodes of 132 patients that underwent surgical lymph node resection (mostly oncologic staging). ISLFL cases were further analyzed

using a FISH assay for detection of t(14;18) and PCR analysis to determine B-cell clonality.

**Results:** 18 lymph nodes of three patients showed ISLFL without any manifest lymphoma up to now. Interestingly, in one patient also a lymph node resected two years ago retrospectively showed ISLFL in a single reactive follicle.

**Conclusions:** Taking into account the incidence of FL of about 1:10,000 inhabitants p.a., our study shows that ISLFL is encountered with a significant higher frequency than expected. Clinical impact of this observation remains to be elucidated.

#### Fr-163

##### microRNAs in malignant lymphomas

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**Aims:** Summary of new data on miRNA-signatures in malignant lymphoma

**Methods:** RT-PCR based microRNA signatures of B-cell non-Hodgkin-lymphomas. Literature search.

**Results:** MicroRNAs are short non coding RNAs that can modulate gene expression. They are involved in diverse biological processes such as cell cycle and apoptosis and are involved in cancer, acting as oncogenes or tumour suppressors. Formalin-fixed and paraffin-embedded tissue samples are a suitable source of microRNAs and can be used for the generation of microRNA signatures. Moreover, microRNAs can be measured in body fluids like serum and urine. Single microRNAs and microRNA signatures correlate with prognosis in malignant lymphomas.

**Conclusions:** MicroRNAs may be used for molecular subclassification of malignant tumours and as tumour markers. Moreover, they may be attractive therapeutic targets.

#### Fr-164

##### Phospho-proteome profiling of malignant thymomas and thymic carcinomas

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**Aims:** Advanced thymomas and thymic carcinomas are often refractory to standard chemotherapeutic treatments. Novel, ideally targeted, treatments are urgently needed. However, little is known about the molecular mechanisms involved in the progression of these tumors.

**Methods:** We analysed protein extracts from 30 advanced malignant thymomas and thymic carcinomas by phospho-protein-arrays covering a large panel of activated receptor tyrosine kinases (RTKs) and MAP kinases.

**Results:** We identified two different major molecular groups. In group one, tumors showed activation of only very few RTKs, in particular EGFR and TYRO3. Tumors in group two showed activation of multiple RTKs, including the insulin receptor. Very preliminary clinical data in three patients suggest that multikinase inhibitors may be more effective in the group of patients with activation of multiple RTKs. Analysis of downstream signalling pathways suggested an important role of stress-activated protein kinases in general of the p38 family in particular.

**Conclusion:** The presented data may help to better define clinical high risk groups among thymoma patients and to develop an experimentally validated rationale for tumor-specific targeted treatments.

## Poster: Gynäko- und Mammopathologie I

### Sa-001

#### COX-2 and HER-2/neu are possible therapeutic targets in Paget's disease of the vulva

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**Aims:** Paget disease (PD) of the vulva is a rare lesion and account for about 1% of vulvar neoplasms. Some lesions are multifocal and the rate of recurrence is about 30%. Evaluating therapeutic relevant molecules PDs were investigated immunohistochemically.

**Methods:** 8 PPC were stained with antibodies against estrogen and progesterone receptors, HER-2/neu (Hercept-Test) and COX-2 with semiquantitative evaluation of the staining results.

**Results:** All tested PD were negative for estrogen and progesterone receptor analysis. 6/8 PDs represented COX-2 overexpression. All vulvar PD showed strong immunoreaction for HER-2/neu (Score 3).

**Conclusions:** Vulvar PD is probably not under hormonal control of estrogens or progestogens. The strong overexpression of COX-2 and HER-2/neu suggest that these both molecules represent possible therapeutic targets.

### Sa-002

#### Frozen section analysis of vulvectomy specimens: results of a 5-year period

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**Aims:** The most important goals of surgical treatment in vulvar cancer (VCX) are complete tumor resection and adequate treatment of inguinal nodes. The status of resection margins and inguinal node involvement, might be subject of intraoperative frozen section examination.

**Methods:** During a study period of five years we determined the frequency of all gynaecologic specimens in between all specimens sent for frozen section evaluation with special focus on VCX. Within cases of VCX we determined the time, necessary for frozen section, its accuracy and describe our practical approach for their handling.

**Results:** In between 7,921 frozen section analyses, 10.6% resulted from vulval gynaecologic surgery. The accuracy rate of vulval frozen section analyses was 100% for the evaluation of Cloquet's node, 98.6% for the margin status. The mean time, necessary for frozen section analysis of the vulvectomy specimens, was 24.5 minutes (range 6–44 min).

**Conclusions:** Gynaecologic specimens are not infrequently submitted for intraoperative consultation. Regardless of the high degree of individualised surgical treatment of VCX some procedures within handling of vulvectomy specimens can be tailored and might be subject of standardisation, including frozen section evaluation.

### Sa-003

#### Liposarcoma of the vagina

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**Aims:** Liposarcoma is the most common soft tissue sarcoma in adults, and it typically occurs in either the retroperitoneum or the extremities. However, this malignant tumor is very rare in the female reproductive system.

**Methods:** Detailed clinical and histopathologic review of a clinical case and review of the literature using PUBMED for publications on liposarcoma.

**Results:** A 50-year-old woman with rapidly growing tumor in the vaginal area is presented. Pathological evaluation of the biopsy specimen showed the presence of well differentiated liposarcoma which was characterized by the proliferation of mature fat cells whose size ranged from small to large, and lipoblasts, which contained multiple vacuoles in the cytoplasm with enlarged, irregular, dense nuclei in fibrous tissue. The tumor measuring 13,6×10,4×6,4 cm was completely removed at operation. Histopathological evaluation of the resected vaginal tumor showed a dedifferentiated liposarcoma of the vagina. Pathological diagnosis was confirmed using ancillary immunohistochemical staining with S-100, MDM 2 and CDK4.

**Conclusions:** By our knowledge this is the first case report of a vaginal liposarcoma. Its diagnosis requires immunohistochemistry and awareness of its possible existence.

### Sa-004

#### No false negative PAP smear test cancer check up in over 90% of cervical carcinomas

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**Aims:** From the beginning of the 70ies to the middle of the 80ies the incidence of the cervical carcinoma shows a clearly declining trend in Germany as well as Europe wide. The drop of the rates has weakened since then. Critical discussions take place about the sensitivity of only 50% of the once-only smear tests in comparison with the cervical carcinoma and its preliminary stages. The following will show retrospectively the participation ratio of women with cervical cancer at smear test cancer check ups as well as the results.

**Methods:** 2008 our Institute diagnosed 12 cervical carcinomas overall. The patients are from Germany, mainly from the North. 8 cases were diagnosed histologically and 5 cases cytologically. Retrospectively we found out if these women took part in check ups for cervical cancer and the results.

**Results:** 9 of 12 women (75%) didn't have a smear test 6 years prior to their cancer diagnostic. Suspicious cytological results were found in 2 women (17%) in the years prior to their illness, but these were obviously never followed up. Merely one woman (8%) had a negativ cytological result. Here another suspicious result was followed up and consequently lead to the cancer diagnostic.

**Conclusions:** As 92% of the cervical carcinoma happened without a false negative cytology, an increase of the participation ratio and the consistent follow up of suspicious PAP smear tests should be a matter of priority to clearly reduce the incidence of the cervical carcinoma.

### Sa-005

#### The potential of infrared microspectroscopic imaging for a molecular characterization of tissue sections originated from the cervix uteri

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**Aims:** Infrared (IR) microspectroscopic imaging has been proposed for automated histological tissue differentiation on unstained specimens, but realistic comparisons with conventional histopathology are missing.

**Methods:** For six sections of cervix uteri encompassing regions mainly with stroma, precancerous structures, and squamous cell carcinoma, an overall of 46 ROIs (0.1–4 mm<sup>2</sup>) were captured using a FTIR spectrometer with focal plane array detector (resolution ~4.2 μm). More than 2.8 million pixel spectra were processed with a fuzzy c-means clustering followed by hierarchical cluster analysis. Found clusters were taken to accomplish image segmentation with respect to different spectral properties. Landmark-based linear image registration was applied to compare segmentation results from IR microspectroscopic imaging vs. manual labelling (res. ~0.7 μm).

**Results:** For recognition of nine different tissue types sensitivities and specificities were determined between 42–91% and 79–99%, respectively.

**Conclusions:** The application of this imaging technique is only possible in combined use with concomitant conventional histopathology.

#### Sa-006

##### **c-met and HGF- $\alpha$ in advanced carcinoma of the cervix uteri (FIGO III and IV) a possible therapeutic target Target ?**

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**Aims:** Der hepatocyte growth factor (HGF; scatter factor) und sein Rezeptor c-met sind bei malignen Tumoren eingebunden in die Zellproliferation, deren Motilität, die Angiogenese und das peritumorale Stromaremodellierung. Rezente Untersuchungen ergaben zudem die Möglichkeit der gezielten Inhibition des c-met/HGF-Systems (Tosche & Jänne 2008).

**Methods:** 140 CX der FIGO-Stadien III und IV wurden immunhistochemisch mit einem polyklonalen anti-c-met und anti-HGF Antikörper untersucht. Die zytoplasmatische Färbereaktion wurde semiquantitativ evaluiert.

**Results:** Unter Verwendung eines cut-off Wertes von 33% (Furukawa et al. 1995) zeigten 83,6% aller CX eine c-met- und 74,3% eine HGF-Expression. Plump infiltrativ wachsende CX wiesen eine signifikant stärkere HGF-Expression auf ( $p=0,019$ ). HGF-positive CX wiesen ein längeres Gesamtüberleben auf als HGF-negative CX (9% versus 20%;  $p=0,04$ ).

**Conclusions:** Möglicherweise spielt das HGF/c-met System keine Rolle bei der malignen Progression des CX. Die Majorität der untersuchten CX zeigten eine c-met-Überexpression. Somit könnten fortgeschrittene CX möglicherweise von einer Inhibition von c-met (z.B. durch AMG102 oder L2G7) bzw. eine Inhibition der MET Kinase Aktivität (z.B. durch PHA-665752 and SU11274) profitieren.

#### Sa-007

##### **LDH 5 expression in endometrial cancer**

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**Aims:** Lactate dehydrogenase 5 (LDH5) is a pacemaker enzyme of anaerobic glycolysis in malignant tumor cells. Recent data in a relative small patient cohort suggest that the expression of LDH5 is an independent prognostic marker in endometrial cancer (EC). Here we tested the impact of LDH5 expression on clinico-pathological parameters.

**Methods:** LDH5 expression was assessed by immunohistochemistry (IHC) in formalin fixed and paraffin embedded EC specimens ( $n=94$ ). Clinico-pathological parameters, including patient survival, were analyzed in relation to LDH5 expression. The EC cohort consisted of 83,3% endometrioid adenocarcinomas (type I) and 16,7% (type II) carcinomas. LDH5 expression was scored quantitatively ranging from score 0 (negative) to score 3 (strong expression).

**Results:** Of the 94 EC, 7 were classified as score 0, 9 as score 1, 24 as score 2 and 54 a score 3. Moderate or strong cytoplasmic expression of LDH 5 was significantly correlated with histological grading ( $p<0,0001$ ). All other parameters tested including survival did not reveal any significant correlation with LDH5 expression.

**Conclusions:** LDH5 is highly expressed in a substantial number of EC indicating that anaerobic glycolysis is the main energy source for EC cells. LDH5 is a highly potential target for new treatment option for EC. In contrast to recently published data, LDH5 fails to prove as a prognostic marker in EC in our study cohort.

#### Sa-008

##### **Peritoneal keratin granulomas in a case of an endometrioid adenocarcinoma of the corpus uteri**

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**Aims:** Endometrioid Adenokarzinome des Corpus uteri haben im Stadium FIGO III eine 5-Jahres-Überlebensrate von ca. 45%. Zum FIGO-Stadium II ergibt sich ein Unterschied von 30%. Der bestimmende Faktor für dieses Tumorstadium ist unter anderem eine peritoneale Metastasierung, die auch durch andere Prozesse vorgetäuscht werden kann.

**Methods:** Histologische Untersuchung eines Hysterektomiepräparates mit beiden Adnexen, systematischer Lymphonodektomiepräparate sowie peritonealer Biopsien aufgrund eines auffälligen intraoperativen Befundes bei einer 68-jährigen Patientin.

**Results:** Die histologische Untersuchung des Uterus erbrachte ein schlecht differenziertes endometrioides Adenokarzinom des Corpusendometriums mit teils vorhornenden Plattenepithelmetaplasien und im Bereich der auffälligen Areale des Peritoneums, bei fehlendem Nachweis peritonealer Tumorfiltate, multiple peritoneale Keratingranulome im Bereich der Fimbrientrichter beidseits, der Ovarialoberfläche beidseits sowie in der Biopsie des rektosigmoidalen Überganges.

**Conclusions:** Keratingranulome des Peritoneums als Folge einer plattenepithelialen Metaplasie eines endometrioiden Adenokarzinoms des Corpusendometriums sind eine seltene Differenzialdiagnose und können Peritonealmetastasen imitieren. Der Nachweis von Keratingranulomen hat keinen Einfluss auf die Prognose des Endometriumkarzinoms.

#### Sa-009

##### **VEGF-A expression is a prognostic factor in serous epithelial ovarian carcinomas after complete surgical resection**

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**Aims:** Clinical stage and achievement of complete surgical resection (Ro-status) are key factors in determining outcome of ovarian carcinoma, but prediction of outcome lacks accuracy. Angiogenesis-related factors might be promising candidates for new prognostic and predictive parameters.

**Methods:** Expression levels of vascular endothelial growth factor (VEGF)-A and hypoxia inducible factor (HIF)1- $\alpha$  were analysed immunohistochemically in a homogeneous group of 112 patients with serous ovarian carcinoma. Vascular density as an indicator of angiogenesis was assessed using the Chalkley eyepiece method after staining for CD34. Survival and multivariate analysis and correlation tests were performed.

**Results:** Ro-resected tumours ( $n=54$ ) with VEGF-A positivity showed an improved progression-free survival in the univariate and the multivariate (FIGO-stage, grading, resection status as fixed variables) analysis. No significant correlation was found between vascular density and VEGF-A expression.

**Conclusions:** VEGF-A is a prognostic marker in serous ovarian carcinoma patients with macroscopically complete resection.

### Sa-010

#### MSH2 and ERCC1 in high grade serous ovarian carcinoma: expression and survival analysis

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The human MutS homolog 2 (MSH2) and excision repair cross-complementation group 1 protein (ERCC1) are suggested to be prognostic markers in adjuvant cisplatin-based chemotherapy in non-small cell lung cancer. We already demonstrated that ERCC1 overexpression is an important prognostic marker in high grade serous ovarian carcinoma (OC).

**Aims:** Using MSH2 and ERCC1 antibodies, we analyzed the expression of both proteins and their correlation with patients' survival.

**Methods:** We analysed the MSH2- and ERCC1-protein expression using ERCC1 antibodies (clone 8 F11) and MSH2 antibody (clone FE11). Immunohistochemistry was performed on multi tissue microarrays (78 patients; median age at diagnosis 64 years; range 32 to 88 years). In all cases a cytoreductive surgery was followed by platinum-based chemotherapy. The immunoreactivity was analyzed in a double blind fashion by two pathologists.

**Results:** The survival of patients with positive MSH2 status and ERCC1 negativity was significantly better (mean survival 49,2 months) compared with the MSH2 negative and ERCC1 positive subgroup (13,4 months;  $p=0.007$ ). MSH2 alone was not a significant prognostic factor whereas ERCC1 alone was significantly associated with the patients' survival ( $p=0.004$ ).

**Conclusions:** ERCC1 protein overexpression is negatively correlated with the outcome of high grade serous OC. The combination of ERCC1 and MSH2 has no further prognostic impact on the outcome of OCs.

## Poster: Gynäko- und Mammopathologie II

### Sa-011

#### Tumor hypoxia occurs irrespective of ovarian cancer histology but does not correlate with patient outcome

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**Aims:** Tumor hypoxia has been identified as a major driver of tumor de-differentiation and progression. In this respect, HIF- and HIF-target proteins are centrally involved in tumorigenesis. We aimed to analyse expression of HIF-1 $\alpha$ , CAIX, GLUT1, and Ki-67 in ovarian carcinomas.

**Methods:** A tissue micro array was constructed to represent eight normal tissues, 36 borderline tumours and 150 cases of ovarian carcinomas. Protein expression of HIF-1 $\alpha$ , GLUT-1 and CAIX was immunohistochemically analysed and semiquantitatively scored (IRS 0–12). Data on Ki-67 was available from preceding studies.

**Results:** The median IRS of HIF-1 $\alpha$  expression in ovarian cancer was 1, only 3% of cases showed a moderate to strong nuclear expression. However, the HIF-1 target genes GLUT1 and CAIX were found at higher levels (42% and 57% respectively). Particularly GLUT1 was highly correlated to HIF-1 $\alpha$ -positivity ( $cc=0.38$ ,  $p=0.001$ ) and higher Ki-67 fractions ( $cc=0.18$ ,  $p=0.025$ ). Interestingly, borderline tumors showed equal rates of expression. No correlation was seen with FIGO stage, tumor grade or other clinico-pathological criteria including tumor histology. In univariate survival analyses, no prognostic significance of the hypoxia markers could be demonstrated.

**Conclusions:** Although markers of hypoxia can be detected in ovarian carcinomas of different histological types, no immediate diagnostic or prognostic value was apparent.

### Sa-012

#### Glycoproteome of ovarian cancer: Diagnostic and therapeutic implications

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**Aims:** Applying a systems biology approach to discover ovarian cancer specific protein signatures for diagnosis and treatment.

**Methods:** Ovarian tissue from a conditional KRAS overexpression and PTEN-knockout mouse model was investigated using selective enrichment of N-glycopeptides and mass spectrometry-based label-free quantification. Mouse tissue signatures were validated in plasma and tissue of humans by selected reaction monitoring (SRM), ELISA, and immunohistochemistry (IHC) techniques.

**Results:** Mouse tissue signatures were verified in human plasma of patients with ovarian cancer. Ovarian cancer tissue microarrays were used to validate plasma signatures in human tissue by immunohistochemistry.

**Conclusions:** The availability of protein biomarkers for the detection and treatment of ovarian cancer will have profound impact on diagnosis and treatment of ovarian cancer.

### Sa-013

#### Serous tubal in situ carcinoma (STIC) and p53-signature in tubal and primary peritoneal carcinomas

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**Aims:** Serous tubal in situ carcinoma (STIC) has been defined as one important precursor of pelvic serous cancer (Folkins et al. 2008, Crum 2009; Lynch et al. 2009). Morphologically, STIC is defined by are cytologic atypia, high proliferative index and strong staining for p53 (Jarboe et al. 2008). Recently, STIC has been reported in about 30% of endometrial serous carcinomas (Jarboe et al. 2009). As a precursor lesion of STIC, characterised by low proliferative index, but p53-accumulation and no cytologic atypia has been defined (Lee et al. 2007).

**Methods:** The present study evaluates the presence of STIC and p53-signature in consecutive cases of 12 prophylactic salpingo-oophorectomy in women with BRCA-1-mutation (BSO), 11 macroscopically inconspicuous tubes of patients with primary tubal cancer (TC) and 9 cases of primary peritoneal cancer (PPC) using immunohistochemistry against Ki-67 and p53 (clone DO-7).

**Results:** The frequency of p53-signature and STIC was 8% and 0% in cases of prophylactic surgery, 9% and 18% in TC and 0% and 33% in PPC.

**Conclusion:** STIC and p53-signature as precursor lesions of pelvic serous cancer is seen in macroscopically inconspicuous Fallopian tubes in unilateral TC in patients with elective BSO and patients affected by PPC. We propose that the sectioning and extensively examining the fimbria protocol be applied to all cases with PPC, TC and in women with prophylactic BSO.

**Sa-014****3 cases of ovarian cancer with a „Sister-Mary-Joseph-nodule“**

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**Aims:** Umbilical metastasis (UM) is a special entity of skin metastasis also being termed „Sister-Mary-Joseph-nodule“ which have no connection to the primary tumour. They are often the only sign of a malignant primary tumour and are associated with a poor prognosis. The aim of this study of three cases is to describe this rare phenomenon which is often misconstrued.

**Methods:** It is about a clinical-pathological description of three own cases of our Institute of Pathology, which had been sent to us for histological investigations. All cases came from clinics of gynecology.

**Results:** 1. 64 year old woman with the leading symptom weeping navel. Primary diagnosis: metastasis of the peritoneal cancer, after surgery: papillary carcinoma with psammom-bodies caused by an ovarian cancer (FIGO IV). 2. 79 year old woman: Ovarian carcinoma (FIGO IV) with an UM. 3. 67 year old woman: 2nd adenocarcinoma of the ovary by a malignant transformation of a teratoma with an UM, primary interpreted as umbilical hernia (FIGO IV).

**Conclusions:** 1. Umbilical metastasis as first sign of ovarian carcinoma (case 1). 2. Correlation with metastasis in „umbilical hernia“ (case 2). About 4% of ovarian cancers metastasize to the skin (case 3). UMs are often misconstrued with endometriosis, nevus, hernia umbilicalis or epidermal cysts as differential diagnoses.

**Sa-015****Adenomatoid tumors: a clinicopathologic and immunohistochemical study of 62 cases emphasizing their site-specific morphologic diversity and the hormone receptor status**

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**Aims:** Adenomatoid tumor is a rare benign neoplasm of probable mesothelial origin with a striking predilection for female and male genital organs. Rare examples may arise from serosal surfaces and in parenchymatous organs.

**Methods:** We reviewed all adenomatoid tumors at our departments with emphasis on histological pattern, expression of mesothelial markers including D2-40 as well as steroid hormones (estrogen, progesterone and androgen receptor).

**Results:** Sixty-two cases were retrieved. Site distribution was as follows: uterus (n=21), fallopian tube (n=12), ovary (n=4), para-ovarian (n=2), epididymis (n=12), testis (n=11) and adrenal gland (n=1). Mean age was 44 and 55 yrs for testicular and epididymal, and 47 and 55 yrs for uterine and tubal tumors, respectively. While most non-uterine lesions had predominantly adenoid/angiomatoid pattern lacking a prominent smooth muscle component, the majority of uterine adenomatoid tumors displayed a mixture of microcystic (lymphangiomatoid) infiltrating spaces amid a prominent leiomyoma-like smooth muscle component that was responsible for the fibroid-like gross appearance in most cases. All lesions expressed Cytokeratin, calretinin, D2-40, and variably (mostly weakly) steroid hormone receptors.

**Conclusions:** Awareness of the morphologic diversity of adenomatoid tumors should prevent mistaking them for infiltrating epithelial neoplasms. The term mesothelial adenomyoma might be more appropriate for uterine tumors.

**Sa-016****Registry for gestational trophoblastic disease of the German Working Group of Gynecological Oncology (AGO) first results**

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**Aims:** The German Working Group for Gynecologic Malignancies (Arbeitsgemeinschaft für Gynäkologische Onkologie e.V. (AGO) which acts under the roof of the German Cancer Society and the German Society of Obstetrics and Gynecology has established a registry for GTD at the Institute of Pathology at the University of Leipzig.

**Methods:** Retro- and prospective analysis of the frequency of GTD and collecting clinico-pathologic data.

**Results:** Between 1996 and 2008 64 cases of GTD were registered. The majority of them (70.9%) were in house cases from the University of Leipzig Hospital; the other ones resulted from consultation cases. Within the GTD, the following diagnoses were seen: exaggerated placental site (EPS; 18.1%), placental site nodule (14.6%), partial moles (15.2%), PSTT (2.4%), partial mole with EPS (1.2%), complete moles (6.4%), complete mole with EPS (1.2%), invasive moles (1.2%) and choriocarcinoma (2.9%).

**Conclusions:** The AGO will please all gynecologists and pathologists to submit their cases to the registry. The registration of the cases within the GTD-registry is free of charge. There will be no refund for the mailing expenses.

**Sa-017****Does “the worst killer” escape? Comparison of screening detected breast carcinomas with tumours in a control group of patients in the same age range**

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**Aims:** We examine the influence of the screening program on selected pathological and prognostic parameters in the beginning of a breast cancer screening program.

**Methods:** The first 97 cases of screening-detected breast carcinomas in the region Neckar-Alb were compared to a consecutive series of 159 primary breast cancer patients of the same age range, who underwent surgery in the Universitätsfrauenklinik Tübingen in the same time period and had not participated in a screening program. In addition to standard clinical and histological parameters we evaluated hormone receptor status and proliferative activity (Mib-1 rate >20%).

**Results:** A significant difference in tumour grade was observed between screening-detected invasive carcinomas and the control group, with more poorly differentiated carcinomas in the control group (7% vs. 31%, p=0,009). In addition, the screening group had a much lower number of tumours with high Mib-1 index (20% vs. 40%, p=0,008), hormone receptor-negative (HR-) tumours (1,3% vs. 15%, p<0,001) and most importantly no triple-negative tumours (HR- and Her2 neg: 0% vs 11%, p>0,001).

**Conclusions:** Screening-detected carcinomas represent a selected group of mostly hormone-receptor positive, small carcinomas. As might be expected, the prognostically unfavourable, poorly differentiated and especially triple-negative tumours are underrepresented in our series in comparison to a control group of patients in the same age.

### Sa-018

#### EGFR1 (7p12) overexpression and aneuploidy in BRCA1 positive and sporadic breast cancer

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**Aims:** The aim of this study was to point out, whether a highly increased EGFR1 expression in BRCA1 associated breast cancer, detected by immunohistochemistry, coincides with amplification or aneuploidy of the EGFR1 gene locus, compared to sporadic breast cancer.

**Methods:** Immunohistochemical and cytogenetic (FISH) analyses have been performed on formalin fixed and paraffin embedded tissue from 20 BRCA1 positive and 48 sporadic breast cancers, using multi tissue arrays. The FISH probes covered the 7p12 region containing the EGFR1 gene locus and the centromeric region of chromosome 7. The results have been scored according to the Her2/neu ASCO/CAP guidelines.

**Results:** 40% of BRCA1 tumors yielded an overexpression of EGFR1, compared to 8% of sporadic carcinomas. 50% of BRCA1 cases and 40% of sporadic tumors showed aneuploidy with 3 to 6 copies of either the EGFR1 gene and the centromeric region of chromosome 7. A concomitant overexpression and aneuploidy of EGFR1 has only been observed in 30% of BRCA1 carcinomas, whereas 40% of those cases showed neither overexpression nor aneuploidy. 20% of BRCA1 cases showed aneuploidy without overexpression. 6% of sporadic tumors yielded coincidental overexpression and aneuploidy, whereas 58% did not show any changes. In 33% of sporadic cases aneuploidy without overexpression has been detected. No real amplification of the EGFR1 gene locus has been found in either group.

**Conclusions:** Overexpression of EGFR1 is not correlated with aneuploidy or amplification in BRCA1 positive and sporadic breast cancer. Whether the EGFR1 expression is directly or indirectly influenced by mutated BRCA1 in a different manner remains to be elucidated in further studies.

### Sa-019

#### Distinguishing medullary carcinoma of the breast from high-grade invasive ductal carcinoma: a comprehensive immunohistochemical approach

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**Aims:** Medullary carcinomas (MCs) represent a rare breast cancer subtype associated with a rather favorable prognosis compared to invasive ductal carcinomas (IDCs). Due to histopathological overlaps MCs are frequently misclassified as high-grade IDCs, potentially leading to overtreatment of MCs. Our present study aimed to establish novel diagnostic markers distinguishing MCs from hormone-receptor negative high-grade IDCs.

**Methods:** 61 MCs and 133 hormone-receptor negative IDCs were analyzed in a comparative immunohistochemical study. Applied markers included a comprehensive panel of cytokeratins (CKs), vimentin, smooth-muscle-actin (SMA), p63, p53, cell adhesion molecules (N-CAM (CD56), Syndecan-1 (CD138), E-cadherin and P-cadherin) and development associated transcription factors (AP-2 alpha, AP-2 gamma).

**Results:** A significantly higher proportion of IDCs displayed increased expression of CK7, AP-2 alpha and HER2 in contrast to MCs (CK7: 91% in IDCs vs. 77% of MC; AP-2 alpha: 77% vs. 57% and HER2: 26% vs. 7%, each  $p < 0.01$ ). Vice versa, MCs were slightly more frequently positive for SMA and vimentin ( $p > 0.05$ ).

**Conclusions:** Hormone-receptor negative high-grade IDCs are significantly associated with luminal differentiation, Her2 and AP-2alpha overexpression, whereas MCs tend to display myoepithelial features. Markers analyzed in this study are of diagnostic value regarding the differential diagnosis of MCs.

### Sa-020

#### C-myc and Topoisomerase IIa alterations in triple negative breast carcinomas

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**Aims:** Triple negative breast carcinomas are nearly always poorly differentiated and are associated with poor clinical outcome. Alterations of Topoisomerase IIa and c-myc genes have possible therapeutic consequence to adjuvant chemotherapy in this cancer group. Here we investigated the presence or absence of gene alterations of these genes and we correlated these findings with differentiation grade and with the presence of basal phenotype.

**Methods:** We examined 30 triple negative breast carcinomas (negative for estrogen (ER) and progesterone receptors (PR) and no amplification of the Her2 gene). The tumours corresponded to pT1b or pT2 and were histologically G2 or G3. Basal phenotype was defined by expression of CK5/6. Top IIa and c-myc gene copy number alterations were detected by Fluorescence in situ Hybridisation (FISH). The expression of hormone receptors and basal cytokeratins (CK5/6) were analyzed by immunohistochemistry.

**Results:** Amplification of the c-myc gene was found in 1 of 30 tumours (6,25%). No amplification or deletion of the Top IIa-gene was detected. 16 cases (53,3%) expressed diffuse basal phenotype in more than 5% of the tumour cells, in 5 cases (16,7%) basal phenotype was present in less than 5% of the tumour cells. The case with amplification of c-myc gene showed expression of CK5/6 in 5% of the tumour cells.

**Conclusions:** The expression of basal cytokeratins was present in a vast majority of triple negative carcinomas of the breast. C-myc gene amplification occurs in a minority of these tumours and seems to be independent from the presence of basal type carcinomas.

### Sa-021

#### Flat epithelial atypia (FEA) is a common B3 breast lesion and associated with non-invasive cancer but not with invasive cancer in final excision histology

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**Aims:** The optimal management of benign breast lesions with uncertain malignant potential (B3 category) found in breast needle core biopsies (NCB) is still under debate.

**Methods:** We reviewed 122 needle core biopsies with B3 lesions and compared the B3 histological subtypes with the final excision histology to determine associated rates of malignancy.

**Results:** The most common histological subtypes in biopsies are FEA in 35.2%, followed by papillary lesions in 21%, and ADH in 20%. Final excision histology was benign in 73 (90.2%) and malignant in 8 (9.8%; 2 invasive cancer, 6 DCIS). Of all B3 subtypes, ADH and FEA were associated with malignancy, whereas only ADH was accompanied by invasive cancer.

**Conclusions:** FEA and ADH are common lesions of the B3-category in biopsies of the breast. Both lesions are associated with malignancy whereas only ADH was related to invasive cancer. We conclude that an excision biopsy after diagnosis of FEA is recommended depending on clinical and radiological findings.

## Poster: Gynäko- und Mammopathologie III

## Sa-022

**Breast cancer screening in the prevalence phase. What are the differences to the era before?**

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**Aims:** The aim of the breast cancer screening program is detecting breast cancer in early cancer stages. From the pathological point of view is the question: Does the screening situation really render our diagnostic results in pTNM classification, grading and hormone receptors status?

**Methods:** Search in 3353 (2004 till 2009) cases of our iPath based breast cancer database including anonymous clinical and surgical pathology information. A comparison was made between cancer cases from the breast cancer screening program and cancers detected in a „curative“ setting. Parameters studied were pT-, pN- und pM-status, grading, hormone receptor status and maximum lesion diameter. The results were tested for statistical significance with Knime® and R®.

**Results:** 1334 full documented cases including 190 from screening could be analyzed. The screening population and the compared population were uniformly distributed. Tumour diameter in the screening population was significant lower than in routine population (14 mm vs. 18 mm,  $p=0,003$ ). Even the grade of tumour was lower (mainly G2) than in the compared population (balanced G2 and G3).

**Conclusions:** The target of the screening program is met in the routine surgical pathology. The size of tumours seems to be diminished, their lymph node status improved and the tumors are better differentiated, however at a lesser extent than expected.

## Sa-023

**Morphology of interval carcinomas in the mammographic screening programme**M. Wöhlke, B. Beese<sup>1</sup>, K. Sobolewski<sup>2</sup>, R. Hinze

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**Aims:** Interval carcinomas (IC) are breast cancers occurring within a negative mammographic screening episode. So far, pathological features of IC are described insufficiently.

**Methods:** Morphology of 16 invasive IC (occurring in a screening period of 31 months; 47.349 screened subjects) was compared with either invasive screening carcinomas (SC) (n=188) or carcinomas detected outside the screening programme (NSC) (n=402) in terms of histological type, grading, tumor stage (pT and pN), ER, PR, HER2 expression. Finally, the radiologists retrospectively reviewed the mammograms of the IC.

**Results:** There was no significant difference in the histological type in all three groups. Compared with SC, IC were more frequently highly proliferative (Ki67 mean: 32%), more frequently high grade (56% vs. 25%,  $p=0,050$ ), nodal positive (63% vs. 28%,  $p<0,001$ ) and triple negative (38% vs. 12%,  $p=0,006$ ). Retrospective review of the preceding screening mammograms of IC revealed that 2 tumors (13%) were missed (observer's error), 11 studies (69%) showed no tumor (true interval carcinomas), and 3 cases (19%) showed subtle signs of malignancy.

**Conclusions:** Interval carcinomas in this series revealed small tumors with a more aggressive phenotype, high grade differentiation, triple negative tumors and higher frequency of lymph node metastases. Thus, breast cancers with assumable poor prognosis are overrepresented in this subset.

## Sa-024

**Determination of the Her2/neu gene amplification status in cytologic breast cancer specimen using automated silver enhanced in situ hybridization**

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**Aims:** Silver enhanced in situ hybridization (SISH) is a new emerging tool for the determination of the Her2 status in breast cancer. SISH is technically comparable to Fluorescence in situ hybridization (FISH) but does not require a fluorescence microscope for its interpretation.

**Methods:** While histological evaluations of SISH are promising, we aimed to evaluate its use for cytologic specimens. Her2 status as routinely determined by FISH was available.

**Results:** SISH signals in cytologic cell blocks and smear specimen were almost constantly easy to evaluate. Lack of tumor cells in the cell block material was the limiting factor. The Her2 status, as determined by the Her2/Chr17 SISH, was basically identical to the results of the Her2 FISH. Interobservervariability was low.

**Conclusions:** Her2/Chr17 SISH is a useful and accurate method for the evaluation of the Her2 gene status in cytologic breast cancer specimens. The advantages of signal permanency and bright field microscopic result interpretation make this technique an attractive alternative to the current FISH based gold standard.

## Sa-025

**The novel HOPE-technique as a promising diagnostic tool for human breast cancer pathology**D.S. Lang<sup>1</sup>, T. Zeiser<sup>2</sup>, H. Schultz<sup>1</sup>, J. Gerdes<sup>3</sup>, E. Vollmer<sup>1</sup>, T. Goldmann<sup>1</sup><sup>1</sup>Klinische und Experimentelle Pathologie, Forschungszentrum Borstel<sup>2</sup>Abteilung für Gynäkologie und Geburtshilfe, Paracelsus Klinik Henstedt-Ulzburg<sup>3</sup>Tumorbiologie, Forschungszentrum Borstel

**Aims:** Despite remarkable progress in the treatment of human breast cancer, the identification of reliably predictive biomarkers is still mandatory to determine more accurately those patients, who will benefit from (neo)adjuvant therapy. The HOPE-(Hepes glutamic acid buffer mediated Organic solvent Protection Effect) technique has already proven to be a fascinating new tool for histology. In order to test its suitability for optimal diagnostics of modern molecular medicine, the detectability of several predictive biomarkers for human breast cancer was analyzed by application of the novel HOPE-fixation and subsequent paraffin-embedding method.

**Methods:** Expression of established (ER-, PR receptors, Her-2) and novel predictive markers (uPA/PAI-1, PTEN) for breast cancer was determined by immunohistochemistry (IHC); Her2 and Topoisomerase II $\alpha$  were also analyzed by FISH and CISH (fluorescence / chromogen in situ hybridization), respectively.

**Results:** The new HOPE-technique provided clear immunohistological detection of all biomarkers in all cases without any thermal denaturation of the specimens. Moreover, the amount of primary antibodies and of the detection-systems could be significantly reduced. Molecular analysis of Her2 - gene amplification was also consistently enhanced as compared to the corresponding formalin-fixed specimens.

**Conclusions and Perspectives:** Due to the reduced denaturation of antigens, the HOPE-technique offers enhanced detection of all biomarkers analyzed. The resulting well preserved morphology and elevated degree of standardization for IHC as well as the possibility of molecular analyses with consistent results represent a substantial improvement for more exact diagnoses, prognoses and even treatment options for patients with human breast cancer.

### Sa-026

#### Cell suspensions as positive and negative controls in immunohistochemistry

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**Aims:** Cell lines are used as controls in immunohistochemistry for more than 10 years. Mostly, the cells are fixed in formalin, mixed with agar and paraffin embedded comparable to routinely processed tissue specimens. The paraffinized cell blocks are cut and mounted on glass slides often together with tissue sections (on-slide controls). To overcome this cutting procedure for the paraffinized cell blocks a cell suspension was dropped onto the slides.

**Methods:** Breast carcinoma cells (SKBR3, MCF-7) were washed in PBS and fixed in formalin 4% for 30 minutes. After centrifugation the cells were resuspended in Tris/HCl 0.05 mol/l and NaN<sub>3</sub> 15 mmol/l at pH 7.2 and stored in a refrigerator at 4°C. For five months these cells were dropped on a slide each week onto which a paraffinized section of breast carcinoma was added. The slides were stained immunohistochemically for HER2 using an immunostainer and an anti-HER2 antibody.

**Results:** For more than five months the SKBR3 and MCF-7 cells dropped onto the slides showed a correct immunohistochemical result. The deparaffinization step for the paraffinized tissue did not harm the non-paraffinized fixed cells.

**Conclusions:** In comparison to the storage of antibodies used in immunohistochemistry it was possible to store formalin-fixed cell lines in Tris/HCl and NaN<sub>3</sub>, drop them onto slides and use them as controls in immunohistochemistry. Dropping the cells onto the slides appears to be more comfortable than to make paraffin sections and to store sections for on-slide control in advance. Moreover, it should be possible to integrate cell suspensions into the automatic staining procedure using special dispensers.

### Sa-027

#### The XbaI G>T polymorphism of the glucose transporter-1 gene (GLUT1) modulates 18FDG uptake and tumor aggressiveness in breast cancer

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**Aims:** We investigated the relevance of single-nucleotide polymorphisms (SNPs) in the glucose transporter 1 (GLUT1) gene on the uptake of 18FDG and tumor aggressiveness in breast cancer.

**Methods:** In 52 individuals with breast cancer, a diagnostic PET/CT was performed, and the standardized uptake value (SUV) was determined as a measure of FDG uptake. Three GLUT1 SNPs (XbaI G>T, HpyCH4 V A>T, and HaeIII T>C) were investigated in genomic DNA that was isolated from paraffin-embedded specimens of all patients.

**Results:** The GG genotype of the XbaI G>T SNP was associated with increased tumor uptake of 18FDG, with a mean SUV of 11.7 (TT/GT genotypes: 5.9, P=0.031). Furthermore, the GG genotype was positively related to enhanced tumor proliferation (mitotic count, P=0.002). In line with this finding, the GG phenotype was absent in grade 1 carcinomas and increasingly prevalent in tumors with higher malignancy (grade 2: 28.0%; grade 3: 50%).

**Conclusions:** This study found that the XbaI G>T SNP of the GLUT1 gene results in an increased 18FDG uptake and is associated with a more advanced tumor grade/growth in breast cancer. Thus, this genetic variant might favor aggressive phenotypes by modulating the efficiency of cancer cells to recruit glucose and escalate growth rate, suggesting the XbaI G>T SNP as a proliferation-related prognostic factor.

### Sa-028

#### PARP expression in primary operable breast cancer translational research in GeparTrio

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**Aims:** The polyadenosine diphosphate [ADP]-ribose polymerases (PARPs) are a large family of multifunctional enzymes. PARP-1 plays a key role in the genomic stability. Increased expression could be associated with resistance to DNA damage-inducing therapeutic agents. PARP inhibitors could lead to an enhanced efficiency of DNA-methylating agents as well as an increased rate of apoptosis. We analysed the PARP expression in correlation to hormone receptor (HR) status and HER2 expression in a cohort of 587 participants of the GeparTrio trial.

**Methods:** The GeparTrio trial was a multicenter prospective randomised phase III trial with the primary goal to evaluate the pCR after different schemes of neoadjuvant chemotherapy. Immunohistochemical stainings of estrogen receptor, progesterone receptor, HER2 and PARP were performed according to standard procedures on a tissue microarray.

**Results:** We evaluated the cytoplasmic and nuclear expression in 587 samples. A cytoplasmic staining correlates significant with less differentiated (G<sub>3</sub>), nodal positive, HR negative and triple negative tumors.

**Conclusions:** A positive cytoplasmic PARP expression shows in contrast to nuclear staining significant correlations with e.g. advanced tumor types. Further studies are necessary to research the potential different functions.

### Sa-029

#### Myoglobin status in human breast cancer: Unexpected expression, novel regulation and putative function

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**Background:** We aimed to analyze expression, regulation and function of myoglobin (Mb) in human breast cancer.

**Methods:** A wide spectrum of methods was applied: Immuno-histochemistry, immunofluorescence, microdissection, electron microscopy, Western blot, qPCR, cell culture (normoxia/ hypoxia), shRNA/siRNA knockdown, high resolution respirometry, proliferation and migration assays.

**Results:** Mb protein is expressed in 71% of breast carcinomas (n=917) in association with the hypoxia inducible factor 2 $\alpha$ , carbonic anhydrase IX and a better prognosis. Expression of Mb in breast cancer was confirmed on mRNA level, irrespective of rhabdomyoid differentiation and was inducible by prolonged hypoxia in breast cancer cell lines. This induction originated from an alternative Mb transcription start site near a non-coding exon upstream of the genuine ATG codon. Stable myoglobin knockdown in MDA-MB468 breast cancer cells was associated with a stimulated O<sub>2</sub> uptake and also conferred a significant retardation of the cells' in vitro motility.

**Conclusions:** Regarding cancer cells, unconventional functions of myoglobin, not directly related to the transport of oxygen need to be envisioned.

**Sa-030****Tumor progression in mouse mammary carcinoma is associated with expression of VE-cadherin and HIF hydroxylases**

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**Aims:** Epithelial mesenchymal transition (EMT) is an important event during carcinoma progression, which contributes to increased invasive and metastatic potential of cancer cells. One of the hallmarks of this transition is the switch in cadherin expression. In this study, the modulation of cadherin expression during EMT was analyzed in an experimental model of breast tumor progression. The second part of the project was dedicated to analyse the level of hypoxia-inducible factor (HIF) hydroxylases in the tumor cells which are representing different stages of tumor progression.

**Methods:** Transfections; proliferation assays; tumor experiments immunofluorescence; immunohistochemistry; RNA interference; RT-PCR and western blot.

**Results:** We show that vascular endothelial (VE)-cadherin is induced during EMT in mouse mammary tumor cells and is aberrantly expressed in invasive human breast carcinomas. VE-cadherin expression enhanced the capacity of fibroblastoid tumor cells to proliferate. Analysis of the signaling mechanisms involved revealed that VE-cadherin expression enhances the transforming growth factor (TGF)-beta signaling pathway which also contributes to malignant tumor cell proliferation. Furthermore we detected that hypoxia-inducible factor (HIF) hydroxylases are differentially expressed in tumor cell lines which are representing different stages of tumor progression.

**Conclusions:** Our findings provide evidence for a novel function of VE-cadherin in tumor progression and reveal a previously unknown molecular link between VE-cadherin expression and TGF-beta signalling. Moreover they suggest that HIF hydroxylases play a role in EMT and breast cancer progression.

**Sa-031****Identification of a metastasis-associated gene expression signature of canine mammary carcinomas and its relevance for human breast cancer**

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**Background and Aims:** Mammary tumors of the female dog are often associated with fatal outcome due to metastatic spread. However, the molecular mechanisms associated with metastatic disease are largely unknown. Furthermore the value of this tumor as a comparative model for human breast cancer has not been extensively investigated yet.

**Methods:** To identify genes associated with metastatic progression, mRNA expression profiles of lymph node positive canine mammary carcinomas were compared with lymph node negative tumors. Differentially expressed genes were examined for their function and associated pathways. The findings were also correlated with published data on human breast cancer.

**Results:** Metastasized carcinomas showed an increased expression of genes related to cell cycle progression, DNA-repair, stress response and apoptosis. In contrast, expression of cell differentiation-, growth factor signaling- and transcription regulation genes was decreased. Interestingly, only few genes associated with angiogenesis, modulation of the extracellular matrix and cell-cell adhesion were differentially expressed between both groups. Literature mining disclosed that almost two third of the differentially expressed genes have also been cited in association with human breast cancer. These included a significant portion of the prognostic 70-gene signature that has been established for human breast cancer.

**Conclusions:** Our study provides new insights into the molecular mechanisms of canine mammary tumor metastasis by identifying gene expression profiles associated with the metastatic phenotype. Furthermore, our data suggest that canine mammary carcinomas are suitable as a translational model for human

breast tumors in order to identify prognostic molecular signatures and potential therapeutic targets.

**Poster: Paidopathologie****Sa-032****Association of rare chronic histiocytic intervillitis of the placenta with assisted reproduction-induced pregnancies**

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**Aims:** Chronic histiocytic intervillitis of the placenta (CHI) is a rare and poorly understood pathological placental lesion which may occur in all trimesters. The most conspicuous feature of CHI is an extensive and diffuse monocytic infiltration of the intervillous space without involvement of the villous parenchyma. Disease recurrence in subsequent pregnancies is a frequent finding and, in the majority of pregnancies, children had higher rates of perinatal mortality or growth retardation. Several associated maternal diseases have been observed, but the aetiology of CHI remains unknown.

**Methods:** Retrospective screening for CHI cases (1994–2008); focus on histological, immunohistological and fluorescence in situ hybridisation-derived findings, foetal status and clinical data for previously unrecognised CHI-associated features.

**Results:** i) 4 cases: maternal age 30–40 years; gestation age 30–36 weeks, 3 female/1 male children assisted reproduction-induced pregnancies in 2/4 cases; ii) Histiocytic intervillous infiltrates of 70%–80% in 3 cases (massive/grade III CHI) and 5% in 1 case (focal/grade I CHI); iii) Foetal growth retardation in all 4 cases, lethal in 1/4 cases.

**Conclusions:** Insemination-induced pregnancy had been performed in 2 of 4 CHI cases, an association which has not yet been reported.

**Sa-033****Monophasic epithelial nephroblastoma as unusual cause of recurrent macrohematuria in a 12 year old girl**

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**Aims:** Aim of the current report is to emphasize the need to take into account the presence of a neoplastic kidney disease as a cause of recurrent macrohematuria, even in the context of a family history of IgA nephropathy and an inconspicuous ultrasound investigation.

**Methods:** Against the background of a positive family history of IgA GN a 12 year old girl with macrohematuria received a kidney biopsy which was investigated by light- and electron microscopy as well as immunofluorescence. No evidence of glomerular disease was found so that a subsequent computer tomography was performed which was suspicious of a renal tumor.

**Results:** Consecutively, the patient underwent unilateral nephrectomy which revealed on macroscopic inspection a 1.5×1.3×0.7 cm tumor in the vicinity of the renal pelvis. Histologically, the rare case of a monophasic, epithelial nephroblastoma was diagnosed despite the relatively high age of the patient. This diagnosis was subsequently confirmed by the German registry for Pediatric tumors. Thereafter, the patient underwent a standard chemotherapy protocol. So far no signs of recurrence have been detected in regular controls.

**Conclusions:** Neoplastic kidney disease must be taken into the differential diagnosis of recurrent macrohematuria in adolescent children.

### Sa-034

#### **Histomorphological tumor progression in pediatric/ syndromic GISTs seems to be comparable to that seen in sporadic GISTs in adults: Presentation of a pediatric GIST and of a new case of Carney triad**

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**Aims:** Carney triad (CT) was first described as an association of gastric leiomyosarcoma (GIST) with pulmonary chondroma and extraadrenal paraganglioma. Pediatric GISTs differ from CT-GISTs by absence of other components of the CT. Both variants metastasize frequently to regional lymph nodes and are wild-type for KIT-/PDGFRA-mutation.

**Methods:** Two girls with gastric GISTs were analysed, with a special focus on histomorphological patterns in the primary tumors and in the metastases.

**Results:** The first patient, a 15-year-old girl with incomplete CT (paraaortal paraganglioma) had a 7 cm multifocal gastric tumor with biphasic spindle and epithelioid histology, while the liver metastases showed an epithelioid morphology. The second patient, a 13-year-old girl, had an 8 cm gastric GIST with biphasic, mostly epithelioid growth pattern in the primary tumor and a hypercellular epithelioid pattern in the lymph node metastases. The epithelioid components each had a tendency for higher proliferation rates and higher mitotic counts. The tumors and metastases in both cases were positive for CD34/c-kit, and had no KIT or PDGFRA mutation.

**Conclusions:** Both pediatric GISTs showed morphological progression within primary tumor and in the metastases similar to that seen in sporadic GISTs with KIT/PDGFRA-mutations, suggesting that morphological progression is independent of the mutational status. Pediatric/ syndromic GISTs may share similar mechanisms of tumor progression seen in adult GISTs.

## Poster: Pneumopathologie

### Sa-035

#### **HIF dependent regulation of vascular S100A4 in COPD**

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**Aims:** Patients with end stage pulmonary diseases such as lung fibrosis, lung cancer, and COPD often suffer from chronic hypoxia. Further, hypoxia-inducible factor (HIF) is a master regulator that controls transcriptional responses to hypoxia and has been linked to pulmonary vascular remodelling. As Ca<sup>2+</sup> binding protein S100A4 plays a role in hypoxia induced vascular remodelling, we hypothesize, that HIF-1 $\alpha$  and HIF-2 $\alpha$  are involved in the regulation of S100A4 in the context of COPD.

**Methods and Results:** A strong upregulation of S100A4 mRNA as assessed by real-time RT-PCR, was shown utilizing murine and human primary PSMCs kept under hypoxic conditions (1% oxygen) for 24 h. Additionally, laser-microdissection combined with real-time RT-PCR analysis revealed a high up-regulation of S100A4 mRNA in remodelled intrapulmonary arteries of COPD lungs. Findings from both cell culture and COPD-lungs were validated by semiquantitative analysis of immunohistochemical data. Two putative hypoxia-inducible response elements (HREs) were identified in the murine and human S100A4 gene applying in-silicon screening of the promoter and enhancer regions of the gene. Pretreatment of human PSMCs with siRNA against HIF-1 $\alpha$  or HIF-2 $\alpha$  attenuated hypoxia-dependent upregulation of S100A4 mRNA. Putative HIF-1 binding to S100A4 HREs were analysed by EMSA.

**Conclusions:** The results let assume a causative link between HIF and S100A4 regulation in vascular remodelling of COPD.

### Sa-036

#### **Angiogenic (micro)environment and vascular remodelling in high grade hypertensive pulmonary disease**

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**Aims:** Our aim was to study the pro-angiogenic microenvironment in patients with primary and secondary pulmonary arterial hypertension and to elucidate its influence on vascular remodelling and patient outcome.

**Methods:** Pro-angiogenic markers (VEGF, GDF-15, etc) were monitored in the peripheral blood of patients with primary and secondary pulmonary arterial hypertension. When end stage patients underwent lung transplantation (n = 17) the extent of vascular remodelling was graded (grade of hypertrophy, occurrence of plexiform lesions, etc) and defined anatomical compartments isolated by laser-assisted microdissection. In addition to the markers monitored in the peripheral blood, mRNA and protein expression of different angiogenic receptors (Tie2, etc) were analyzed and correlated with clinical data.

**Results:** Expression profiles measured in the peripheral blood could be confirmed in the explanted lungs. Plexiform lesions differed significantly with regard to expression of VEGF, Angiopoietin2, Tie2, sm-actin, FGF2, Caspase 9 from other vessels with intimal fibrosis in the same lungs as well as from controls.

**Conclusions:** Anatomical changes in primary and secondary pulmonary hypertension are characterized by distinct alteration in angiogenic regulation. Mediating cytokines can be measured in the lung and correlate with peripheral blood findings.

### Sa-037

#### **S100 protein positive (sustentacular) cells in pulmonary carcinoids and thoracic paragangliomas: differential diagnostic and prognostic evaluation**

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**Aims:** Paragangliomas have a classical histomorphology comprising a so-called „Zellballen“ or nesting pattern with surrounding S100 protein positive sustentacular cells (SC). The prevalence of SC is inversely associated with the patients' outcome. However, there are reports describing sustentacular cells in pulmonary carcinoids as well. We aimed to get more insight into the prevalence as well as the prognostic and differential-diagnostic value of sustentacular cells in tumorlets, pulmonary carcinoids and in thoracic paragangliomas.

**Methods:** Pulmonary carcinoids (n=147), tumorlets (n=26) and thoracic paragangliomas (n=10) were immunohistochemically stained against S100. All S100 positive cells with detectable cellular processes were scored in relation to the tumor cells. The prognostic impact of the scores for pulmonary carcinoids was evaluated by Kaplan-Meier analyses.

**Results:** All pulmonary carcinoid patients with a bad outcome had low numbers of SC or no SC. Moreover, we found a similar distribution of sustentacular cells in thoracic paragangliomas and in pulmonary carcinoids.

**Conclusions:** We provide evidence that distribution of SC alone does not represent a reliable differential-diagnostic criterion to distinguish thoracic paragangliomas and pulmonary carcinoids. However, the distribution of SC may aid in the prognostic assessment of pulmonary carcinoids, especially in biopsies.

**Sa-038****Downregulation of Desmocollin1 and CDX2 is associated with shorter patients survival in colorectal cancer patients**

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**Purpose:** Genomewide expression profiling has identified a number of genes expressed at higher levels in colorectal carcinomas (CRCs) than in normal tissue. Our objectives in this study were: 1) to test whether genes were also distinct on the protein level 2) to evaluate these biomarkers in a series of well characterized CRCs 3) to correlate the expression with clinicopathological data.

**Methods:** Tissue microarrays (TMAs) from 409 patients were constructed to evaluate the genes desmocollin 1 (DSC1), DSC2, DSC3, PLTX1, CDX2, E-cadherin, CDK4, MDM2, FaktorH, and TLE1. The staining was scored semi-quantitatively as -, negative; +, weak; ++, moderate; and +++, strong positive. Furthermore mRNA expression of DSC1 was evaluated in 8 colon cancer cell lines. Demethylation test was performed by treatment with 5-aza-2'-deoxycytidine in 5 colon cancer cell lines.

**Results:** Kaplan Meier analysis showed that downregulation of DSC1 (63%) and CDX2 (59%) was significantly associated with poor survival and thus could serve as a prognostic marker. On mRNA level one out of 7 cell lines has expression of DSC1. Four out of 5 colon cancer cell lines showed restoration of Desmocollin 1 expression after demethylation test.

**Conclusions:** Downregulation of DSC1 and CDX2 is related with tumor progression and was a statistically independent prognostic marker. Downregulation of desmocollin 1 could be explained by DNA hypermethylation in colon cancer cells.

**Sa-039****2-D-electrophoresis and peptide mass fingerprinting using HOPE-fixed non small cell lung cancer specimens**

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**Aims:** HOPE-fixed, paraffin-embedded tissue was subjected to high resolution two dimensional electrophoresis (2-DE), 2-DE Western immunoblotting and MALDI-TOF mass spectrometry analyses.

**Methods:** Non Small Cell Lung Cancers were processed and paraffin-embedded using the HOPE-technique. Isopropanol- deparaffinized sections were protein-extracted and 200 µg of protein were applied to 2-D-electrophoresis and a blotting system. 2-DE results from HOPE-fixed tissues were compared to formalin-fixed and fresh frozen material. Immunoblotting targeted marker cytokeratin. Blot- corresponding protein spots were excised out of coomassie-stained twin gels for MALDI-TOF mass spectrometry analyses.

**Results:** HOPE-fixed, paraffin-embedded tissues can be used for high resolution 2-DE and subsequent immunoblotting of 2-DE-separated proteins. Tryptic digest and mass spectrometric fingerprinting are feasible.

**Conclusions:** For proteomic applications, the feasibility is directly connected to the quality of tissue fixation. In this study is shown that formalin- and ethanol-free fixation of tissues enables high quality proteome investigations using HOPE-fixed material. Further, the HOPE-technique meets the requirements for various other molecular and biochemical investigations (immunohistochemistry, RT-PCR, DNA/RNA in situ hybridization, transcription arrays, all blotting techniques). Therefore multi-methodical investigations of scientific problems can be performed using one single donor material.

**Sa-040****Epigenetic inactivation of insulin-like growth factor binding protein 7 (IGFBP7) in human lung cancer**

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**Aims:** Insulin-like growth factor binding protein 7 (IGFBP7) was considered a tumor suppressor gene in lung cancer. However, the mechanism responsible for the downregulation of this gene has not yet been fully understood. The aims of this study were: 1) to analyse the expression of IGFBP7; 2) to explore the mechanism for the gene silencing; 3) to investigate the regulation of IGFBP7 expression in lung cancer.

**Methods:** RT-PCR was carried out for the expression analysis of IGFBP7 in 16 lung cancer cell lines and normal lung cells (HBEC and SAEC). Protein expression of IGFBP7 was analyzed in 60 primary lung tumors by immunohistochemistry. Demethylation test by using 5-aza-2'-deoxycytidine, bisulfite sequencing (BS), and methylation-specific-PCR (MSP) were performed to evaluate the methylation status of IGFBP7 in 10 lung cancer cell lines and 60 primary lung tumors. Furthermore, three lung cancer cell lines (H82, H2170, and H1299) were treated with P53 inducing drug adriamycin alone, or in combination with 5-aza-2'-deoxycytidine, to investigate the regulatory role of P53 on IGFBP7. Transfection of lung cancer cell lines with a P53-Wild-type expression vector is planned.

**Results:** IGFBP7 was downregulated in 11 out of 16 lung cancer cell lines, and in 9 out of these 11 cell lines methylation in exon/intron 1 of IGFBP7 was found by BS and MSP. In primary lung tumors, 27 out of 60 samples exhibited no expression of IGFBP7, among these samples, MSP showed that 59,2% harboured IGFBP7 methylation, which is statistically significant ( $P=0,02$ ). However, the methylation status of IGFBP7 was not related to any of the clinicopathological data including age, gender, tumor stage, and grading, as well as overall survival. Treatment with anti-cancer drug adriamycin alone increased the expression of IGFBP7 in the unmethylated cell line H82 but neither in the P53-null cell line H1299 nor in the IGFBP7 methylated cell line H2170. Interestingly, treatment with adriamycin in combination with 5-aza-2'-deoxycytidine induced the expression of IGFBP7 in H2170.

**Conclusions:** IGFBP7 was downregulated in lung cancer, being explained by DNA hypermethylation. Induction of endogenous P53 expression might increase the expression of IGFBP7.

**Sa-041****Downregulation of desmoplakin in human lung cancer is related to DNA hypermethylation**

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**Aims:** Desmoplakin (DSP) is one of desmosomal components involved in carcinogenesis. However, the role of DSP in human lung cancer has not yet been well understood. The aims of this study were: 1) to analyse the DSP expression in lung cancer; 2) to explore the mechanism for downregulation of DSP; 3) to investigate the functional role of DSP in lung cancer cells.

**Methods:** Real-time PCR and Western blot analysis were performed to analyse the expression of DSP in lung cancer cell lines. The protein expression of DSP in primary lung tumors was evaluated by immunohistochemistry. To investigate methylation status of DSP in lung cancer, demethylation test, bisulfite sequencing (BS), and methylation-specific-PCR (MSP) were carried out in lung cancer cell lines and in 41 primary lung tumors. Stable transfection of DSP expression vector into lung cancer cell lines is planned.

**Results:** DSP was downregulated in 15 out of 19 lung cancer cell lines. More than 50% of primary lung tissues exhibited no expression of DSP protein. Treatment with demethylation agent 5-aza-2-DC restored the DSP expression in 6 lung cancer cell lines, and the methylation status of DSP in intron 1 was confirmed by BS. In primary lung tumors, methylation of DSP was detected in 23 out of 41 samples. Currently the condition for transfection with DSP expression vector into lung cancer cell line H1299 was optimized and stable transfection will be done.

**Conclusions:** DSP is downregulated in lung cancer which could be caused by DNA hypermethylation.

### Sa-042

#### **Pneumocytoma (sclerosing haemangioma) in a 21-year-old male patient with hereditary non-polyposis colon carcinoma with hepatic metastasis a case report**

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**Introduction:** Pneumocytomas (sclerosing haemangiomas) are rare tumors in Western countries with unknown etiology. Normally middle-aged patients with a female predominance (5 times higher) are affected. Usually the patients do not have any symptoms. Mostly the tumors are solitary, peripheral, and well circumscribed with an average size of 2.8 cm. Histologically they demonstrate two cell types (round stromal cells and surface cells) in a papillary, sclerotic, solid or haemorrhagic pattern. Immunohistochemically the tumors are among others positive for TTF1, EMA and Progesterone. Pneumocytomas behave in an enigm manner. Few described cases with lymph node involvement did not have a worse prognosis.

**Case report:** We report a case of a 21-year-old man with an HNPCC (hereditary non polyposis colon carcinoma) with heterogenous MSI-H pattern and wildtype BRAF- and KRAS-genes. In the course of staging suspect findings were detected in liver and lung. The liver specimen showed a single metastasis of the known colon cancer. In contrast, the lung tumor, which was 7 mm in diameter, demonstrated a miscellaneous neoplasm. After histology and immunohistochemistry the tumor could be classified as pneumocytoma. First genetic analyses generated MSS-H in both, liver and lung.

**Conclusions:** The diagnosis of a pneumocytoma is not ordinary, because of its rarity and its numerous differential diagnoses. In such a case immunohistochemistry might be very supportive.

### Sa-043

#### **MicroRNAs regulating neo-angiogenesis and vascular smooth muscle cell proliferation are differentially expressed in plexiform lesions of pulmonary artery hypertension**

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**Aims:** MicroRNAs are involved in the regulation of proliferative vascular diseases. Neo-angiogenesis and vascular smooth muscle cell (VSMC) proliferation are key factors in vascular remodelling in pulmonary arterial hypertension. Therefore we investigated the expression of selected miRNAs in pulmonary arterial compartments in patients with severe pulmonary arterial hypertension.

**Methods:** Formalin fixed and paraffin embedded lung samples from patients with pulmonary arterial hypertension were analyzed. Plexiform lesions and neighboring pulmonary arteries were laser-micro-dissected. Expression of the VSMC-specific miR145 regulating VSMC proliferation and the endothelial-specific pro-angiogenic miR126 was measured by real-time PCR.

**Results:** MiR126 was significantly up regulated in plexiform lesions while miR145 significantly down regulated compared to neighboring pulmonary arteries and controls (p<0.05).

**Conclusions:** MiRNAs regulating neo-angiogenesis and vascular smooth muscle cell proliferation appear to be involved in the vascular remodelling process leading to the formation of plexiform lesions. Endothelial-specific microRNAs are over-expressed in plexiform lesions, while VSMC-specific microRNAs are down-regulated.

## Poster: Varia I

### Sa-044

#### **A simple and very flexible top pin tissue arrayer using loose pins**

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**Aims:** Paraffin tissue microarrays (PTMAs) are constructed by injecting paraffin tissue core biopsies (PTCBs) into preformed holes of a recipient block. Devices to pour the holes normally consist of a modified steel mold with steel pins at the bottom. So, the size of the recipient block and the arrangement and the number of the holes are determined by the steel mold. To make the system more flexible I designed a device which uses ordinary steel molds of different size, a spacer plate with loose steel pins and a perforated plate.

**Methods:** 375 holes (diameter: 1.05 mm; distance of the holes: 0.3 mm) were drilled into a spacer plate and a perforated plate. Depending on the desired number of holes in the future PTMA up to 375 steel pins (diameter: 1.0 mm; length: 30 mm) were put into the holes of the spacer plate. The steel pins had a 1.2 mm swelling at one end to prevent gliding through the holes. The spacer plate equipped with the desired number and arrangement of steel pins, the perforated plate, a plastic cassette and an ordinary steel mold (tissue-tek) were put together like a sandwich and filled with liquid paraffin. After solidification, the sandwich was disassembled to release the recipient block. Paraffin tissue core biopsies were manually injected into the holes of the recipient block.

**Results:** PTMAs with different size and with different numbers and different arrangements of the PTCBs were successfully constructed.

**Conclusions:** With the novel device consisting of a spacer plate, a perforated plate and loose steel pins it is possible to pour recipient blocks using ordinary steel molds of different size making the system very flexible.

### Sa-045

#### **Ventricular unloading is associated with increased 20S proteasome protein expression in the myocardium**

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**Aims:** The ubiquitin-proteasome system (UPS) breaks down numerous proteins including cyclins involved in cardiac hypertrophy. Since congestive heart failure (CHF) instigates cardiac hypertrophy, indicative of increased protein synthesis and/or impaired breakdown and ventricular unloading decreases cardiac hypertrophy ("reverse cardiac remodelling"), we tested the hypothesis that unloading alters myocardial UPS.

**Methods:** In 23 myocardial specimens before and after unloading the protein expression of ubiquitin, 20S proteasome and cyclin D1 were immunohistochemically investigated and morphometrically quantified in relation to cardiomyocyte hypertrophy, DNA content and cyclin D1 protein expression.

**Results:** Ventricular unloading is associated with a significant increase of 20S proteasome and a significant decrease of ubiquitin compared to CHF and correlated inversely with cardiomyocyte size, DNA content and cyclin D1.

**Conclusions:** The UPS is depressed in CHF, but this is reversed by ventricular unloading and associated with decreased cardiomyocyte hypertrophy, mean DNA content, and cell cycle regulatory proteins, suggesting that the UPS is involved in both the pathogenesis of cardiac hypertrophy and „reverse cardiac remodelling“ after ventricular unloading.

**Sa-046****Reversible regulation of the Retinoblastoma protein (Rb)/E2F-1 pathway during “reverse cardiac remodelling” after ventricular unloading**J. Wohlschlaeger, J. Stypmann<sup>1</sup>, C. Schmid<sup>2</sup>, H.A. Baba

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**Aims:** Cyclin D1, the retinoblastoma protein (Rb) and the E2F transcription factors are involved in the pathogenesis of cardiac hypertrophy. Ventricular unloading is associated with reversible regulation of molecular systems and decreased hypertrophy. The hypothesis whether the Rb/E2F-1 pathway is altered by ventricular unloading was tested and correlations with the cyclin D1 protein expression and cardiomyocyte diameters were explored.

**Methods:** In 21 myocardial samples prior to and after unloading from patients with congestive heart failure (CHF), the protein expression of cyclin D1, pRb, p107 and p130 (pocket proteins) and E2F-1 was immunohistochemically investigated and morphometrically quantified.

**Results:** Cyclin D1 and the proteins of the Rb/E2F-1 pathway were significantly increased during CHF and significantly decreased after unloading. Cyclin D1-, pRb- and p130 protein expression correlate significantly with cardiomyocyte diameters. A significant positive correlation between the pocket proteins, E2F-1 and cyclin D1 was noted.

**Conclusions:** Increased protein expression of phosphorylated (inactivated) Rb and the pocket proteins is associated with cardiomyocyte hypertrophy in CHF. Rb inactivation might be explained by phosphorylation by increased numbers of cyclin D1/cdk4 complexes associated with cardiomyocyte hypertrophy. However, this process can be reversibly regulated by ventricular unloading. These data underscore the importance of cell cycle regulatory proteins in the pathogenesis of CHF-associated (maladaptive) cardiomyocyte hypertrophy.

**Sa-047****SPIDIA: an European initiative to minimize pre-analytical biomarker variations**B. Ergin<sup>1</sup>, R. Langer<sup>1</sup>, J. Slotta-Huspenina<sup>1</sup>, R. Rosenberg<sup>2</sup>, H. Friess<sup>2</sup>,H. Höfler<sup>1</sup>, K.-F. Becker<sup>1</sup><sup>1</sup>Institut für Pathologie TU München<sup>2</sup>Klinikum rechts der Isar, Chirurgie, München

Molecular in vitro-diagnostics will play an important role in future health care practise. However, the potential effects of tissue processing, including time of vessel ligation, excision, transport to pathology, fixation, and storage, on protein biomarker expression and stability are not known in detail. SPIDIA, standardisation and improvement of generic pre-analytical tools and procedures for in-vitro diagnostics, is a 4-year project, funded by the European Union FP7 programme and involving 16 partners from 11 different European countries ([www.spidia.eu](http://www.spidia.eu)). One of the aims of SPIDIA is to establish potential influences of tissue processing on protein biomarker stability and expression in order to provide guidelines for more reliable biomarker measurements in the routine clinical setting. Initially, we will focus on non-malignant and malignant colon tissues. Immediately after surgery we will collect resected tissue samples in the operating theatre and define which of the tissue processing steps mostly influence protein expression and stability by comparing frozen and formalin-fixed samples at different time points during tissue processing. We will use Western blot, protein microarrays, and immunohistochemistry. Based on our approach, we will: (1) assess critical pre-analytical steps for variability of protein biomarker expression and stability; (2) identify surrogate protein markers that indicate tissue quality suitable for molecular analysis; (3) evaluate novel tissue fixatives for preservation of tissue architecture and maintaining the molecular content reflecting the disease state.

**Sa-048****Prolyl-hydroxylase 4 over-expression inhibits tumor growth by stimulating non functional angiogenesis**

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**Aims:** Hypoxia plays a major role in tumor vascularization and progression. The cellular hypoxia response is mediated by hypoxia-inducible factors (HIFs) which in turn are regulated by HIF prolyl-hydroxylases (PHDs). The goal of our project is to elucidate the function of PHD-4 in tumor progression.

**Methods:** Generation of stable PHD-4 over-expressing LM8 osteosarcomas and embryonic endothelial progenitor cells (eEPCs). In vitro: analysis of the HIF stability by Western blot; microarray analysis and angiogenesis/sprouting assay. In vivo: PHD-4 over-expressing LM8 tumor cells were subcutaneously injected in the back of C3H mice. Tumor growth was monitored and isolated tumors were histologically analyzed.

**Results:** PHD-4 over-expressing LM8 tumor cells and eEPCs reduced the HIF2 $\alpha$  protein level under hypoxia, whereas there was no effect on HIF1 $\alpha$  stability. In a syngeneic mouse tumor model, PHD-4 over-expression in LM8 osteosarcomas reduced tumor growth, due to an increased, but non-functional neoangiogenesis. This can be explained by an increased TGF $\alpha$  expression in LM8 cells over-expressing PHD-4, which increases endothelial cell side-sprout branching.

**Conclusions:** PHD-4 over-expression in tumor cells reduces tumor growth by the up-regulation of TGF $\alpha$ , which leads to an excessive, but non-functional angiogenesis. These data indicate that PHD-4 plays an important role in tumor progression.

**Sa-049****Expression of proteinase-activated receptors (PAR) 1–4 in human cancer**

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**Aims:** Proteinase activated receptors (PAR 1–4) are membrane receptors with a unique way of activation. In cancer, PARs induce angiogenesis, cell proliferation, motility and stimulate metastasis. They are activated or silenced by proteinases like thrombin, trypsin and matrix metalloproteinases. To evaluate the significance of expression and co-expression of PAR in cancer we performed a survey on published data and analyzed tumors by immunohistochemistry (IHC) of tissue microarrays (TMAs).

**Methods:** A Pubmed literature search was performed using the terms “PAR, thrombin, cancer”. 50 out of 160 publications were selected for systematic review based on the availability of the following parameters: tumor type, material type, detection method, specification of positive cases. IHC was performed on 414 samples of colon adenocarcinomas and 132 carcinomas of the pancreas.

**Results:** In the literature, PAR-1 was found in 83.6% of malignant samples (n=711), PAR-2 in 91% (n=336), PAR-3 in 37.5% (n=83) and PAR-4 in 26% (n=134). PAR-1 and -2 were present in adenocarcinomas, melanomas, osteosarcomas, glioblastomas, meningiomas, leukaemias and squamous cell carcinomas. Presence of PAR-3 was limited to kidney and liver cancer. PAR-4 was detected by immunohistochemistry and flow cytometry, but not RT-PCR or Northern Blot. Our own IHC results confirmed widespread expression of PAR-1 and PAR-2 in colon and pancreas carcinomas. PAR-3 was also widely detectable however showing a weaker and sometimes negative staining patterns.

**Conclusions:** The data highlights widespread expression of PAR-1 and -2 in human tumors suggesting an important role in tumorigenesis and providing potential targets for tumor therapy. However, coexpression is frequent and should be considered. PAR-3 and -4 are less frequently detectable, their expression and potential role in tumorigenesis require further investigation.

### Sa-050

#### Tippling the balance of PHD-2 leads to tumor growth retardation

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**Aims:** The right amount of nutrients and oxygen are crucial for a tumor to develop. Therefore, angiogenesis can be induced by HIF as a response to deprivation of oxygen in the tumor. The HIF-prolyl hydroxylases strictly regulate these processes, but for the moment not many in vivo experiments have been conducted demonstrating the role of PHDs in the tumor cell and highlighting their cross-talk with the endothelium. Therefore, our research group is studying in detail the modulation of PHD-2 in different tumor cell lines in mice.

**Results:** We demonstrate here that knocking down PHD-2 expression in a mouse osteosarcoma cell line, using independent shRNA constructs, resulted in a significant induction of vessel density in vivo, as could be expected. Surprisingly however was the observation that these tumors hardly grew and in some cases even completely disappeared in time; a feature that was accompanied by a reduced proliferation rate of the tumor cells rather than necrosis. On the other hand, over-expression of hPHD-2 in the same cell line significantly reduced vessel density, again accompanied by retardation of tumor growth and reduced cell death in comparison to their wild type counterparts. In search for the molecular background of these findings we found that the TGF-beta pathway was activated in all PHD-2 modulated cells during all stages of tumor development. On the other hand, we could only significantly reverse tumor growth retardation and proliferation, induced by TGF-beta in sh-PHD2 tumors. In addition, we were able to identify the proteins that are involved in the activation of TGF-beta.

**Conclusions:** In conclusion, modulating PHD-2 in this mouse osteosarcoma cell line (and others) always results in reduced tumor growth and is, at least in cells where PHD-2 is knocked down, independent of angiogenesis.

### Sa-051

#### The HOPE<sup>®</sup> fixation technique – a promising alternative to common biobanking approaches

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**Aims:** The availability of well-annotated human tissue samples through biobanks is key for translational research. Whilst fresh-frozen tissue is most applicable to a broad spectrum of molecular analyses, its storage and handling is complex and cost-intensive. Formalin-fixed, paraffin-embedded specimen (FFPE) are easy to handle and economic to store, but their applicability for modern analysis methods is restricted. The recently introduced, formalin-free HOPE<sup>®</sup> fixation method is a promising alternative, which might have the potential to unite the benefits of FFPE and fresh-frozen specimen. Aim of our study was to compare fresh-frozen samples, HOPE<sup>®</sup>-fixed samples, and FFPE samples for their application to different molecular methods.

**Methods:** We preserved 20 prostate cancer samples each with HOPE<sup>®</sup>, formalin, and liquid nitrogen. All tissue samples were H&E stained, and assessed by immunohistochemical markers (i.e. PSA, AMACR, AR) and a FISH assay (i.e. ERG rearrangement). Furthermore, DNA and RNA was extracted, quantified, and assessed for DNA/RNA integrity.

**Results:** Of the HOPE<sup>®</sup> samples, quality of H&E sections, immunohistochemical staining, and the FISH assay was at least equal to FFPE tissue, and significantly better than the fresh-frozen specimen. On the molecular basis, RNA and DNA analysis of the HOPE<sup>®</sup> samples provided similar results compared to the fresh-frozen specimen. Expectedly, FFPE-samples were insufficient for most of the molecular analyses.

**Conclusions:** This is the first study, assessing in parallel the pros and cons of these three techniques on the same tissue specimen. HOPE<sup>®</sup>-fixed tissue

combines the benefits of FFPE- and fresh-frozen specimen. Results of this study have the potential to expand on contemporary biobanking approaches.

### Sa-052

#### Hypoxia-induced down-regulation of microRNA-449a/b abrogates control over targeted PAI-1 mRNA in transplant-related tissue remodeling

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**Aims:** Tissue repair and replacement of damaged cells by connective tissue in transplanted organs provokes a response of fibroblasts to cellular stress factors such as hypoxia. MicroRNAs (miR) are small non-coding RNA molecules which bind to their mRNA targets which eventually lead to repression of translation. The cellular response of fibroblasts to transplant-related stress factors on the miR level is largely unknown.

**Methods:** MiR profiling in primary fibroblasts cultured under hypoxia, low density array profiling of fibrosis-related genes, transfection of miR-449a inhibitors/mimics, real-time PCR and immunohistochemistry in kidney transplants.

**Results:** We identified down-regulation of miR-449a/b expression in hypoxic fibroblasts. Functional studies using specific miR inhibitors and mimics showed direct evidence for targeting the plasminogen activator inhibitor-1 (PAI-1) by miR-449a/b leading to PAI-1 up- and down-regulation, respectively. In kidney transplants showing fibrosis and allograft remodeling decreased miR-449a/b expression was paralleled by increased PAI-1 levels. PAI-1 protein expression could be located predominantly in areas of active tissue remodeling.

**Conclusions:** We present a novel mechanism comprising hypoxia-induced decrease of miR-449a/b, subsequent increase of targeted PAI-1 and promotion of tissue remodeling and fibrosis.

### Sa-053

#### Removal of the methodological “bottleneck” formalin by implementation of the HOPE-technique into molecular tumor pathology

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**Aims:** To introduce an alternative and novel tissue fixative compared to conventional formalin which overcomes the obstacles concerning molecular tumor pathology.

**Methods:** Besides different stains used in routine diagnostics including histochemistry as well as immunohistochemistry many common molecularbiological techniques on the nucleic acid level (RNA and/or DNA) on HOPE fixed and paraffin embedded tissues were compared to formalin fixation.

**Results:** We have shown that the HOPE-technique results in a comparable morphological preservation to formalin fixation (up to now for more than ten years experience); furthermore that DNA, RNA and proteins are protected. Thus, the HOPE technique permits a successful application of all common molecular techniques such as in situ hybridization targeting either DNA or RNA, immunohistochemistry without antigen retrieval and for formalin-refractory antigens, PCR, RT-PCR, Western blot, Northern blot, and transcription microarrays etc.

**Conclusions:** Taken together, the HOPE-technique to date represents the best alternative fixation that is in contrary to other procedures, well documented and broadly scientifically analyzed. Therefore, new possibilities are opened up especially within the rapidly growing field of molecular pathology.

**Sa-054****Evaluation of autopsy reports in children at the Charité in the 20th Century**C. Proch<sup>1</sup>, R. Meyer<sup>1</sup>, T. Schnalke<sup>2</sup>, M. Dietel<sup>3</sup>, R. Hetzer<sup>1</sup><sup>1</sup>Klinik für Herz-, Gefäß- und Thoraxchirurgie, Deutsches Herzzentrum Berlin<sup>2</sup>Berliner Medizinhistorisches Museum der Charité<sup>3</sup>Institut für Pathologie, Charité-Universitätsmedizin Berlin, Campus Mitte**Aims:** We analysed the changes of death in children recorded in autopsy reports at the Charité **between 1900 and 1999**.**Methods:** A total of 21.343 autopsy reports in children (<18 years) were evaluated in terms of gender distribution, cause of death and age at death. The data were analysed using SPSS (Version 17.0).**Results:** The number of autopsy reports declined considerably during the study period. During the total study period the gender distribution was approx. 56% male to approx. 44% female children. In only 0,42% of autopsies the gender was not given. The number of stillbirths fell from 28% to 15%, with a mean by approx. 16%. This figure is subject to great fluctuations, which are mainly determined by external influences. The main cause of death is dependent on age: in newborn infants respiratory disease and infections are the main cause; at a later age tumors are the leading cause of death. Fatal malformations of different kinds constitute only a small proportion of total deaths and require separate study.**Conclusions:** For the period 1900–1910 no evaluable data were available. The last decade of the century, from 1990 to 1999, is characterized by important changes that make comparison of the results difficult.**Sa-055****Extracellular matrix remodelling and re-expression of Fibronectin and Tenascin-C splicing variants in human myocardial tissue of ischemic and valvular heart disease**M. Franz, P. Richter<sup>1</sup>, K. Grün, B.R. Brehm, D. Neri<sup>2</sup>, H. Kosmehl<sup>3</sup>, A. Berndt<sup>1</sup>  
Dept. of Internal Medicine I and <sup>1</sup>Institute of Pathology, University Hospital Jena, Germany<sup>2</sup>Institute of Pharmaceutical Sciences, ETH, Zurich, Switzerland<sup>3</sup>Institute of Pathology, HELIOS-Klinikum Erfurt, Germany**Aims:** Cardiovascular diseases are accompanied by changes in the cardiac extracellular matrix (ECM) including the differential re-expression of fibronectin (Fn) and tenascin-C (Tn-C) splicing variants. Using human recombinant SIP format antibodies, a molecular targeting of these proteins might be of therapeutic interest. **Methods:** Tissue samples of the right atrial auricle from patients with ischemic and valvular heart diseases were analysed by PCR based ECM gene expression profiling. Moreover, the re-expression of Fn and Tn-C splicing variants was investigated by immunofluorescence labelling and confocal Laser Scanning Microscopy.**Results:** 1) There were distinct changes in ECM gene expression depending on histological damage or the underlying cardiac disease with an increased expression of Fn and Tn-C mRNA in association to a rising histological damage and in valvular compared to ischemic heart disease. 2) There was a distinct re-expression of the Fn ED-A and the Tn-C A1 splice domain detectable with human recombinant SIP format antibodies in diseased myocardium. ED-A containing Fn showed a clear vessel positivity and A1 containing Tn-C a particular positivity in areas of interstitial and perivascular fibrosis.**Conclusions:** The human right atrial auricle is a valuable model to investigate cardiac ECM remodelling. Human recombinant SIP format antibodies are novel tools for an antibody-mediated targeted delivery of diagnostic or therapeutic agents in cardiac diseases.**Sa-056****Analysis of carcinoma vessel heterogeneity concerning fibronectin and tenascin-C incorporation**K. Galler, R. Köllner, P. Richter, A. Göhlert, J. Brandt<sup>1</sup>, M. Franz<sup>2</sup>, R. Heller<sup>3</sup>, D. Neri<sup>4</sup>, H. Kosmehl<sup>5</sup>, A. Berndt

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<sup>1</sup>Institute of Pharmacology and Toxicology, University Hospital Jena, Germany<sup>2</sup>Dept. of Internal Medicine I, University Hospital Jena, Germany; Center for Molecular Biomedicine, FSU, Jena, Germany<sup>4</sup>Institute of Pharmaceutical Sciences, ETH, Zurich, Switzerland<sup>5</sup>Institute of Pathology, HELIOS-Klinikum Erfurt, Germany**Aims:** Tumour angiogenesis is associated with the occurrence of oncofetal fibronectin (oncFn) and tenascin-C (oncTn-C) variants which are putative structures for an antibody-mediated targeted drug delivery. Knowledge on the distribution of these proteins is crucial for understanding vessel formation and for therapy planning.**Methods:** Human SIP antibodies against the oncFn ED-A, oncFn ED-B and the oncTn-C A, C, and D splice domains were used for immunofluorescence in different carcinomas, a RCC xenograft and in HUVEC cell culture. The spatial relation to vascular basement membrane (BM) was analysed combining SIP's with CD31 and laminin  $\alpha$ 4 chain antibodies. oncFn and oncTn-C mRNA expression was assessed in VEGF stimulated HUVEC.**Results:** 1) Carcinoma entities and subtypes show a differential Fn and Tn-C incorporation in the vessel wall. 2) Vessels of a given tumour type differ in their Fn and Tn-C incorporation with a stratified organization in relation to the BM. 3) Results from xenograft and HUVEC indicate oncFn as a product of endothelial cells and oncTn-C as a product of carcinoma cells.**Conclusions:** Composition and spatial reorganization of oncFn and oncTn-C in tumour vessels depend on tumour type and may reflect an individual tumour stroma interaction or different stages of vessel development. Vascular deposition patterns may be of diagnostic importance. Concerning therapy, oncFn or oncTn-C based targeting must be adapted to the individual patient.**Sa-057****Radiological and pathological findings of a metastatic extra-adrenal composite paraganglioma with neuroblastoma a case report**F.R. Fritzsche<sup>1</sup>, P.K. Bode<sup>1</sup>, S. Koch<sup>2</sup>, T. Frauenfelder<sup>3</sup><sup>1</sup>Institut für Klinische Pathologie, Universitätsspital Zürich<sup>2</sup>Klinik für Radioonkologie, Kantonsspital Winterthur<sup>3</sup>Institut für Diagnostische Radiologie, Universitätsspital Zürich**Case report:** We report the case of a 61 year-old man who was referred to the hospital due to unclear thoracic pain. An abdominal CT scan and ultrasonographic examination detected a retroperitoneal tumour comprising two different tumour components. Via fine needle aspiration a neuroendocrine tumour was diagnosed and the final histopathological workup revealed an extra-adrenal composite paraganglioma with neuroblastoma. The patient died 10 months after the initial diagnosis from tumour associated complications. Composite paragangliomas with neuroblastomas are extremely rare tumours. Here we describe imaging characteristics together with corresponding pathological findings of this tumour. Apart from any component-specific imaging findings, the hallmark of this entity is indeed the presence of different radiologically and histologically distinguishable components.**Sa-058****Ultrastructural cell preservation for immunogold labelling and transmission electron microscope analysis of ACSL-5**J. Bornemann, H. Königs, N. Gaßler<sup>1</sup>

Electron Microscopic Facility (EMF), Medical Faculty, RWTH Aachen University

<sup>1</sup>Institute of Pathology, Medical Faculty, RWTH Aachen University**Aims:** To select proteins for immunogold labelling within transmission electron microscopy two ways of resin embedding are established: by progressive

lowering of temperature (PLT) or by freeze-substitution. The focus of interest is the cell preservation for detailed ultrastructural analysis and localization of the antibody binding. Here we describe a method for a very good cell preservation by PLT to localize ACSL-5, an important enzyme of lipid metabolism.

**Methods:** All steps are carried out at  $-35^{\circ}\text{C}$  except alcoholic dehydration at  $-15^{\circ}\text{C}$  and UV-polymerisation at  $-45^{\circ}\text{C}$ . After embedding in lowcryl HM20 HepG2-cells were cut into 80–100 nm thick slices and immunolabelled with 10 nm gold particles. The samples were analyzed with a PHILIPS EM 400 T at 60 kV and micrographs were taken by an OLYMPUS CCD-Camera MO-RADA.

**Results:** The analyses of immunogold labelled cells show distinct distribution of gold particles and good cell organelle preservation. The quality of morphology allows to localize ACSL-5 to the mitochondrial membrane.

**Conclusions:** The method as demonstrated here is a useful tool for protein localisation by good ultrastructural cell preservation. The localization of ACSL-5 at the mitochondrial membrane is an important cognition to understand the general procedures of lipid metabolism and intracellular operations.

### Sa-059

#### Frequent and differential expression of lipid droplet-associated PAT-proteins marks lipogenic phenotype in malignant tumors

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**Aims:** In many human cancers, lipogenic pathways are activated; in some tumors, this is reflected by the presence of visible lipid droplets (LDs). Yet, the biology of neoplastic steatogenesis is largely unknown.

**Methods:** We comprehensively investigated expression of LD-associated proteins of the PAT-family (perilipin, adipophilin, TIP47) in neoplastic steatogenesis with immunohistology, electron microscopy, protein biochemical and molecular biological methods.

**Results:** By staining for PAT-proteins, LD-accumulation was found to be a frequent phenomenon of carcinoma cells. Whereas adipophilin and TIP47 stained almost ubiquitously the rim of LDs in various tumor types, especially those with clear cell phenotype, perilipin was restricted to LDs of hepatocellular, sebaceous, and lipomatous tumors. In HCC, perilipin, adipophilin, and TIP47 were coexpressed, and showed regional heterogeneity with a predominantly mutually exclusive localization pattern. In stepwise carcinogenesis, adipophilin expression correlated with the proliferation rate and was upregulated during early tumorigenesis, whereas perilipin was often lost during hepatocarcinogenesis.

**Conclusions:** By far more carcinomas contain (PAT-positive) LDs than expected by light microscopy. PAT-proteins may support diagnostic considerations and help establish hepatocellular origin in metastases of unknown primary. Since inhibition of lipogenesis has been shown to exert anti-neoplastic effects, PAT-proteins may represent targets for interventional strategies.

### Sa-060

#### DNA image cytometry of fine needle aspiration biopsies of salivary gland tumours improves diagnostic accuracy

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**Aims:** The preoperative information whether a tumour of the salivary glands is benign or malignant is important for planning the surgical procedure. Fine needle aspiration biopsy (FNAB) of salivary gland tumours is a minimal invasive and accepted method for preoperative diagnosis. The detection of DNA-aneuploidy by DNA image cytometry is strongly associated with malignancy. The concern of our study is to determine if DNA image cytometry could improve preoperative diagnosis of malignancy.

**Methods:** A series of 151 FNABs of salivary gland tumours sent to the Institute of Cytopathology during April 1995 and September 2005 is enrolled in this

study. 96 FNABs yielded sufficient material for DNA image cytometry and DNA-ploidy was analysed. Histological and clinical follow up was available for 77 FNABs, including 58 adenomas, one with enhanced proliferation, one chronic sialadenitis and 18 malignant tumours (carcinomas, metastases and others).

**Results:** Regarding to malignancy, cytological diagnosis achieved a sensitivity and specificity of 88,9% and 91,5%, respectively. Combination with detection of DNA-aneuploidy by DNA image cytometry increase sensitivity to 89,5% concerning the identification of malignancy and specificity to 100%.

**Conclusions:** Application of DNA image cytometry to FNABs of salivary gland tumours enhances the diagnostic accuracy and enables better preoperative therapy planning.

### Sa-061

#### Regulation of apoptosis by Notch: molecular mechanisms of tumor cell resistance

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**Aims:** Signaling via Notch receptors is an evolutionary ancient and highly conserved pathway which is involved in tissue homeostasis and maintenance of stem cells. Since deregulated Notch signaling can contribute to cellular resistance and cancer development, we investigated the regulation of apoptotic cell death by Notch1 in malignant tumor cells.

**Methods:** Adenoviral and retroviral siRNA transfer, quantitative PCR analysis, immunoblot analysis, immunohistochemistry, apoptosis and proliferation assays.

**Results:** Notch1 is highly expressed in colon carcinomas, renal cell carcinomas, and glioblastomas. Ectopic over-expression of Notch1 results in increased expression of the anti-apoptotic proteins Mcl-1, Bcl-2, and Survivin. Conversely, adenoviral knockdown of Notch1 leads to decreased expression levels of Mcl-1 and Bcl-2 which is accompanied by an increased susceptibility to mitochondrial apoptosis. Notch1-mediated down-regulation of Mcl-1 occurs at a posttranscriptional level. The anti-apoptotic properties of Notch1 might be caused by the Notch1-mediated inhibition of GSK-3 $\beta$  which is known to promote the proteasome-dependent degradation of Mcl-1.

**Conclusions:** Activation of the Notch signalling cascade results in the up-regulation of anti-apoptotic members of the Bcl-2 family, thereby blocking apoptotic cell death in cancer cells. Notch-antagonizing compounds such as  $\gamma$ -secretase inhibitors and novel ADAM17 inhibitors might represent promising new tools to overcome tumor cell resistance to apoptosis.

### Sa-062

#### Expression pattern of argininosuccinate-synthetase (ASS) in normal and tumor tissue as a marker for susceptibility to arginine-deiminase (ADI) therapy

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**Aims:** Enzymatically induced amino acid deprivation of malignant tumors (e.g. asparaginase in acute leukemia) lacking specific enzymes is a well established therapeutic concept for malignant disease. Arginine-Deiminase (ADI) treatment is based on a similar concept. Tumor cells lacking argininosuccinate-synthetase (ASS) are arginine-auxotrophic and depend on extracellular arginine uptake. ADI treatment lowers blood arginine levels and tumor cell death is induced by amino acid deprivation. However, little is known about the presence of ASS in normal and tumor tissues on the actual protein level.

**Methods:** A novel monoclonal antibody (mAb 195-21-1) to ASS and tested for specificity and its suitability in IHC. Subsequently, panels of normal organs and tumours were analyzed by IHC for the presence of ASS.

**Results:** MAb 195–21–1 worked well in paraffin embedded tissue using heat-based antigen retrieval techniques. In normal tissues, ASS was widely distributed and present in most normal organs of the genitourinary, respiratory and GI tract and other tissues. Interestingly, particular cells remained negative such as pancreatic islets, melanocytes, as well as adrenal medulla. In most analyzed tumours such as carcinomas of the lung, GIT, kidney and breast, as well as various sarcomas ASS was expressed ranging from 25%–100% ASS-positive tumour cells, though single tumours showed only focal ASS expression.

**Conclusions:** In normal organs, ASS is widely distributed showing expression in most tissues. Its presence in tumours parallels its expression in normal tissue with colorectal, kidney and breast cancer being mostly ASS-positive though occasional neoplasms show little ASS expression. However, SCLC, melanomas, and neuroendocrine carcinomas show little to none ASS expression. Our study indicates that melanoma, small cell lung cancer, and neuroendocrine carcinomas are potential candidates for ADI-based cancer therapy.

## Poster: Varia II

### Sa-063

#### Regression of the capillary deficit in the heart of subtotaly nephrectomized rats

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**Aims:** Remodeling of the heart in patients with chronic renal failure is characterized by hypertrophy, fibrosis and capillary/myocyte mismatch. It was the purpose of the present study to clarify whether treatment with the angiotensin receptor blocker Losartan and the mineralocorticoid receptor blocker Spironolactone reverses the capillary deficit in subtotaly nephrectomized rats.

**Methods:** Sprague-Dawley rats were subjected to subtotal renal ablation (SNX) or sham operation. Eight weeks after surgery, they were either euthanized or allocated to a treatment with vehicle, Losartan (250 mg/kg/day), Spironolactone (15 mg/kg/day), their combination, or unspecific antihypertensive treatment (Dihydralazine: 20 mg/kg/day) for the subsequent 4 weeks. Heart morphology was evaluated by stereology. Immune staining was performed for VEGF, angiopoietin 1 and 2.

**Results:** Systolic blood pressure was significantly higher in SNX compared with sham-operated animals and decreased in all treatment groups. Albuminuria was significantly higher in SNX compared with sham-operated animals and decreased in animals treated with Losartan and Losartan+Spironolactone. Compared with week 8 after SNX ( $3.4 \pm 0.5$  m/mm<sup>3</sup>) (period 1), the capillary density was not significantly lower in untreated SNX at week 12 ( $2.8 \pm 0.5$ ) (period 2). The capillary density was improved in period 2 compared to period 1 in SNX treated with Losartan+Spironolactone ( $3.9 \pm 0.4$ ), but not with Losartan alone ( $2.9 \pm 0.8$ ) or Dihydralazine ( $2.9 \pm 0.7$ ). Staining for VEGF was less intense in vehicle treated SNX at week 8 (score  $1.4 \pm 0.3$ ) and 12 ( $1.3 \pm 0.1$ ) compared with sham-op ( $2.1 \pm 0.4$ ); it was significantly more marked in SNX treated with Losartan ( $2.1 \pm 0.7$ ), Losartan+Spironolactone ( $2.0 \pm 0.6$ ), Spironolactone ( $1.8 \pm 0.4$ ), and Dihydralazine ( $1.9 \pm 0.6$ ). There was no difference in staining for angiopoietin 1 and 2.

**Conclusions:** The study documents regression of the capillary deficit in the heart of subtotaly nephrectomized rats by combined treatment with Losartan and Spironolactone, but not with the monotherapies.

### Sa-064

#### Non-coding RNA biomarkers in breast and liver cancer

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**Aims:** Reliable biomarkers for prognosis and therapy response are lacking in many tumor entities. Past research almost exclusively focused on protein-coding genes, while recent genome-wide analyses revealed that the vast majority of the human transcriptome is non-coding. Our study aims to identify differentially expressed non-coding RNAs (ncRNA) as potential biomarkers and characterize their molecular and cellular functions in breast and liver cancer.

**Methods:** For breast cancer, we profile the expression of 17000 ncRNAs and 22000 coding RNAs in parallel using microarray technology. For liver cancer, we used a candidate approach to find differentially expressed ncRNAs and uncover their cellular and molecular functions in cell culture experiments and aptamer-based RNA affinity purification strategies.

**Results:** RNA from normal breast tissue and primary mamma carcinoma tissues are profiled for ncRNAs and coding genes and the expression patterns are correlated to therapy response and survival to establish novel biomarkers. In liver cancer, at least two ncRNAs are significantly overexpressed in hepatocellular carcinoma compared to normal liver which are functionally characterized.

**Conclusions:** Our data will allow conclusions about the suitability of ncRNAs as biomarkers in direct comparison to coding RNAs. Beyond their potential use as biomarkers, uncovering differentially expressed ncRNAs also enables the analysis of their molecular and cellular functions and thus provides deeper insights into the biology of tumor cells.

### Sa-065

#### Increased senescence in plaques of A. carotis interna in clinical symptomatic patients

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**Aims:** High grade carotid artery stenosis is one of the main causes for stroke. Operation for asymptomatic patients as stroke prophylaxis is recommended for high grade stenosis but there are no reliable signs for individual stroke risk prediction. Several lines of evidence indicate that vascular cell senescence is relevant to acceleration of atherosclerosis.

**Methods:** p16(INK4a), p21 and p53 immunostaining was performed on intact CEE plaques from 20 patients with high-grade (>70%) stenosis undergoing carotid endarterectomy (CEA) (group I, asymptomatic, n=10; group II, symptomatic, n=10). Both groups were matched for gender, age, risk factors and degree of carotid artery stenosis. Plaques were measured using a semiquantitative score system in a blinded fashion by two observers. The atherosclerotic lesions were divided in margin to calcified-, lipid- and mixed type of plaque formation.

**Results:** There was significantly elevated expression of p16(INK4a) and p21 in margin of lipid- and mixed lesions in symptomatic compared to both asymptomatic and control zones. Comparison of the margin zones showed significant higher expression of all three markers in the margin of mixed plaques.

**Conclusions:** Recent guidelines recommend operation in patients with high grade carotid artery stenosis to prevent neurological events. This study showed correlation of senescence markers and clinical course of patients with atherosclerotic lesions of the carotid artery. Further investigations in this field bare the potential to get a more individual indication protocol and decreasing of NNT.

### Sa-066

#### **MLDP/ perilipin 5 is a lipid droplet-associated protein expressed in various human tissues**

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**Aims:** Eukaryotic cells store excess neutral lipids in lipid droplets. Lipid droplet-associated proteins of the so called PAT-family (perilipin, adipophilin, TIP47), fundamentally regulate lipid storage and utilization. In mouse and cell culture models, MLDP ("myocardial lipid droplet-protein", perilipin 5), the newest member of this protein family, is reported to be expressed in highly oxidative tissues such as heart, red muscle and liver during fasting. Yet, we could show, that PAT-proteins show differences in expression between species as in the case of perilipin de novo expression in human hepatocyte steatosis.

**Methods:** Therefore we started to analyse MLDP expression pattern in human tissues with protein biochemistry, immunofluorescence microscopy and immunohistochemistry.

**Results:** Using a pan tissue microarray, MLDP staining was detected at extremely small lipid droplets in heart, striated and smooth muscle, brown adipose tissue, and hepatocytes of steatotic liver, but in minor amounts also in other epithelia of the gastrointestinal tract. MLDP staining visualized lipid droplets of a size which were not visible in conventional light microscopy. In different patient samples, the amount of MLDP-positive lipid droplets varied highly. In different human tissues, MLDP and the other PAT-proteins perilipin, adipophilin and TIP47 were differentially expressed.

**Conclusions:** MLDP represents a lipid droplet-associated protein with predominant expression in steatotic hepatocytes, in myocytes, and brown adipocytes. However, our data indicate that MLDP may have a broader distribution pattern in human implicating a more general function in lipid droplet storage.

### Sa-067

#### **Expression of senescence markers in accelerated atherogenesis of uninephrectomized ApoE<sup>-/-</sup> mice**

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**Aims:** To evaluate the hypothesis that young uninephrectomized (UNX) ApoE<sup>-/-</sup> mice show similar vascular alterations when compared to old sham-op ApoE<sup>-/-</sup>.

**Methods:** Sixty-four ApoE<sup>-/-</sup> mice receiving normocholesterol diet were divided into 4 groups: UNX and Sham-op, observation period of 16 or 32 weeks (wk) post-operative. UNX was performed at 8 weeks of age. Hearts and aortas were harvested for analysis through stereology and immunohistochemistry. Aortic p16, p21, p53 and telomerase were accessed through RT-PCR.

**Results:** Compared to Sham-op (16 and 32wk) aorta and heart remodeling were more pronounced in UNX 32-wk animals, which presented a significant increase in wall to lumen ratio, plaque size and a reduced heart capillary length density despite no significant alterations in blood pressure. Plaque collagen content was overall low and similar between the groups. RT-PCR of aortic material showed a higher expression of p21 in the UNX 32wk animals compared to all other groups (10.4±4.6 vs. 3.1±0.43, 4.5±3 and 6.2±2.3 ratios/HPRT, p<0.05) and a lower telomerase expression compared to Sham-op 16 and 32wk.

**Conclusions:** UNX promoted significant aortic and cardiac remodelling, which was paralleled by increased expression of p21 and reduced telomerase activity after a 32 week observation period.

### Sa-068

#### **IgG4-positive plasma cells under diverse inflammatory conditions: Is it possible to histologically diagnose IgG4-related systemic disease with certainty?**

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**Aims:** IgG4-related systemic disease is an emerging concept encompassing a heterogeneous group of chronic fibroinflammatory disorders of unknown etiology. The major medical conditions attributed to this disease are autoimmune pancreatitis, sclerosing sialadenitis (Küttner tumour), sclerosing cholangitis, retroperitoneal fibrosis and others. The occurrence and number of IgG4-expressing plasma cells in common chronic inflammatory conditions not known to be IgG4-related has not been investigated yet.

**Methods:** Cases from common, plasma cell-rich inflammatory lesions (chronic gastritis, chronic inflammatory bowel disease, radicular /odontogenic cysts, chronic synovitis, sigmoid diverticulitis, tumour-associated inflammatory infiltrates) were analyzed immunohistochemically using CD138, IgG and IgG4. The ratio of IgG: IgG4-positive cells was assessed semi-quantitatively.

**Results:** The results were heterogeneous among different lesions and also among different cases within the same condition. A high number of IgG4-positive plasma cells were seen in oral odontogenic cysts and apical granulomas. Cases of chronic synovitis with features suggestive of rheumatoid disease revealed a varying number of positive cells ranging from significant counts to almost absent IgG4-positive cells.

**Conclusions:** Varying numbers of IgG4-positive plasma cells are common component of chronic inflammation at different anatomic sites and generally do not imply a diagnosis of IgG4-related systemic disease. The role of these cells in such disorders remains to be investigated.

### Sa-069

#### **The proteoglycan biglycan acts both as enhancer of antigen presentation via TLRs and as autoantigen to trigger experimental autoimmune perimyocarditis**

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**Aims:** Biglycan (BGN) is a leucine-rich proteoglycan ubiquitously present in extracellular matrix. Here we focus on the role of BGN as an endogenous TLR-ligand on antigen (Ag) presentation and we address the capacity of BGN to trigger autoimmune response.

**Methods:** Bone-marrow derived dendritic cells (DCs) from WT, TLR2-, TLR4-, MyD88- or TRIF- deficient mice were pulsed with ovalbumin (Ova) and exposed to LPS, BGN or vehicle. The DCs were further co-incubated with B3Z, OTI, Bo17 or OTII cells. Secreted IL-2 and IFN-γ and T cell proliferation were measured using CBA and CFSE assays. Ova uptake by DCs was quantified by both flow cytometry and confocal microscopy. For in vivo experimental autoimmune perimyocarditis induction, DCs were pulsed in vitro with Troponin I, CD40 ligand and LPS, BGN or vehicle and injected i.p. into mice. On day 7, mice were sacrificed and intensity of perimyocarditis was evaluated and scored 0-4.

**Results:** BGN strongly enhanced DC Ova presentation by 80% or more to all applied T cell types in both TLR2 - and TLR4 - dependent manner. In our in vivo experiments, BGN induced autoimmune experimental perimyocarditis when applied as TLR-ligand in a comparable extent to LPS (average scores 1.2 and 1.4, respectively) showing additionally a moderate autoantigenic capacity (average score 0.5).

**Conclusions:** Biglycan may enhance Ag presentation via TLR2 and TLR4 receptors and may trigger autoimmunity demonstrating both TLR-stimulatory and antigenic characteristics.

**Sa-070****Structural requirements of research tissue banks as essential platforms for molecular tumor research**

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**Aims:** Tissue banking is the decisive and rate limiting resource and technology platform for basic and translational molecular tumor research. Thus it is essential to plan and structure tissue banking and allocate resources according to research needs, but little is known about the actual requirements. Thus, we have analyzed the complete structured and prospective registration and tracking records from all projects of the tissue bank of the NCT Heidelberg for relevant structural information that may support tissue bank planning.

**Methods:** All projects of the NCT tissue bank (n=449; 2005 until 10/2009) involved fresh frozen (-80°C) (116 projects) and paraffin embedded tissues (333 projects), that were provided as probes, sections or derivatives (multi-tissue-arrays (n=37; 105 projects)). In selected projects immunohistology (70 projects) and image acquisition (VM) was performed by the tissue bank. Recorded in all projects were: project leader, type and amount of material provided, type (basic/translational) and size of project, project fulfilment, and provision of clinical/follow-up data; projects were tracked in a standardized manner after 90 and 180 days for sample usage and satisfaction with material quality and handling time.

**Results:** Of 461 research projects 449 (97%) were successfully completed by material transfer agreement; 55% were basic research, 45% were translational research projects. Most projects were bilateral (one PI with tissue bank); 5% of projects required and included clinical/follow-up data. Project sizes split into 95 small, 283 medium, and 71 large size projects. Project tracking demonstrated that all projects had started after 90 and satisfaction with provided material exceeded 99% of the projects.

**Conclusions:** Standardized registration and tracking provides valuable structural information for planning and financing of tissue banks and resources allocation. High project number and completion rates as well as high user satisfaction rate demonstrate that research oriented structuring of tissue banks is preferable and highly efficient; significant numbers of paraffin and fresh frozen tissue as well as TMA based projects argues in favor of a broad spectrum platform approach in order to fulfill research needs.

**Sa-071****Impact of accreditation on tissue bank definition and quality management**

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**Aims:** The tissue bank of the NCT Heidelberg, founded in 2005, has implemented a structured quality management (QM) system in 2006. To provide an objective, externally reviewed, transparent, and documented structure for QM, the process of accreditation of the NCT tissue bank was initiated in 2007 and successfully completed in early 2009. Aim of this analysis was to define the impact of the accreditation process on tissue bank definition and its QM system.

**Methods:** Parameters analysed were interface definition prior and past accreditation process, definition of resources allocation, structure and regularity of QM measures and novel development arising after accreditation.

**Results:** The accreditation process of the NCT Tissue Bank led to a clear definition of its previously not formalized interface with diagnostic pathology (tissue acquisition, archiving, reporting) and cooperation partners, defining its duties and necessities of funding. It enforced regular QM measures (audits, further education) supporting internal transparency and outside reviewing and motivated partners for participation.

**Conclusion:** Accreditation of a tissue bank, although representing a major effort, has positive effects on structural definition of the tissue bank, and its acceptance among its partners. Accreditation of the NCT-tissue bank provides a framework for further tissue banks to come and a solid basis for full cost calculation and definition of its duties.

**Sa-072****Linking population-based epidemiological studies with molecular biology research a new quality of collectives for biomarker analyses**

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**Aims:** Linking specifically composed tumor tissue collectives with relevant histological, clinical, and follow-up data is essential for correlative molecular analysis in basic and translational tumor research. Nevertheless this approach fails in uncovering pre-existing, environmental, and life-style conditions (e.g. smoking, nutrition habits) contributing to carcinogenesis and specific molecular phenotypes. This will only be possible by developing tissue collectives that are linked to epidemiological studies collecting pertinent risk factor data. For this purpose the cooperation of the NCT-tissue bank Heidelberg with large scale population based case-control and cohort studies was analysed for its potential to build up relevant collectives.

**Methods:** Via the NCT long standing cooperations of the tissue bank with epidemiological partners were initiated including several large scale population based epidemiological studies, e.g. DACHS, ESTHER, and EPIC. Due to sample size and comprehensive risk factor data (detailed risk factor data, clinical data and, follow-up data), the DACHS-study was chosen as proof of principle. Prospectively collected data, tumor frequencies, study-associated tumor tissue retrieval, multi-tissue array composition, and rational molecular grouping were analysed.

**Results:** Overall 1564 participants recruited in the population-based case control study in the Rhine-Neckar region developed colo-rectal cancer; study associated and supported tumor tissue (FFPE) retrieval was realized for 1329 (85%) patients. All retrieved colo-rectal cancer samples were reviewed (exclusion rate <5%) and a multi-tissue array consisting 1262 samples was composed. TMA allowed rapid and rational preliminary molecular grouping using an immunohistochemical algorithm.

**Conclusions:** Cooperation with long term population-based epidemiological studies is able to build up unprecedented collectives for molecular analyses and provides a unique possibility to systematically expand the questions addressed at biospecimen to preexisting, environmental and life-style factors. The approach provides less biased molecular prevalence data, and is able to solicit so far non-identified subcollectives for further exploration. Thus it is essential for academic pathology and biomarker-directed molecular tumor research to intensely cooperate with epidemiological studies including ab initio inclusion of biobanking into study protocols, consent forms, and funding schemes. It underscores the need to further optimise molecular technology for FFPE-tissues.

**Sa-073****Autopsy results from a patient with fatal novel human influenza A (H1N1) infection**

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**Aims:** The 2009 pandemic swine-origin influenza A (H1N1) virus (S-OIV) is composed of a combination of human, swine, and Eurasian avian strains and has quickly spread to all continents. Mortality in western countries is relatively low and only few autopsy results have been published so far. Here, we present the autopsy results of a case with fatal S-OIV infection.

**Methods:** A 64 year old male was admitted to the hospital and diagnosed with flu and severe pneumonia. PCR analysis displayed the presence of the S-OIV. Within a week, the patient's condition worsened and ultimately died from acute respiratory distress syndrome and multi-organ failure.

**Results:** Macroscopically, lungs were heavy, consolidated, and diffusely edematous with marked hemorrhage. Histologically, we found exsudative diffuse alveolar damage (DAD) with extensive hyaline membranes and a massive alveolar and interstitial edema with few capillary microthrombi. Extrapulmonary findings included adipositas, generalized arteriosclerosis and circumscribed terminal myocardial re-infarction.

**Conclusions:** This post mortem case report presents the pathological findings of a fatal case with proven S-OIV infection. Our findings are consistent with the data from a larger autopsy cohort recently published by Mauad et al. (Am J Respir Crit Care Med, 2009) who identified DAD as the major cause of death in patients with S-OIV infection.

#### Sa-074

##### **Tissue reaction on vascular implants in dependence of different glycerine-coatings of vascular grafts**

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**Aims:** Aim of this study was to improve the flexibility of vascular implant materials, by increasing the glycerine content of their coating. The glycerine content of the coating of silver-coated polyester-vascular implants may possibly influence the tissue reaction on clinically used implant material.

**Methods:** Three different glycerine coatings on silver-coated vascular implants were analyzed histologically to estimate possible changes improving their biocompatibility. We analyzed three different coatings (15%, 30%, 45%) with respect to the resorption-time of the coating, the induction of periimplant fibrosis, inflammation and foreign body reaction (giant cell induction) after 1 and 3 months of implantation in a rat model.

**Results:** After 1 month a significantly reduced resorption of the 45%-glycerine coating was found which could not be demonstrated after the 3 month period. Significant differences in quality and quantity of periimplant fibrosis and chronic periimplant inflammation were not visible. The higher glycerine content induced a mild increase in foreign body giant cells whereas the 30%-glycerine-coating did not induce any difference compared to the standard coating (15%).

**Conclusions:** The use of an increased glycerine content of vascular implants up to 30% does not induce significant changes in the biocompatibility of the implant but provides a higher flexibility of the implant resulting in an improvement of clinical characteristics.

#### Sa-075

##### **Comparison of morphological parameters of UniGraft Synthetic-Implants in dependence of the polymer content of the endoluminal coating of vascular prosthesis**

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**Aims:** In order to reduce the possible risk of infection resulting from gelatine coated vascular implants and to improve the characteristics of the coating of vascular implant materials a new device (Unigraft Synthetic) was developed.

**Methods:** Two different endoluminal coatings on polyester-based vascular implants were analyzed histologically to assess their biocompatibility. We analyzed two different coatings (29%, 49% polymer-content) with respect to the resorption-time of the coating, the induction of periimplant fibrosis, inflammation and foreign body reaction (giant cell induction) after 3 months of implantation in a sheep carotis-implant model.

**Results:** After an implantation period of 3 months a significant change in both groups regarding all analyzed tissue reaction-parameters (neointima induction, endothelialization, periimplant fibrosis, inflammation and giant cell reaction)

was not visible. Comparison of both groups showed a significant acceleration of resorption of the implant coating with the lower (29%) polymer content.

**Conclusions:** The reduction of the polymer content of the vascular implant coating from 49% to 29% in polyester-based vascular endoprosthesis is associated with an significantly accelerated resorption of the coating and does not show any alterations of the biocompatibility of the implant material.

#### Sa-076

##### **Automatic sample processing in molecular pathological approaches**

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**Aims:** Automatic sample processing is an increasing approach in many laboratory disciplines. However, in molecular pathology, handling formalin fixed and paraffin embedded (FFPE) tissues, automatization is rare and difficult. In the present study, we have established an automatic DNA isolation method and its linkage to automatic DNA analyses by Real Time PCR set up.

**Methods:** DNA from FFPE materials were prepared by using the Agentcourt FormaPure system on a Biomek automatic work station (Beckman Coulter, Krefeld, Germany). The DNA isolation and the pipetting process was linked to a 2-dimensional bar code reader (Thermo Biosciences), recognizing each tube out of 96, and a decapper (Fluid X, UK). Furthermore, a fluorometer and spectral photometer was integrated into the system.

**Results:** Automatic DNA isolation resulted in DNA yields comparable to manual isolation. DNA quantification proceeds automatically by pico-green measurement and values were used for automatic sample normalisation. In order to enable efficient sample tracking, isolated DNA were filled by the roboter into 2D-bar-coded tubes. Normalized DNA volumes were pipetted by the roboter system and applied to various real time PCR assays. The pipetting layout was transferred to the real time cyler and the data were analysed using automatic sample attribution.

**Conclusion:** Automatic sample processing is also possible in approaches of molecular pathology handling FFPE materials. It efficiently saves time and improves sample tracking and quality control.

#### Sa-078

##### **Differences in proinflammatory cytokine gene expression in the NZB/W mouse model of systemic lupus erythematosus (SLE)**

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**Aims:** Cardiovascular disease is a major clinical problem in patients with systemic lupus erythematosus (SLE) accounting for up to 30% of deaths in this population. In particular, the impact of the concomitant renal disease is still controversial. Differentially regulated genes most notably involved in the immune response pathways were already identified in the heart.

**Methods:** NZB/W F1 mice with SLE-like disease were grouped according to low level (NZB/W LL), middle level (NZB/W ML) and high level (NZB/W HL) of active lupus nephritis. After RNA isolation from heart tissue and reverse transcription, cDNA was used for realtime PCR with primers for IL-15, IL-10 and TNF $\alpha$ . Furthermore, hearts were investigated for T- and B-cell infiltration (CD3 and CD20) and TNF $\alpha$  expression in immunohistochemistry (IHC).

**Results:** In realtime PCR IL 15 gene expression was significantly upregulated in all NZB/W groups compared to NZW controls (NZB/W LL 4-fold, NZB/W ML 3.2-fold and NZB/W 2.7-fold). There was no significant difference in TNF $\alpha$  and IL 10 gene expression between the NZB/W groups and NZW controls just as well as CD3, CD20 and TNF $\alpha$  IHC staining showed no significant differences.

**Conclusions:** Our results indicate that the heart muscle itself constitutively overexpresses IL-15 in systemic lupus erythematosus and proinflammatory gene regulation appeared to be selective since comparable expression of TNF $\alpha$  was observed in SLE and non-SLE strains.

**Sa-079****Is the RAS-pathway of sinonasal adenocarcinoma of intestinal type (ITAC) affected as in colorectal adenocarcinoma?**

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**Aims:** Histologically, ITAC bear a strong resemblance to colorectal adenocarcinoma. For our analysis, we therefore used mutational of KRAS- and BRAF1-gene to investigate if the RAS- MAPK-pathway is affected as in colorectal adenocarcinoma.

**Methods:** Tumor tissue from 30 cases of ITAC (grade 1 grade 3) were isolated by laser microdissection (Leica) from paraffinized slides. For the analyses of the KRAS mutation in the codons 12/13 we used the low density microarray 1.4 (Chipron). The V600E mutation in the BRAF1-gene was analyzed by sequencing.

**Results:** Among 30 cases of ITAC only few adenocarcinomas revealed a KRAS mutation of codon 12 and 13 respectively. A V600E mutation of the BRAF-gene could not be detected in our group of 30 ITAC, neither in cases of low grade nor in high grade malignancy.

**Conclusions:** The very low rate of KRAS mutations and the absence of BRAF1 mutations indicates that the RAS signal transduction pathway is not involved in the genesis of ITAC. Thus colorectal adenocarcinoma is molecular quite different from ITAC on molecular level although being quite similar histologically. Our results underline the histological observation that ITAC has no adenomatous precursor lesion.

**Sa-080****Robust microRNA expression profiling of specific cells in complex archival tissue stained by immunohistochemistry**F. Hlubek, C. Schuster, J. Budczies<sup>1</sup>, T. Kirchner

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**Aims:** Molecular diagnostics in modern pathology has been limited by the use of formalin-fixed, paraffin-embedded (FFPE) tissues in current routine diagnostic procedures due to modification and degradation of nucleic acids. In particular, molecular analysis of specific cell types potentially important for basic research and biomarker identification is largely prevented in highly complex, solid tissues routinely used in histopathology. Our objective was to establish a sensitive and robust procedure to quantify miRNA expression in specific cells from complex archival colorectal tumor tissue identified by immunohistochemistry.

**Methods:** We combined classical immunohistochemistry and isolation of specific tissue cells by laser microdissection with the molecular analysis based on miRNA-preamplification and expression analysis by quantitative real-time PCR.

**Results:** We show reliable detection of miRNA expression profiles determined from limited amounts of colorectal cancer FFPE-tissues after routine staining procedure. The combination of routinely used FFPE-specimens stained by immunohistochemistry with the molecular analysis of laser microdissected complex tumor tissue resulted in robust miRNA expression patterns exclusively obtained from epithelial tumor cells.

**Conclusions:** The data demonstrate the feasibility of molecular analysis of a specific cell type within complex, solid tumor tissues identified by immunohistochemistry enabling new perspectives in research and biomarker identification for individual molecular diagnostics.

**Sa-081****Sudden death of an immunocompetent young adult caused by novel (swine origin) influenza A/H1N1-associated myocarditis**G. Gdynia, P. Schnitzler<sup>1</sup>, R. Kandolf<sup>2</sup>, H. Bläker, P.A. Schnabel, P. Schirmacher, W. Roth

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**Aims:** The main cause of death from novel (swine origin) influenza A H<sub>1</sub>N<sub>1</sub> infection is acute respiratory distress syndrome. Most fatal cases are immunocompromised patients or patients with a severe underlying disease. Here, we report a fatal case of acute interstitial myocarditis associated with novel influenza A/H<sub>1</sub>N<sub>1</sub> infection in an immunocompetent young woman.

**Methods:** Autopsy, (nested) RT-PCR analysis, sequencing, immunohistochemistry, electron microscopy.

**Results:** A previously healthy 18-year-old woman experienced malaise, diarrhea, and fever for several days before a sudden collapse at home. Autopsy revealed an acute interstitial myocarditis in absence of a significant respiratory tract infection. Infection with novel (swine origin) influenza A (H<sub>1</sub>N<sub>1</sub>) was confirmed by PCR analysis of blood as well as myocardial tissue. Influenza-caused diarrhea with consecutive hypokalemia potentially contributed to the fatal outcome of the myocarditis leading to ventricular fibrillation. **Conclusions:** Sudden death by myocarditis may be a rare complication of novel influenza A H<sub>1</sub>N<sub>1</sub> infection in otherwise healthy individuals, even in the absence of significant respiratory tract infection.

**Vorträge: Mammopathologie: HER 1 und KAI 1****Sa-082****Her2 ASCO-Guidelines – der Weisheit letzter Schluss?**

G. Sauter

Hamburg

**Sa-083****Prätherapeutische HER2-Testung beim Mammacarcinom: Die Rolle der Immunhistochemie**

J. Rüschoff

Kassel

**Sa-084****HER2 genetic heterogeneity according to ASCO/CAP criteria is frequent in invasive breast carcinomas with equivocal (2+) HER2 immunohistochemical score**

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**Aims:** Intratumoral heterogeneity of HER2 gene amplification may contribute to inaccurate assessment of the HER2/neu status in breast carcinoma. Recently published ASCO/CAP guidelines define HER2 genetic heterogeneity (GH) as the presence of >5%, but <50% of tumor cells with a HER2/CEP17 ratio >2.2. The aim of this study was to determine the frequency of HER2 GH in invasive breast carcinomas with equivocal (2+) HER2 immunohistochemical (IHC) score.

**Methods:** HER2 FISH results of 231 invasive breast carcinomas with equivocal (2+) HER2 IHC score were analysed for HER2 amplification (HER2/CEP17 ratio >2.2), HER2 gene clusters, chromosome 17 polysomy, and HER2 GH, using ASCO/CAP criteria. For each carcinoma, single cell scoring of 60–100 carcinoma cells was performed.

**Results:** HER2 amplification, HER2 gene clusters, chromosome 17 polysomy, and HER2 GH were found in 14%, 11%, 6%, and 37% invasive breast carcinomas with equivocal (2+) HER2 IHC score, respectively. Among the 199 carci-

nomas lacking HER2 amplification, we found 83 (42%) tumors with HER2 GH.

**Conclusions:** HER2 GH is frequent in breast carcinomas with equivocal (2+) HER2 IHC score. Tumors with HER2 GH may respond to trastuzumab treatment (despite a HER2/CEP17 ratio <2.2), since they harbour a significant proportion of tumor cells with HER2 gene amplification. Single cell scoring of the HER2/CEP17 ratio is necessary to detect carcinomas with HER2 GH.

#### Sa-085

##### **PTK6 and HER receptor signaling in breast cancer**

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**Aims:** The cytosolic protein tyrosine kinase 6 (PTK6) is over expressed in two-thirds of primary breast tumours and is involved in EGF receptor signal transduction. Its significant role in the malignancy of breast carcinomas is obvious but still unclear. IHC analyses on tumour samples show co-expression of PTK6 with HER receptors, and several signaling proteins. Using RNA interference, the down regulation effect of PTK6 on signaling molecules and interaction partners can indicate its role in signal transduction and display as a new target in cancer therapy.

**Methods:** For IHC, FFPE tissue samples from breast carcinoma were incubated with different antibodies, and the results were correlated with histological and follow-up data. For RNAi experiments, breast cancer cell line T47D was transfected with specific siRNAs and lentiviral vectors containing corresponding shRNAs. Extracted proteins of transfected and control cells were analysed with Western blot and immunoprecipitation (IP). To investigate the migratory behaviour of transfected and non-transfected cells, a wound healing assay was performed.

**Results:** IHC analyses identified HER2, HER3, HER4, MAPK, Sam68, PTEN, co-expressed with PTK6 in tumour tissues, and an important role of PTK6 in survival analysis of patients. Reduced PTK6 protein levels in T47D cells lead to reduced expression of phosphorylated MAPK, STAT3, PTEN, Paxillin and to increased levels of HER2, HER3 and HER4. IP's also indicate reduced interaction of PTK6 and identified interacting partners. Moreover, reduced migration rate of transfected cells was observed.

**Conclusions:** This study demonstrates PTK6's role in breast cancer and that RNA interference can reduce the malignancy of breast carcinoma cells due to reduced signal transduction via phospho-MAPK or -STAT3. Decreased levels of phospho-Paxillin and reduced migration in transfected cells can influence metastasis.

#### Sa-086

##### **High concordance of HER-2 status in hematogeneous breast cancer metastases**

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**Aims:** Only about 60% of patients with metastatic breast cancer respond to anti-Her2 treatment with trastuzumab (Herceptin). The reasons for therapy failure are still largely unknown. The aim of the current study was to search for differences of the HER2 status between primary tumors that are used for clinical HER2 testing and the hematogeneous metastases which are the target of the therapy.

**Methods:** A tissue microarray (TMA) with 4 samples, each from different areas from 58 primary tumors and 757 distant metastases from 160 patients was analyzed by fluorescence in situ hybridization (FISH) and immunohistochemistry (IHC).

**Results:** A good concordance between IHC and FISH was found. 94.3% of the IHC 3+ and 40.0% of IHC 2+ samples showed amplification. For FISH, heterogeneity between different tissue samples from one tumor was seen in 2.1%, between different metastases in 6.2% but not between primary tumors and their metastases. All cases with discrepant FISH data had borderline FISH findings that were scored differently in separate analyses and did not reflect true heterogeneity. The IHC findings were more often heterogeneous (within one tumor in 5.1%, between different metastases in 12.8% and between primary tumors and their metastases in 17.4%). Remarkably, the corresponding FISH data did not provide evidence for genomic heterogeneity in IHC heterogeneous cases. These findings indicate a considerable impact of technical issues on IHC.

**Conclusions:** Our results suggest a low frequency of true HER2 heterogeneity in primary and metastatic breast cancer. The vast majority of cases with conflicting results are either caused by varying interpretation of borderline FISH findings or by false (negative or positive) IHC results. HER2 testing on primary tumors is largely reflective of the HER2 status of all hematogeneous metastases of a patient.

#### Sa-087

##### **Profiling signalling pathways in formalin-fixed and paraffin-embedded breast cancer tissues**

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**Aims:** The aim of our study was to develop and optimize methods for relative and absolute protein quantifications in formalin-fixed, paraffin-embedded (FFPE) tissues with special emphasis on HER mediated pathways in breast cancer.

**Methods:** Using a recently developed technology for extraction of full-length proteins from FFPE tissues, we evaluated >50 commercial antibodies for specificity using Western blots and reverse phase protein arrays (RPPA). Purified HER receptor proteins were used to determine absolute protein concentrations in FFPE tissue extracts.

**Results:** We confirmed specificity of 35 commercially available phosphospecific and non-phosphospecific antibodies using Western blots with protein extracts from cell lines and tissue extracts from breast cancer patients. Spiking known amounts of purified HER receptor proteins in HER receptor negative tissue extracts allowed us to precisely measure abundances of HER-receptors in FFPE breast cancer tissues using RPPA technology. Adequate controls were designed.

**Conclusions:** Our results will provide a basis for the development of diagnostic techniques for the quantitative analysis of deregulated HER receptors and downstream signalling proteins in typical clinical tissues, e.g. formalin-fixed samples, for widespread use in most hospitals world-wide.

#### Sa-088

##### **Expression and regulation of the KAI1 metastasis suppressor gene in human breast cancer**

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**Aims:** The cell-surface protein KAI1 suppresses metastasis in animal models. Downregulation of KAI1 has been implicated in the progression of cancer. This study investigated the expression and regulation of KAI1 in human primary breast cancers and distant metastases from breast cancer.

**Methods:** KAI1 expression was analyzed on tissue microarrays comprising primary breast tumors (n=209) and distant metastases (n=92). Human breast cancer cell lines were employed to study the regulation of KAI1 expression.

**Results:** KAI1 immunoreactivity was lost in 77% of primary breast cancers. Strikingly, KAI1 was preferentially lost in estrogen receptor (ER) positive cases (p<0.001). This was validated by real time RT PCR analyses showing a 75 fold downregulation of KAI1 mRNA in ER positive relative to ER negative tumors (p=0.028). Notably, this was also corroborated by independent Af-

fymetrix microarray expression data of an additional cohort of 49 breast cancers. In distant metastases from breast cancer, KAI1 was also preferentially lost in ER-positive cases but was retained in a large proportion of ER-negative cases ( $p=0.005$ ). Exposure of ER positive breast cancer cells to fulvestrant, an ER antagonist that reverses ER mediated gene repression, induced a significant upregulation of KAI1 and inhibited cell migration.

**Conclusions:** KAI1 is a target of ER mediated gene-repression and thus, it is down regulated in ER positive breast cancers. Importantly, KAI1 expression is frequently retained in primary as well as metastatic lesions of ER negative mammary carcinomas.

## Vorträge: Mammopathologie I

### Sa-089

#### Molecular grading and molecular subtyping by gene expression profiling

J.F. Reis-Filho  
London

### Sa-090

#### Genexpressionsanalysen zur Prädiktion und Prognoseabschätzung beim Mammacarcinom

A. Schneeweiss  
Heidelberg

### Sa-091

#### Predictive factors in neoadjuvant chemotherapy in breast cancer classical histology and new diagnostic tests

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**Aims:** The neoadjuvant chemotherapy in breast cancer leads to a complete response in 15–20% of cases. Several classical histological parameters for complete response have been described. In this study, we focused on the contribution of the immune system to chemotherapy response and the presence of a lymphocytic infiltrate in cancer tissue as a predictor of response to neoadjuvant chemotherapy.

**Methods:** We investigated lymphocytes in tumor tissue in a total of 1058 pre-therapeutic breast cancer core biopsies from two neoadjuvant anthracycline-taxane-based studies (GeparDuo,  $n=218$ , training-cohort (TC) and Gepar-Trio,  $n=840$ , validation-cohort (VC)). Molecular parameters of lymphocyte recruitment and activation were evaluated by kinetic PCR in formalin-fixed paraffin-embedded tumor samples.

**Results:** The percentage of intratumoral lymphocytes was a significant independent parameter for pathological complete response (pCR) in a multivariate regression analysis including all known predictive clinicopathological factors. Lymphocyte-predominant breast cancer (LPBC) responded with a pCR rate of 40–42%. In contrast, those tumors without any infiltrating lymphocytes had a pCR rate of only 3–7%. The expression of inflammatory marker genes and proteins was linked to the histopathological infiltrate and a significant association of the T-cell-related markers CD3D and CXCL9 with pCR was observed. Based on these markers a new approach for molecular prediction has been developed, that could be used as a new diagnostic test. This diagnostic test is currently evaluated prospectively in the PREDICT study in a cohort of 300 patients.

**Conclusions:** The presence of tumor-associated lymphocytes in breast cancer is a new independent predictor of response chemotherapy. This is the basis for molecular approaches to identify a subgroup of patients with a high benefit from neoadjuvant chemotherapy.

### Sa-092

#### Possible subtypes of triple negative breast carcinomas defined by gene expression signatures of receptor tyrosine kinases

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**Aims:** In the present study we investigated gene expression levels of different receptor tyrosine kinases (RTK) and associated biomarkers in triple-negative breast carcinomas with the aim to define heterogeneous expression patterns and possible carcinoma subtypes.

**Methods:** Gene expression levels of 30 triple-negative breast cancer tumor samples (FFPET) were investigated by RT-PCR analysis. Based on a panel of 31 tumor relevant biomarkers (primarily of the IGF- and EGF-receptor family as well as associated biomarkers of the downstream-signaling) generation of a gene expression profile was performed. Pearson correlation based hierarchical clustering of tumor expression profiles was used for tumor subtyping.

**Results:** Triple-negative breast carcinomas can be divided into 2 subtypes by gene expression signatures. 19 out of the 31 biomarkers show significant differences between the two subtypes.

**Conclusions:** Gene expression signatures characterize two subtypes of triple-negative breast cancer by different expression of members of the IGF- and EGF-receptor family and associated biomarkers of the downstream-signaling. This definition of subtypes by signaling heterogeneity could be relevant for the development of targeted therapies.

### Sa-093

#### Evaluation of prognostic and predictive value of standardized immunohistochemical analysis of breast cancer as a surrogate for identification of molecular cancer subtypes

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**Aims:** In order to be not dependent on molecular identification of breast cancer subtypes as an indicator for overall prognosis and prediction of response to neo-adjuvant chemotherapy, the aim of this study was to determine the validity of a standardized immunohistochemical analysis as a diagnostic surrogate.

**Methods:** A retrospective single-center study was performed with breast cancer tissue of 3179 patients, sampled between 1995 and 2008. Cancers with ER+/low Ki-67 phenotype were assigned to the molecular subtype Luminal A, ER+/high Ki-67 to Luminal B, Her2+ cases to the Her2 subtype and triple negative cases to the basal subtype. Of these, 587 patients received neo-adjuvant chemotherapy.

**Results:** 41% of all cancers corresponded to the Luminal A subtype, 30% to Luminal B, 15% to the Her2 subtype and 13% to the Basal subtype, with the highest 10-year-survival in Luminal A types (77%), followed by Luminal B (70.3%) and Her2 (67%) and basal subtype (67%). The basal and Her2+ subtypes had significantly higher rates of complete remission after neo-adjuvant chemotherapy (46% and 36% respectively) compared to the Luminal subtypes (3% for Luminal A and 8% for Luminal B).

**Conclusions:** Immunohistochemical analysis to determine subtypes of breast cancer with different prognosis and different response rates to neo-adjuvant chemotherapy is a valid surrogate for molecular classification. Her2+ and basal subtypes had significant higher response rates to neo-adjuvant chemotherapy compared to Luminal A and Luminal B subtypes. However, this does not equal a better prognosis, as Luminal A and Luminal B subtypes showed longer overall survival.

## TNM 2010: Was ist neu?

### Sa-094

Ch. Wittekind  
Leipzig

## Vorträge: Mammapathologie II

### Sa-095

**Flache epitheliale Atypie: Morphologie und molekulare Pathologie**  
F. Moïnfar  
Graz

### Sa-096

**Micrometastasis for prognostication in breast cancer – robust and solid or rubbish**  
P.J. Van Diest  
Utrecht

### Sa-097

**Reciprocal induction of hypoxia-inducible factor 1 and 2 mediates a dynamic hypoxia response in breast cancer**  
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**Background:** Hypoxia-inducible factors (HIFs) mediate the adaptation of hypoxic cells. Prolyl-4-hydroxylase domain (PHD) oxygen sensors modify HIF-1 $\alpha$  subunits, leading to their rapid proteasomal destruction. Interestingly, prolonged hypoxia leads to a specific downregulation of the HIF-1 $\alpha$  isoform in a variety of human cancer cell lines, while HIF-2 $\alpha$  levels increase with chronic hypoxia. We aimed to analyse HIF-1/2- $\alpha$ , to screen HIF target genes and to verify selected target genes in a medium sized cohort of clinically characterized breast cancer cases (n=346).

**Methods:** HIF/PHD isoforms were stably downregulated by short hairpin RNA interference in MCF-7 breast carcinoma cells. Temporal gene expression patterns of 40 known HIF target genes were analyzed for HIF-1 and HIF-2 dependency by quantitative PCR in cells exposed to 1% oxygen for 4–72 hours. Four HIF2 target genes were validated by immunohistochemistry in concert with HIF-1/2- $\alpha$ .

**Results:** While the induction kinetics of genes involved in metabolic adaptation to hypoxia was mainly HIF-1 $\alpha$  dependent, only few genes were selectively activated by HIF-2. These included transmembrane proteins and secreted factors, suggesting a role of HIF-2 in regulating intercellular communication. Also, expression of HIF2 and its target genes were associated with longer patient survival times.

**Conclusions:** Breast cancer progression might be dampened by a high HIF-2/HIF-1 ratio, possibly involving auto- and paracrine action of HIF-2 dependent growth factor secretion.

### Sa-098

**Identification of self-renewal pathways in breast cancer stem cells**  
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<sup>3</sup>Frauenklinik, Universitätsklinikum Tübingen

**Aims:** Cancer stem cells (CSC) or cancer initiating cells (CIC) are rare tumor cells capable of initiation and propagation of the malignant disease. Because CSC display specific stem cell features they can be isolated from the main tumor. In this study, embryonic molecular pathways in primary breast

carcinomas are characterized by an integrated and interdisciplinary approach.

**Methods:** Fresh tissue was obtained from breast carcinoma resection specimens, submitted to primary culture and induced to form mammospheres, a characteristic feature of breast cancer stem cells. ALDH1, Sox2, Nanog, Oct4 and Notch1 gene expression was analyzed on mRNA-level. In addition protein expression was detected by immunohistochemistry in paraffin embedded specimens of the same cases and of an independent group of consecutive cases.

**Results:** We identified the expression of embryonic factors in fresh and paraffin embedded breast tumor specimens. Immunohistochemistry showed a variable expression within different regions of individual tumors. Parallel analysis of the markers reveals, that combinations of factors can be expressed separately and alternating.

**Conclusion:** Embryonic factors are expressed in breast carcinoma samples in variable patterns. This finding supports the hypothesis, that different embryonic molecular pathways can alternatively be activated in breast cancer. Future efforts will concentrate on using this model to understand the molecular regulation and function of embryonic factors in breast CSC, as well as on evaluation of their role as prognostic markers.

### Sa-099

**ITIH5 in human breast cancer: functional characterization of a novel tumor marker**

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**Aims:** In recent years, several novel putative tumor markers have been described but their usefulness has not been further validated. ITIH5, a novel member of the ITI gene family and predictive marker in human breast cancer, is lost during breast cancer progression. ITIH5 loss correlates significantly with clinical parameters of metastasis and a prognostic value indicating poor clinical outcome. In order to support its putative relevance as a breast tumour biomarker, we aimed to further analyze its biological function in human breast cancer using an in vitro model.

**Methods:** We have created a model of human breast cancer by stably transfecting a full length ITIH5 cDNA in the highly metastatic MDA-MB-231 breast cancer cell line mirroring basal-type breast cancer. Subsequently, we performed a series of in vitro experiments including migration and invasion assays as well as morphological analysis followed by in vitro differentiation studies.

**Results:** As a result of ITIH5 expression we observed a remarkable decrease of invasive capabilities in vitro. Consequently, ITIH5-transfected tumour cells exhibited a significantly enhanced cell-matrix adhesion, a notable reduction in cell growth and, moreover, a significant retardation of in vitro motility and invasiveness when compared with empty vector control cells. Matching these findings, we observed a switch from a mesenchymal to an epithelial phenotype correlating with the re-expression of the characteristic epithelial markers ZO-1, Desmoplakin-1 and Claudin-1.

**Conclusions:** Our data indicate that loss of ITIH5 is implicated in epithelial-mesenchymal transition (EMT), a process intimately correlated with tumour invasion and metastasis, thereby supporting the putative role of ITIH5 as metastasis repressor gene in human breast cancer. Moreover, our in vitro data support the potential use of ITIH5 as a predictive tumour marker even though this demands further studies. Based on this premise, we intend to determine ITIH5's prospective usefulness as a diagnostic and prognostic tumour marker analysing a wide range of clinical samples in the near future.

## Vorträge: Aktuelle Methoden der Molekularpathologie II

### Sa-100

#### Mikrosatelliten-Instabilität – ein neuer prädiktiver Marker

W. Dietmaier  
Regensburg

### Sa-101

#### DNA-Methylierung – von der Grundlagenforschung zur Routine-diagnostik

U. Lehmann  
Hannover

### Sa-102

#### DNA-Sequenzierung in der molekularen pathologischen Diagnostik – potential, pitfalls and the future

R. Penzel  
Heidelberg

### Sa-103

#### mRNA-Quantifizierung – die nächste Herausforderung in der Routinediagnostik

A. Jung  
München

## Vorträge: Aktuelle Habilitationen

### Sa-104

#### Prognostic evaluation of molecular genetic parameters in gastrointestinal stromal tumors (GISTs)

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Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors of the gastrointestinal tract. Prognostication is currently based on the clinico-pathological parameters anatomical localisation, size and mitotic count, whereas molecular genetic parameters will provide additional information with prognostic impact. A series of 120 primary GISTs with long-term follow-up was analysed for prognostic molecular genetic parameters using mRNA expression analysis, western blot analysis, reverse phase protein arrays, tissue microarrays and CGH. Accordingly, upregulation of KIT/PDGFR $\alpha$  signal transduction and deregulation of cell cycle control were identified to be early and late events in tumor progression, respectively. As an example for early tumor progression, KIT exon 11 deletions lead to stronger activation of intracellular signalling cascades, emerging to higher cyclin D1 expression. In contrast, loss of p16INK4A expression and upregulation of E2F1 are late events in tumor progression, and were highly correlated with tumor progression and metastasis. Comparing a broad spectrum of GISTs including small GIST tumorlets (<1 cm in size) to biphasic GISTs with a highly malignant phenotype, a concerted model for tumor progression was developed. This model will help to improve prognostication in GISTs.

### Sa-105

#### Migration of allogeneic T lymphocytes after hematopoietic stem cell transplantation

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**Aims:** Acute graft-versus-host disease (GVHD) remains a major complication of allogeneic hematopoietic stem cell transplantation. To understand behav-

our of donor lymphocytes in vivo extensive migration analyses have been performed. Translational therapeutic aspects were pursued using various knockout models and antibody homing-blockades combined with splenectomy.

**Methods:** Allogeneic bone marrow transplantation was performed after myeloablative pre-conditioning (800 cGy) using a transgenic luciferase expressing (luc+) donor mouse (FVB). Wildtype recipient mice (BALB/c) as well as Peyer's patch (PP) deficient mice and Lymphotoxin alpha -/- mice (B6 back-ground) were transplanted with 4x10<sup>6</sup> luc+ splenocytes. To block lymphoid homing anti CD62L and anti MADCAM-1 antibodies were utilized. T cell migration was analyzed by in vivo Bioluminescence imaging (BLI), flow-cytometry and immunofluorescence.

**Results:** In vivo BLI and IFM displayed a very defined distinction between initiation phase (day 0-3) and effector phase (>day 4) of acute GVHD. PP and LN deficient mice (LTalpha-/-) showed, that the spleen alone was sufficient to cause severe aGVHD with a time course similar to that of wildtype mice. Strikingly, treatment of splenectomized recipients with blocking antibodies against the lymphoid homing receptors L-selectin and MADCAM-1 prevented GVHD with 100% survival (>120 d, p<0.0001).

**Conclusions:** Multiple priming sites are involved in GVHD initiation, the spleen compensating for the lack of PP and mesenteric LN, and vice versa. Homing blockade combined with splenectomy prevented acute GVHD long-term and offers potential for future therapeutic strategies.

### Sa-106

#### Molecular mechanisms of tumor progression in human hepatocarcinogenesis

K. Breuhahn  
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**Aims:** Hepatocellular carcinoma (HCC) is already the fifth most prevalent human malignancy worldwide and shows increasing incidence. Because the current therapeutic options for HCC patients are sobering, there is a great need to analyze molecular oncogenic mechanisms in order to determine novel targets for specific systemic therapy.

**Methods:** Genome-wide expression profiling and comparative genomic hybridization of human HCCs were performed to identify dysregulated genes and cellular pathways. Expression of potential target genes was analyzed in normal livers and HCCs. The functional relevance of these genes was determined in vitro and partly in vivo.

**Results:** Activation of the insulin-like growth factor (IGF)-II pathway as well as overexpression of the microtubule-destabilizing factor stathmin and the transcription factor far upstream-element (FUSE)-binding protein (FBP-1) were frequently observed in HCCs. Surprisingly, FBP-1 is a transcriptional activator of stathmin expression. All identified pathways and factors support tumor growth and tumor cell dissemination and therefore represent promising therapeutic target structures. In case of IGF-II/IGF-1R-signalling, administration of the tyrosine kinase inhibitor picropodophyllin (PPP), significantly reduced IGF-II-dependent proliferation, anti-apoptosis, and migration. In addition, PPP diminished IGF-II-driven tumor growth in a xenograft transplantation model.

**Conclusions:** Based on comprehensive screening approaches in part novel therapeutic structures in human hepatocarcinogenesis have been identified. Especially, the IGF-1 receptor is currently the target of several investigational agents in clinical and pre-clinical development.

### Sa-107

#### About the significance of angiogenesis in cartilage tumors and the influence of interleukin-1 $\beta$ and hypoxia on angiogenic signaling in a cell culture model

Thomas Kalinski  
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**Aims:** Angiogenesis is an important factor in neoplastic tissue. In contrast to other tumors, cartilage tumors obviously consist of a special angiogenic phenotype. The aim was to elucidate angiogenesis in cartilage tumors morpho-

logically, and to investigate angiogenic signaling, and its factors of influence in a cell culture model.

**Methods:** The angiogenic phenotypes of enchondromas conventional chondrosarcomas, and dedifferentiated chondrosarcomas were specified, and the expression of angiogenic molecules was analyzed morphologically. Furthermore, the influence of interleukin (IL)-1 $\beta$  and hypoxia on angiogenic signaling in chondrosarcoma cells was investigated using immunohistochemistry, (quantitative) RT-PCR and Western Blot.

**Results:** Cartilage tumors exhibit a heterogeneous, but predominantly mature angiogenic phenotype with differential proliferative activity, which is positively correlated with differential VEGF-A expression. Angiogenic signaling in chondrosarcoma cells is influenced by hypoxia, and especially by IL-1 $\beta$ . IL-1 $\beta$  induced angiogenic signaling can be sufficiently blocked at the IL-1 receptor by Curcumin in chondrosarcoma cells.

**Conclusions:** In contrast to normal cartilage, which is avascular, angiogenesis is characteristic of cartilage tumors. Angiogenic signaling can be influenced, which may provide another therapeutic option, even in this robust tumor model.

## Vorträge: Molekulare Tumorpathologie: Urothel/Niere

### Sa-108

#### Stand des Wissens zur molekularen Pathologie des Urothelcarcinoms

A. Knüchel-Clarke  
Aachen

### Sa-109

#### Stand des Wissens zur molekularen Pathologie des Nierenzellcarcinoms

H. Moch  
Zürich

### Sa-110

#### Novel DNA methylation biomarkers for early detection and prediction of progression in bladder cancer

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**Background:** Though the genetic changes occurring in the development of bladder cancer have been intensely studied in recent years, knowledge of epigenetic modifications, especially DNA methylation changes, is still limited. Thus, the aim of the current project is the identification and molecular characterization of novel DNA methylation markers which could be of potential use in early detection as well as treatment stratification in bladder cancer patients.

**Methods:** cDNA array based expression profiling identified 24 candidate genes showing significant downregulation in different types of bladder cancer and harbouring a CpG island in their promoter region. Methylation frequencies of these genes in normal bladder cell lines, cancer tissues, and urine samples are being analyzed using methylation-specific PCR (MSP) and pyrosequencing. In order to further prove epigenetic silencing, in vitro demethylation is carried out by aza-cytidine/trichostatin A treatment. DNA methylation based multi-marker panels will be optimised using receiver operator characteristics (ROC) curve analysis. Such panels will be applied to collected urine samples in order to assess their viability in predicting disease course and for diagnostic reliability. Furthermore, selected candidate genes will be functionally analyzed in cell line models to determine their effect on tumor genesis and growth.

**Results:** Both MSP and pyrosequencing analysis revealed that eight of the 24 candidate genes exhibited aberrant promoter methylation in bladder cancer cell lines (n=5), in clear association with loss of mRNA expression, the latter being restored in vitro by demethylation. In human bladder cancer, two can-

didate genes showed frequent methylation in non-invasive (n=30) and invasive bladder carcinomas (n=30) compared with a set of normal urothelial samples (n=15). Interestingly, we characterised candidate genes preferentially exhibiting promoter DNA methylation in progressive disease, i.e. invasive bladder cancer, whereas non-invasive tumours presented unmethylated or weakly methylated promoters, indicating a putative tumour suppressive function of these genes.

**Conclusions:** This project started just 15 months ago but has already defined several interesting novel genes that are epigenetically silenced in various types of bladder cancer. Subsequent evaluation of these genes as DNA methylation markers, as well as their functional analysis in vitro should reveal signalling pathways in bladder cancer affected by aberrant DNA methylation.

### Sa-111

#### EMMPRIN (CD147): a potential new prognostic marker for tumor progression in bladder cancer

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<sup>3</sup>Institute of Pathology, University Hospital Erlangen

**Aims:** The objective of this study is to characterize the value of EMMPRIN, a transmembrane protein that has been associated with tumor progression and metastasis, as a prognostic marker in bladder cancer patients and its functional relevance in regulating tumor growth and cell migration.

**Methods:** EMMPRIN expression was analyzed on two tissue microarrays (TMA) with superficial and muscle invasive urothelial bladder cancer specimens from a total of 516 patients. For functional analysis cell proliferation, metabolism, apoptosis, migration and tumor growth in vivo was characterized dependent on EMMPRIN expression.

**Results:** EMMPRIN expression correlates with tumor stage and tumor differentiation. In patients with locally advanced bladder cancer it is an independent negative prognostic factor for overall survival. Silencing of EMMPRIN expression resulted in reduced cell proliferation (15–20%) and migration (40%). In vivo, tumor growth was significantly reduced by 50% in cells lacking EMMPRIN expression.

**Conclusions:** The results suggest the use of EMMPRIN as a prognostic molecular marker for locally advanced bladder cancer patients, irrespectively of the mode of adjuvant chemotherapy. EMMPRIN expression influences tumor progression and growth in bladder cancer cell lines and might represent a new target molecule in bladder cancer therapy.

### Sa-112

#### Loss of inducible nitric oxide synthase (iNOS) expression in the mouse renal cell carcinoma cell line RENCA is mediated by microRNA miR-146a

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**Aims:** Production of nitric oxide (NO) in low or high concentrations by the inducible nitric oxide synthase (iNOS) expressed in tumor-associated macrophages (TAMs) or in tumor cells leads to either angiogenesis or cell death, respectively. In this study we wanted to investigate mechanism regulating the protein expression of iNOS, the production of NO and the effects on tumor growth.

**Methods:** Determination of nitrites with Griess reaction, Western blots analyses, Quantitative real-time PCR analyses, In vivo mouse model, Immunohistochemistry, Reverse Transfection and Inhibition of miR-146a.

**Results:** We show that the mouse tumorigenic renal cell carcinoma RENCA cell line has completely lost its ability to express iNOS protein upon stimulation in vitro, but expressed high levels of iNOS mRNA. This was mediated by the microRNA molecule miR-146a, as its inhibition restored iNOS expression and NO production ( $4.8 \pm 0.4$  vs.  $0.3 \pm 0.1 \mu\text{M}$  in uninhibited RENCA cells,  $p < 0.001$ ). In vivo, RENCA tumor cells did not express iNOS, while TAMs exhibited high iNOS expression but reduced NO production. Restoring iNOS protein expression in RENCA cells using miR-146a inhibitor increased macrophage-induced cell death by 73% ( $p < 0.01$ ) in vitro, and almost completely prevented tumor growth in vivo. Injections of high NO-producing M1-activated macrophages or high concentrations of the NO-donor NOC-18 reduced tumor growth rate (cytostatic effect).

**Conclusions:** These results suggest that macrophages alone are ineffective in killing tumor cells, and production of NO by the tumor cells, in addition to macrophages, is required to confer their death. Thus, tumor cells may use miR-146a to reduce endogenous NO production and escape macrophage-induced death, suggesting that inhibition of miR-146a may render tumor cells susceptible to adoptive transfer of M1-activated macrophages.

### Sa-113

#### Molecular analyses of multilocular cystic renal cell carcinoma (MCRCC)

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**Aims:** MCRCC is characterized by multiple, non-communicating cysts separated by septa that contain small aggregates of low-grade clear cells. Patients diagnosed with this tumor have an excellent outcome. Nevertheless, the molecular background of this RCC is not yet characterized. In this study, molecular mechanisms that promote cyst formation and suppress tumor progression were investigated in 12 MCRCC.

**Methods:** VHL gene mutation analysis was performed using a whole genome amplification approach. The expression status of the known factors for cyst formation p-GSK3 $\alpha$  and PAX2, the tumor suppressors PTEN and p27, as well as the cCRCC marker CD10 were immunohistochemically assessed.

**Results:** VHL gene mutations were identified in all 8 tumors that were analyzable. p-GSK3 $\alpha$  and PTEN were not or only weakly expressed, whereas a strong positivity of PAX2 and p27 was observed in the majority of the tumors. CD10 was present in only one-third of the MCRCC.

**Conclusions:** Combined inactivation of VHL, PTEN and GSK3 $\alpha$  as well as PAX2 re-activation appear to be critical steps that contribute to cyst formation in MCRCC. The strong nuclear and cytoplasmic expression of cyclin-dependent kinase inhibitor p27 indicates its suppressive role for tumor progression in this tumor type.

## Vorträge: Molekulare Tumorpathologie II

### Sa-114

#### Molekularpathologie der Sarkome: Stand des Wissens

R. Büttner

Bonn

### Sa-115

#### Adoptive immunresponse to Rhabdomyosarcoma

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**Aims:** Rhabdomyosarcoma (RMS) are the most common soft tissue sarcoma of childhood and adolescence. Recent efforts to enhance overall survival of RMS patients have failed (Dontonello et al.: J Clin Oncol 2009 Mar 20,

27(9):1446–1455). To improve eradication of RMS we applied adoptive transfer of chimeric T-cells with specificity for the gamma subunit of the fetal acetylcholine receptor (AChR), a cell surface antigen specifically expressed by RMS.

**Methods:** We generated an improved chimeric receptor by adding a CD28 domain to our previous chimeric receptor composed of a human anti-fAChR antibody, an Fc hinge region, a human T-cell receptor zeta chain. After transduction of PBMCs with the immunoreceptor we use FACS analysis to determine the number of transduced cells. We find that those chimeric T-cells are able to interact with RMS cells and use MTT tests to determine cell survival after cocultivation of RMS and chimeric T cells. To examine the immunoreceptor activity we use an IFN- $\gamma$  elisa. Simultaneously we check RMS for different surface molecules by FACS and qRT-PCR analysis.

**Results:** In spite of optimized strategies to generate chimeric T-cells, killing efficiency of RMS by chimeric T cells is unusual low compared to killing of lymphoma or CEA-expressing adenocarcinoma cell lines by respective chimeric T-cells. Considering that RMS are generally „ignored“ by the immune system of affected patients, we wondered whether resistance to killing might be due to a lack of co-stimulatory, presence of immunosuppressive surface molecules on RMS cells or expression of anti-apoptotic features. By testing the expression of different surface molecules (including MHC class I, CD80, CD86, ICAM-1/CD54, CD200, PD1/PD-H1, HLA-G) in nine RMS cell lines, we find that RMS-cell lines exhibit low to absent expression of crucial co-receptors (e.g. ICAM1 and CD86) and increased expression of immunosuppressive proteins such as HLA-G.

**Conclusions:** Besides immunosuppressive surface molecules we assume, that inhibitors of apoptosis (like cIAP1, cIAP2) play a crucial role in resistance of RMS cells against the immunosystem. Our findings suggest that induced expression of fetal AChR (i.e. the target of chimeric T-cells) and inhibition of anti-apoptotic pathways will be necessary to improve the treatment of RMS by conventional as well as immunotherapeutic strategies. Alternative strategies may comprise pharmacological blockade of „survival cascades“ (e.g. of the NF $\kappa$ B or PI3 K/AKT pathway) and interruption of (eventual) death receptor/ligand interactions (e.g. with neutralizing anti-FASL antibodies). Such experiments are underway.

### Sa-116

#### The R248C FGFR3 hotspot mutation affects cell growth, apoptosis and attachment of human HaCaT keratinocytes

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**Aims:** FGFR3 mutations have recently been identified in several benign epidermal skin tumors. The functional consequences of these mutations in human skin are yet unknown. In this study we analyzed the functional effects of the most common FGFR3 mutation in skin, the R248C FGFR3 hotspot mutation, in human HaCaT keratinocytes.

**Methods:** The cells were stably transduced with either the R248C or wildtype FGFR3 IIIb sequence using a retroviral vector system. The functional consequences of the R248C mutation were characterized with regard to cell growth, apoptosis and migration.

**Results:** FGFR3 mutant and wildtype cells showed similar growth rates. However, at confluence FGFR3 mutant keratinocytes revealed a significant higher cell growth than wildtype cells. FGFR3 mutant cells showed a significantly decreased apoptotic rate and a significantly decreased attachment to fibronectin. The mutant HaCaT keratinocytes did not show a significantly different migration capacity. In addition, an enhanced phosphorylation of ERK1/2 and PLC-gamma in confluent R248C mutant HaCaT was found.

**Conclusions:** Our results suggest that an increased cell growth at density along with a decreased apoptosis rate may contribute to the development of acanthotic tumors in FGFR3 mutant skin in vivo.

### Sa-117

#### Podoplanin positivity in HPV-associated carcinomas of tonsils and base of tongue: relevance of a new hypothetical concept of collective tumour cell invasion?

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**Aims:** Podoplanin-associated collective tumour cell invasion has recently been postulated especially in squamous cell carcinomas as an alternative to single cell invasion with epithelial-mesenchymal transition (EMT). This concept might be relevant in HPV-associated oral carcinomas without signs of EMT but with plump invasion and early lymphatic metastasis.

**Methods:** 16 cases of (often very small) HPV-associated carcinomas of the tonsils and base of tongue (proven by positive p16 and molecular studies) with cervical lymph node metastases, and 20 controls of tumor-free tonsils were studied by immunohistochemistry for podoplanin and b-catenin.

**Results:** 12/16 carcinomas showed at least moderate podoplanin staining with predominance at the invasion front, while all cases showed preserved strong membranous staining for b-catenin. In the basal cell layer of non-neoplastic cryptal epithelium staining for podoplanin was constantly positive, being negative in regular squamous epithelium.

**Conclusions:** Most HPV-associated carcinomas of the tonsils and base of tongue display podoplanin positivity and plump tissue infiltration, thus representing characteristic features of a newly postulated concept of podoplanin-associated collective tumour cell invasion. Further studies are needed to substantiate the hypothetical correlation to podoplanin-positive cryptal epithelium and to investigate a possible association to early lymphatic metastasis, typical for these carcinomas.

### Sa-118

#### VEGFA amplification in different neoplastic entities: tissue microarray analysis on 2292 tissue samples

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**Aims:** Angiogenesis plays an important role in progression of several tumor types. Evidence from preclinical and clinical studies indicates that vascular endothelial growth factor (VEGFA) is the predominant angiogenic factor. The aim of this study was a systematic investigation of VEGFA amplification in a large survey of solid human tumors in tissue microarray format.

**Methods:** FISH analysis of the VEGFA gene was performed in a multi tumor array (n=2292) including 132 different tumor categories and 31 normal tissue types. Additionally VEGFA gene amplification was evaluated in a further large series of sporadic CRC resections (n=1280) and the obtained data were compared to relevant clinico-pathological features.

**Results:** VEGFA amplification was detected in carcinoma of colon (n=39; 3%), gall bladder (n=5; 13.2%), pancreas (n=3; 6.5%), prostate (n=6; 15.8%), stomach (n=6; 14.3%), testis seminoma (n=4; 8.5%) and colon adenoma (n=7; 9.2%). VEGFA amplification in CRC significantly correlated with higher T stage and higher tumor grade, presence of vascular invasion, right sided location and with worse survival in univariate and multivariable analysis.

**Conclusions:** Albeit rare, VEGFA amplification can be detected in several different tumor entities. In CRC it highlights a small subset of CRCs with aggressive phenotype. Additional studies are needed to evaluate its significance in other neoplastic entities.

### Sa-119

#### Spectrum of Phosphatidylinositol-3-Kinase pathway gene alterations in GIST

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**Aims:** The Phosphatidylinositol-3-kinase (PI3 K) signaling pathway plays a crucial role in proliferation and survival. To date nearly nothing is known about the frequency and distribution of PI3K-pathway alterations in GIST.

**Methods:** DNA was prepared from 72 primary formalin-fixed paraffin-embedded GIST tissues. Mutations in the Kras gene were determined by pyrosequencing whereas mutations in Nras, BRAF, PI3 K and FGFR3 genes were analyzed by PCR-sequencing technique. The c-kit and PDGFR mutation status was available.

**Results:** BRAF mutations (V600E) were detected in 3 of 72 tumours (4.2%). All BRAF mutated GISTs did not show any c-kit or PDGFR mutation, had heterogeneous histology and were localized at different origins. There was only one case showing a PI3 K mutation (H1047L) and in addition a 15-bp deletion in exon 11. No mutations were detected in the Kras, Nras, and the FGFR3 genes. 61/72 GIST (84.7%) showed mutations in the c-kit or PDGFR gene: six in exon 9 (8.3%), 39 in exon 11 (54.2%), one in exon 13 (1.4%), none in exon 17, six in exon 12 (8.3%) and 9 in exon 18 (12.5%).

**Conclusions:** BRAF mutations play a role in KIT/PDGFR wild-type GIST that predominantly affect older females. The PI3 K pathway also seems to be relevant in GIST tumours, therefore it is important to assess these interactions in future studies.

## Vorträge: Molekulare Tumorpathologie: Endokrin/Varia

### Sa-120

#### Molekularpathologie der Schilddrüsentumoren

K.W. Schmid  
Essen

### Sa-121

#### New development in intracellular signalling in neuroendocrine tumors

H. Sasano  
Tohoku

(This presentation represents the exchange program between the Japanese Society of Pathology and the German Society of Pathology)

### Sa-122

#### Gene expression analysis identifies MAPK, AKT/mTOR, WNT/Beta-catenin, and Jak/STAT cascades as potentially driver pathways in insulin-induced liver carcinogenesis

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**Aims:** We developed a rat model of experimental insulin-induced hepatocarcinogenesis to investigate the molecular pathogenesis of human hepatocellular carcinoma (HCC) associated with diabetes mellitus and metabolic syndrome. In this model, pancreatic islets of donor rats are transplanted into the

liver of recipient diabetic rats, with resulting local hyperinsulinism that leads to the development of preneoplastic lesions and HCC.

**Methods:** Genome-wide microarray analysis was applied to kryo-preserved and laser-microdissected preneoplastic lesions and HCC.

**Results:** We identified gene signatures that accurately reflect the pathological progression of the liver disease at each stage. AKT/mTOR and MAPK pathways were progressively upregulated from preneoplastic lesions to HCC, whereas WNT/Beta-catenin and Jak/Stat cascades were induced at the neoplastic stage. Progressive induction of the MAPK cascade from preneoplastic lesions to HCC was due to upregulation of several upstream inducers of this cascade (EGFR, ERBB2, HSF1, DYRK1A) and downregulation of SPRY2 (an ERK inhibitor), implying the existence of a selective genetic pressure toward MAPK unconstrained activation in this rat model.

**Conclusions:** These findings provide a comprehensive molecular portrait of genomic changes in insulin-induced HCC, and might be useful to unravel the molecular mechanisms of HCC development in patients affected by diabetes mellitus and metabolic syndrome.

### Sa-123

#### **SIAH-1 promotes HCC cell proliferation by inducing the transcription factor FBP-3**

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**Aims:** We have recently demonstrated that overexpression of the transcription factors far upstream element (FUSE) binding protein (FBP)-1 and FBP-2 induces hepatocellular carcinoma (HCC) cell proliferation and cell motility. Here we aimed to analyze the expression, functional relevance, and regulation of the third FBP family member (FBP-3) in HCC tissues and HCC cells.

**Methods:** The expression of FBP-3 was analyzed at the transcript and protein levels in normal livers, premalignant lesions (dysplastic nodules) and HCCs. Functional consequences of reduced FBP-3 expression (RNAi) on proliferation and viability were analyzed in HCC cell lines. Correlative analyses were used to identify upstream regulators of FBP-3.

**Results:** A strong overexpression of FBP-3 was observed in 74% of all analyzed HCCs compared to healthy livers. Nuclear expression of FBP-3 significantly correlated with tumour dedifferentiation, tumor cell proliferation ( $r < 0.001$ ), and poor cumulative survival ( $p < 0.05$ ). In HCC cells, FBP-3 predominantly supported proliferation. The nuclear accumulation of the ubiquitin ligase seven in absentia homologue (SIAH)-1 correlated with FBP-3 expression in dedifferentiated HCCs ( $p < 0.01$ ). Indeed, the knock-down of nuclear SIAH-1 as well as treatment with proteasome inhibitors reduced FBP-3 transcript and protein levels. Inhibition of SIAH-1 not only diminished cell proliferation and migration, but also moderately induced tumor cell apoptosis.

**Conclusions:** Overexpression of pro-proliferative FBP-3 is frequently observed in human hepatocarcinogenesis. Indirect mechanisms mediate SIAH-1-dependent overexpression of FBP-3 in HCCs. SIAH-1 promotes tumor growth and tumor cell dissemination by at least partly employing FBP-3.

### Sa-124

#### **Identification of a distinctive multi-gene signature in cutaneous melanoma using qRT-PCR based microfluidic cards**

S. Nambiar, U.R. Hengge, S.T. Liffers, B. Verdoodt, M. Vogt, A. Tannapfel, A. Mirmohammadsadegh  
Institute of Pathology, Ruhr-University of Bochum

**Aims:** Oligonucleotide microarray-based comparison of gene expression profiles of a series of nevi, primary cutaneous melanomas and cutaneous melanoma metastases have yielded several differentially regulated genes that are potential determinants in melanomagenesis and progression. However, high-throughput qRT-PCR based validation and generation of multigene prediction models in melanoma have received limited attention. However, high-throughput qRT-PCR based validation and generation of multigene prediction models in melanoma have received limited attention.

**Methods:** A selection of 62 genes previously found to be differentially expressed in melanoma progression was used in a novel qRT-PCR platform called TaqMan low density array (LDA) or microfluidic card.

**Results:** 30 well-characterized clinical specimens (5 benign, 5 dysplastic nevi, 10 primary melanomas and 10 cutaneous melanoma metastases) were analyzed. Consequently, a 4-gene signature of ASK/Dbf4 and Tpr in combination with the established markers melanoma cell adhesion molecule (MCAM/MUC18) and hepatocyte growth factor receptor (c-MET) was generated that could distinguish benign and atypical nevi from malignant melanomas

**Conclusions:** Our study reports a qRT-PCR-validated novel 4-gene signature that differentiates nevi from melanoma and may complement histopathologic findings.

### Sa-125

#### **microRNA-29 regulates the expression of profibrogenic mediators**

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**Aims:** microRNAs (miRNAs) are short noncoding, endogenous RNAs that posttranscriptionally regulate gene expression. Recently, we have shown that miR-29a and miR-29b inhibit collagen expression. In the present study, we now investigated the role of miRNA-29 in profibrogenic growth factor expression as a central mechanism of fibrosis.

**Methods:** miR-29 expression was analysed by Real Time PCR in rat livers after four weeks bile-duct obstruction and in 84 human biopsies with chronic hepatitis C, representing different stages of fibrosis. Transcripts, putatively targeted by miR-29, were screened using algorithms of Miranda, Targetscan and Pictar databases and then analysed by reporter assays.

**Results:** miR-29, previously shown to repress collagen expression, was significantly decreased in 84 biopsies of patients with chronic hepatitis C and after experimental fibrosis of BDO treated rats. Screening of the various databases revealed that in addition to collagen 1A and 4A mRNA, the transcripts of PDGF receptor B, PDGF-B, PDGF-C, IGF-I and VEGF-A also contain putative miR-29 binding sites. Treatment of HSC with miR-29 resulted in inhibited reporter expression of PDGF-B, PDGF-C, IGF-I and VEGF-A 3'-UTR but not of the corresponding mutated sequences. Thus, we identified transcripts of PDGF-B, PDGF-C, IGF-I and VEGF-A as novel targets of miR-29.

**Conclusion:** Members of miR-29 family, downregulated during experimental and human fibrosis, are antifibrogenic mediators not only by targeting collagen biosynthesis but also by interfering with profibrogenic cell communication.

## Sitzung: AG Dermatopathologie

### So-001

#### **Cutaneous vasculitides – a clinicopathological approach 2009**

B. Zelger

Department of Dermatology & Venerology, Medical University Innsbruck

Vasculitis is defined as inflammatory cells in and around the vessel wall and vascular damage. Damage may be recognized by leukocytoclasia, endothelial and muscle cell necroses, fibrin and thrombi, or collagen and tissue degeneration. In contrast, coagulopathies are defined as vascular disorders with partial or complete occlusion of one or more vessels due to imbalanced ("hyperreactive") coagulation, in particular by thrombi and emboli. Coagulopathies are no primary vasculitis, but a frequent secondary finding in vasculitides, but also independent thereof, e.g. in wound healing. The present approach uses the skin and subcutis as a model for vasculitis and clinicopathological correlation as the basic process for classification. Thereby, we use an algorithmic approach with pattern analysis. One first differentiates between small and large vessel vasculitis. In a second step one differentiates the subtypes of small (capillaries or postcapillary venules) and large (arterioles/arteries or veins) vessels. In the final step one differentiates the

vascular process according to the predominant type of cells into leukocytoclastic, lymphocytic and/or granulomatous vasculitides. Thereby, leukocytes and their debris are most characteristic of authentic vasculitis, lymphocytes indicative of a regenerative process ("secondary" vasculitis) due to coagulopathies, and macrophages in granulomas typical of vasculitis with significant connective tissue damage. This presentation will due to time limitations focus on vasculitides and comment on coagulopathies only as much as it is essential for meaningful understanding.

#### So-002

##### Systemic involvement in cutaneous vasculitis – a pathological approach 2010

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Histological findings of vasculitis, vasculopathies and thrombosis are findings of different underlying diseases as primary vasculitis (immune-mediated and infectious), but also so-called secondary vasculitis as well as coagulopathies. The present approach gives a definition and overview of the most common forms of vasculitis including direct infection as well as immune-mediated types for example giant cell (temporal) arteriitis, polyarteriitis nodosa and Wegener granulomatosis. More in detail the morphology of systemic involvement in classical cutaneous vasculitis is discussed with an emphasis on the question: Can we use the algorithmic approach with pattern analysis used in skin and subcutis as a model for vasculitis elsewhere in the human body? Thereby, we use an analogous diagnostic algorithm to the skin differentiating first between small and large vessel vasculitis, second defining the vessel type and in a final step differentiating the vascular process according to the predominant type of cells into leukocytoclastic, lymphocytic and/or granulomatous vasculitides. The limitations of the histopathological diagnosis in different diseases as well as the importance of clinicopathological correlation are discussed.

#### So-003

##### Disseminated gonococemia – a molecular diagnosis?

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<sup>1</sup>Molekularpathologie Trier

<sup>2</sup>ZHZMD Trier

**Aims:** As we know Gonorrhea is a STD. Systemic distribution of gonococci is an uncommon event. In literature some cases with systemic disseminated disease in 1–3% of patients with gonorrhea have been described.

**Methods:** We analysed tissue specimens of dermal lesions of two male patients (67 and 45 years old) presenting with round, ill-defined pink macules with central pinpoint vesiculation of the upper and lower extremities. The cutaneous lesions were analyzed histologically and by molecular detection methods for infections with *Mycobacteria spec.*, *Leishmania spec.*, *Borrelia burgdorferi*, *Treponema pallidum* as well as *Neisseria gonorrhoeae*.

**Results:** Molecular analysis of the tissue samples of both patients using a STD-Array demonstrated an infection with *Neisseria gonorrhoeae* with the consecutive diagnosis of "disseminated gonococemia". Healing of the dermal lesions following specific antibiotic treatment indirectly confirmed our molecular diagnosis.

**Conclusions:** Regarding the diagnosis of disseminated gonococemia molecular analysis is an appropriate diagnostic method for rare cutaneous lesions which are suggestive for infectious disease.

#### So-004

##### KIT status in cutaneous lesions fails to predict cutaneous mastocytosis versus indolent systemic mastocytosis

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**Aims:** In adult mastocytosis in the skin (MIS) underlying (indolent) systemic mastocytosis (SM) is determined by evaluation of a bone marrow (BM) biopsy according to WHO-defined diagnostic criteria, i.e. compact mast cell (MC) infiltrates, atypical MC-morphology, CD25 expression in MC, detection of activating KIT codon 816 mutations, especially D816 V, and elevated serum tryptase. In the present study, we investigated whether KIT mutational status in skin lesions predicts BM involvement of SM.

**Methods:** Skin and BM biopsies of 24 adult patients with the clinical diagnosis of MIS were tested for the mutation KIT D816 V by LNA-mediated PCR-clamping followed by melting point analysis of amplification products. Enhanced sensitivity and specificity was assured by analysis of microdissected tryptase+ MC.

**Results:** Applying most sensitive molecular methods, BM biopsies of all 24 patients showed mutated KIT (n=24), even when/if the WHO diagnostic criteria for SM were not fulfilled (n=2). However, KIT D816 V was only detected in cutaneous biopsies in 15/22 patients with SM (68%).

**Conclusions:** In adult patients with cutaneous symptoms of mastocytosis BM biopsies are indispensable for the diagnosis or exclusion of SM. KIT codon 816 genotyping of MC obtained from skin biopsies does not predict BM involvement of SM.

#### So-005

##### Syringocystadenoma papilliferum clinically mimicking epidermal cyst: immunohistochemical characterization

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**Case Report:** A 67 year-old man presented with a nodule at his lower occiput clinically appearing as a bulging cystic tumor and thus reminiscent of an epidermal cyst. Histopathology revealed a well-circumscribed adenomatous dermal lesion showing bulbous projections and papillae that extended into a cup-shaped cavity connected with the epidermis. The epithelial lining revealed the presence of two cell rows, with decapitation secretion of the luminal cell row. The stroma showed a plasma cell-rich inflammatory infiltrate. Immunohistochemistry revealed diverse keratin phenotypes as well as tight junction proteins in tumor cells, and stromal cells were also characterized. The diagnosis of syringocystadenoma papilliferum was raised.

**Conclusions:** This tumor belongs to the group of benign adnexal neoplasms. It is often considered as a hamartoma and seen in association with a nevus sebaceus. Scalp and neck are the preferred localizations. The surface typically is verrucous and sometimes crusted. Syringocystadenoma papilliferum is a cystic proliferation of probably apocrine differentiation but histogenesis and biology of this tumor are not yet fully clear. The panel of antibodies used here also could not discriminate between apocrine and eccrine lineage. Clinically, this case was unusual because of the relatively old age of the patient, the lack of a verrucous surface which here was smooth but scaly, and the lack of associated alopecia.

**So-006****Influence of extracellular basal membrane proteins on human mesenchymal stem cells in a modified three-dimensional culture system of skin**

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**Aims:** Multipotent adult mesenchymal stem cells (MSC) are able to differentiate into several mesodermal cell types. MSC cultured at the air-liquid interface on dermal equivalents (DE) under conditions of skin did not differentiate across germinal boundaries into epidermal cells, but acquired a myofibroblastic phenotype (Schneider et al.: Differentiation, 2008). In this study, we analysed the influence of extracellular basal membrane proteins on potential epidermal differentiation and on the process of matrix remodelling during MSC differentiation.

**Methods:** DEs were produced as described in Schneider et al., 2008. Before seeding MSC from bone marrow (BM) or umbilical cord (UC) on the DEs, DEs were coated with collagen IV and laminin, respectively. Then, DEs were cultured for 28 days under culture conditions of skin at the air/liquid interface. We analysed the epidermal and mesodermal fate of MSC and the extracellular matrix remodelling by routine histology stains and immunohistochemistry.

**Results:** The partially stratified growth of the MSC under air exposed conditions indicates their adaptation to the organotypic culture conditions of skin. Our results imply that the basal membrane proteins inhibit MSC migration into the DEs and influence the growth of MSC as well as considerably the intensity of matrix remodelling.

**So-007****Detection and visualization of Merkel Cell Polyomavirus (MCPyV) in Merkel Cell carcinoma (MCC)**A. Schmidt, M. Baumann, A. Haesevoets<sup>1</sup>, J. Diebold<sup>2</sup>, P. Hofmann<sup>3</sup>, M. Kurrer<sup>4</sup>, N. Willi, F. Offner<sup>5</sup>, G. Sauter<sup>6</sup>, V. Zsikla, E.-J. Speel<sup>1</sup>, G. Cathomas

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**Aims:** The recently detected MCPyV can be detected by PCR in 75% to 100% of MCC. Aim of the study was the analysis of MCPyV in MCC using various methods.

**Methods:** Paraffin tissue of MCC were analyzed by PCR. Using a 5 kb MCPyV Probe, viral DNA was visualized by FISH using tyramide amplification and ApoTome microscopy. Immunohistochemistry (IHC) was performed with mab CM2B4 (provided by Y. Chang).

**Results:** A total of 44 (72.1%) of 61 MCC were positive for MCPyV by PCR. 34 (77.3%) of 44 PCR positive MCC were positive by MCPyV FISH and 28 (63.7%) by IHC. In contrast, none of the 17 PCR negative tumors were positive by FISH and in a single PCR negative tumor, a weak positivity was observed by IHC. By FISH, 16 (47.1%) PCR positive MCC revealed a single dot signal, in 8 tumors associated with an additional granular pattern, indicating a clonal integration of viral DNA. In contrast, in 18 (52.9%) PCR positive MCC, exclusively a granular FISH pattern of MCPyV was observed.

**Conclusions:** Using various techniques, our data confirms the association of MCC and MCPyV. FISH and IHC show a strong positive and negative concordance. In a subset of PCR positive tumors, no virus could be detected by FISH or IHC, suggesting persistent MCPyV infection unrelated to the tumor. Only about half of the MCPyV positive MCC show a dot like pattern by FISH, as expected for clonal viral integration, one half of FISH positive MCC showed exclusively a granular signal, indicating a non-clonal type of viral persistence. Our data indicate different types of MCPyV persistence, indicating different pathways of MCC development.

**So-008****Regulation and expression analysis of erythropoietin receptor in malignant melanoma**

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**Aims:** Recombinant human erythropoietin (Epo) is used to prevent and treat tumor-related anemia and improve quality of life in cancer patients. Recent evidence suggested that Epo may adversely affect the survival of selected cancer patients by promoting tumor growth, inhibition of apoptosis, and induction of migration. Epo unfolds its effect on the Epo receptor (EpoR). We investigated the expression of EpoR in malignant melanoma specimens and analyzed the effect of rHuEpo on proliferation and apoptosis of melanoma cells in vitro.

**Methods:** We show significantly increased EpoR expression in clinical melanoma metastases and primary melanomas in comparison with different sets of nevi by rt-qRT-PCR, immunohistochemistry, and western blot analysis.

**Results:** When assessing the functionality of the EpoR-signaling pathway, recombinant human Epo led to the phosphorylation of JAK-2, signal transducers and activators of transcription3 (STAT3), and ERK1/2 in several of the melanoma cell lines that were analyzed. Besides, Epo counteracted cisplatin-induced cell death in BLM and MV3 cells. Finally, Epo promoted cell migration of MV3 cells, whereas inhibition of the JAK/STAT and ERK1/2 pathways reduced Epo-mediated migration.

**Conclusions:** We show the overexpression of functional EpoR expression in about half of the analyzed clinical melanoma metastasis specimens and show anti-apoptotic as well as pro-migratory effects of Epo, which is of importance for the treatment of anemia in advanced melanoma.

**So-009****Atypical intradermal smooth muscle neoplasms: clinicopathologic analysis of 85 cases**S. Kraft<sup>1,2</sup>, C.D.M. Fletcher<sup>1</sup><sup>1</sup>Department of Pathology, Brigham & Women's Hospital, Boston, MA, USA<sup>2</sup>Harvard Dermatopathology Program, Boston, MA, USA

**Aims:** Atypical or mitotically active dermal smooth muscle neoplasms are commonly termed „cutaneous leiomyosarcomas“. However, preexisting – mostly small – series of these rare lesions suggest a low risk of aggressive behaviour. In this study, we undertook a detailed morphologic and immunohistochemical analysis of 85 cases and obtained clinical follow-up data.

**Methods:** The cases were retrieved from the authors' consult files and institutional files. H&E sections were examined, immunohistochemistry was performed, and clinical details were obtained from referring physicians.

**Results:** There was a male:female preponderance (4.3:1), with a mean age of 56 yr (range 6–82). Tumors measured 1.3 cm in average and were predominantly located on the trunk (32) and lower extremities (31). The others occurred on the upper extremities (17) or head & neck (4). Histologically, tumors were confined to the dermis or showed only superficial sub-cutaneous extension. The majority showed an infiltrative growth pattern with fascicles of atypical spindle cells ramifying between collagen fibers. Necrosis was seen in 10%. All cases showed cytologic atypia. Primary tumors showed a mean mitotic rate of 6/10 HPF. Recurrent tumors showed 14/10 HPF (and greater cytologic atypia). All tumors were positive for SMA; 98% expressed desmin, 90% caldesmon, 27% pan-keratin (scattered), and 1 focally S-100. Follow-up ranged from 5–156 months. No metastases or tumor-related deaths were observed. Local recurrences were observed in 18 cases. All recurrences had developed prior to consultation, after a mean of 43 months. No recurrences were observed after consultation (mean follow-up 32 months). 13 recurrent lesions showed positive margins in the primary excision and 3 showed margins <0.2 cm. Margin status was not available for 2 cases. The primary excisions of tumors which later recurred showed no increase in cellular atypia, necrosis, or mitotic rate when compared to those which did not recur, nor were there clinical differences.

**Conclusions:** These tumors show a male predilection and predominantly occur on the trunk and lower extremities. When confined to the dermis, they appear to carry no risk of metastasis, hence the designation „sarcoma“ is inappropriate. Margin status is the most important predictor of recurrence. Upon excision with clear margins, the risk of local recurrence is low. Hence, we propose the term „atypical intradermal smooth muscle neoplasm“.

#### So-010

##### **Methylthioadenosine phosphorylase is a predictive marker for response to interferon therapy in patients with malignant melanoma**

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**Aims:** To investigate whether expression of methylthioadenosine phosphorylase (MTAP), a recently suggested biomarker of malignant melanoma, is prognostic/ predictive for interferon responsiveness in melanoma patients.

**Methods:** Tissue microarrays assembling 465 nevi, primary melanomas and metastases were used.

**Results:** MTAP expression was significantly reduced in melanomas and metastases compared with nevi. In melanomas, loss of MTAP expression was significantly related to Clark level, tumor thickness and nodal status. Subgroup analysis of patients with tumor thickness of 1.5 to 4.0 mm revealed a significant survival benefit from interferon treatment regarding recurrence free survival in patients with MTAP-positive versus MTAP-negative melanomas. According to Cox analysis, MTAP was an independent prognostic marker.

**Conclusions:** MTAP represents a highly promising immunohistochemical marker for prognosis and interferon response of patients with malignant melanoma.

## Sitzung: AG Gynäko- und Mammopathologie

#### So-011

##### **Internet-based assessment of observer variability for endometrial intraepithelial eoplasia (EIN)**

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**Aims:** Endometrial Intraepithelial Neoplasia (EIN) is a diagnostic schema for premalignant endometrial lesions that has been developed by histopathologic correlation with clinical outcome, molecular changes, and objective computerized histomorphometry. Using an internet-based quiz we assessed observer variability in the diagnosis of EIN to better understand the diagnostic pitfalls.

**Methods:** An online quiz consisting of 18 cases of endometrial biopsies considered difficult was prepared. Each case contained clinical history and at least 3 microscopic images. Answer choices included: 1) EIN, 2) Polyp, 3) Benign endometrium (proliferative, secretory, disordered, tubal metaplasia, lower uterine segment) and 4) Adenocarcinoma.

**Results:** The online quiz was completed by 51 participants with percentage agreement with the authors ranging from 22 to 100% (mean 55%). The percentage agreement was highest with benign/polyp cases: tubal metaplasia (86%) polyp (86%), and secretory change (79%). Participants performed the worst with cases containing morular metaplasia (29%) and EIN arising in a polyp (52%). The percentage agreement for EIN without these features was 71%, and those participants who reviewed an online tutorial performed better than those who did not (60% vs 49%).

**Conclusions:** Reproducibility of EIN criteria is good and improved with training. A subset of biopsies with morular metaplasia and EIN in polyps are problematic and likely require consensus review.

#### So-012

##### **PPH3 expression in endometrial cancer is an independent predictor of patient outcome**

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**Aims:** In order to test the prognostic impact of Phospho-Histone H3 (PPH3) expression in endometrial cancer (EC) we analyzed the number of PPH3 positive tumor nuclei and correlated the results with clinico-pathological parameters including survival. PPH3 is a marker for proliferation detecting mitosis in the late G2 phase.

**Methods:** 104 formalin-fixed and paraffin embedded tissues of EC were tested for PPH3 expression by immunohisto-chemistry. Mitoses were counted in 10 high power fields (HPF). The significance of PPH3 as a prognostic factor was evaluated by univariate, multivariate and Kaplan-Meier survival analyses.

**Results:** The number of PPH3 positive cells significantly correlated with histological grading ( $p < 0,001$ ) and the T-stage ( $p < 0,027$ ). Less mitotic figures were detected in estrogen dependent EC (Type 1) compared to non-estrogen dependent Type 2 EC ( $p < 0,001$ ). Kaplan-Meier analysis revealed a significant correlation of PPH3 positive tumor cells and patient survival in combination with other parameters. Nodal negative EC with  $< 15$  PPH3 positive nuclei/10 HPF demonstrated a better survival than nodal negative EC with  $> 15$  PPH3 positive nuclei/10 HPF. Furthermore multivariate analysis identified PPH3 expression as an independent prognosticator of patient survival in EC patients.

**Conclusions:** The quantification of PPH3 positive mitotic figures in EC predicts patient outcome especially for nodal negative patients.

#### So-013

##### **Aberrant promoter methylation of the Synemin gene is associated with early tumour relapse of breast cancer patients**

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**Aims:** Synemin (SYNM) is a type IV intermediate filament (IF) that forms heteropolymeric filaments with major IF proteins, such as desmin and vimentin. SYNM links the extracellular matrix and the IF-network and is thereby involved in cytoskeletal assembly and cytodynamics of mammalian cells. SYNM has recently been associated with cancerous processes in a few tissues including liver and brain.

**Methods:** A dot blot array (including cDNAs from 50 matched normal and tumorous breast samples), ten human breast cancer cell lines and 36 human breast cancer specimens were analysed for SYNM expression using realtime PCR and immunohistochemistry. SYNM promoter methylation analysis in breast cancer specimens ( $n=195$ ) was done by methylation specific PCR and pyrosequencing. We further performed in vitro demethylation experiments in breast cell lines and tested for association between SYNM methylation and prognostic patients' characteristics.

**Results:** SYNM mRNA was downregulated in 86% of matched pairs of breast cancer. SYNM loss was confirmed in 31 non-matching breast cancer specimens ( $P < 0,0001$ ). SYNM protein was clearly expressed in normal myoepithelial cells, whereas it got completely lost in 57% of invasive breast cancer ( $P < 0,001$ ). SYNM promoter methylation was detectable in 27% of breast cancer specimens and in 20% of breast cancer cell lines. Aberrant SYNM promoter methylation was tightly associated with SYNM expression loss ( $P < 0,001$ ) and was re-inducible after in vitro demethylation of originally methylated tumour cell lines. Interestingly, SYNM promoter methylation was independently associated with unfavourable recurrence free patient survival,

higher risk for tumour relapse (hazard ratio: 2.86) and positive lymph node status as well as tumour de-differentiation.

**Conclusions:** We present the first study on the epigenetic regulation of the SYN1 gene in a cancer entity, i.e. breast cancer, proposing that SYN1 could be a novel tumour suppressor, which is epigenetically silenced in human breast cancer. SYN1 promoter methylation could be useful as new predictive biomarker to identify patients with poor clinical outcome.

#### So-014

##### Platelet derived growth factor-C is a therapy target and a novel independent prognostic marker of breast cancer

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**Background:** PDGF-C has been described in tumor associated fibroblasts, yet little is known about its expression in human breast cancer.

**Methods:** We have generated and characterized a monoclonal antibody (clone 6B3) against human PDGF-C which was used to analyze the expression patterns of PDGF-C in 890 human invasive breast cancer cases. To further explore the molecular mechanism of the clinical data, an orthotopic mouse model using the breast tumor cell line MDA-MB-231 was established.

**Results:** PDGF-C shows a diffuse cytoplasmic immuno-reactivity in epithelial and stromal cells including tumoral capillaries. A gradual increase of expression from normal tissue via in situ carcinomas to invasive cancer was noted. In invasive carcinomas epithelial PDGF-C correlates significantly and positively to tumor grade, CK5/6, Her2 and Ki-67 fraction. Negative significant correlations were noted for ER/PR and menopausal status. In a multivariate survival analysis, PDGF-C was demonstrated as an independent prognostic marker of overall patient survival. In the mouse model, treatment with clone 6B3 inhibited the tumor growth significantly in comparison to the controls and decreased the vessel density in the tumor mass.

**Conclusions:** PDGF-C might play an important role in tumor progression of breast cancer, particularly of basal phenotype. Targeting PDGF-C using monoclonal antibodies may offer a new therapy option for this tumor subgroup.

#### So-015

##### Overexpression of the Hedgehog (Hh) transcription factors Glioma-associated oncogene homolog (GLI) 1 and Forkhead Box (FOX) M1 is associated with progressive stages of breast tumours as well as unfavourable overall patient survival

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**Aims:** Aberrant Hh signaling has been found in a variety of human cancers. Interestingly a number of Hh inhibitors have already been described raising the options for new innovative cancer therapeutics. To broaden our knowledge about Hh involvement in human breast cancer we systematically analysed the expression of GLI1 and FOXM1 in a large number of breast tumours. Expression data were further correlated to histopathological and clinical characteristics of the tumours in order to clarify the prognostic relevance for this disease.

**Methods:** Applying real-time PCR and immunohistochemistry (IHC) we analysed GLI1 and FOXM1 expression in human invasive breast carcinomas in comparison to normal breast tissues. Messenger RNA expression was furthermore studied in a set of normal and malignant breast cell lines. IHC data were statistically interpreted using SPSS version 14.0.

**Results:** GLI1 and FOXM1 were found to be overexpressed in breast cancer cell lines as well as in breast cancer tissues. High levels of GLI1 protein significantly correlated with advanced tumour stage but also with high numbers of affected lymph nodes. Furthermore, we found a significant association be-

tween increased nuclear FOXM1 expression and high abundance of the HER2 receptor. More interestingly, overexpression of both molecules was associated with unfavourable overall patient survival.

**Conclusions:** Our study indicates that GLI1 and FOXM1 protein expression measured e.g. by an IHC based scoring system might have an implication in future multi-marker panels for human breast cancer prognosis or molecular subtyping. Furthermore, the positive significant association between FOXM1 overexpression and HER2 status of the analysed tumours may point to a potential role of FOXM1 as a new drug target in HER2 resistant breast tumours. We will analyse this in more detail in our upcoming studies.

#### So-016

##### Prognostic and predictive impact of expression of the angiogenic markers CD105 and CD31 expression in patients with high risk breast cancer (HRBC)

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**Purpose:** Several angiogenic factors have been reported to influence prognosis of breast cancer and spread to regional lymph nodes. CD105 (Endoglin) is expressed by human vascular endothelial cells and plays a major role in angiogenesis. Similarly, CD31 is a platelet endothelial cell adhesion molecule involved in normal and pathologic vessel function. In the prospective randomized WSG-AM-01 trial HRBC patients with more than 9 positive lymph nodes derived particular benefit from tandem high-dose (HD) rather than dose-dense (DD) chemotherapy with regard to both event-free (EFS) and overall survival (OS), particularly among tumors carrying a basal-like or HER2 molecular phenotype. The goal of the present study was to examine the interaction between angiogenic factors and efficacy of different dose intensification strategies in the WSG-AM-01 trial among both unselected patients as well as among distinct molecular disease subtypes.

**Methods:** Immunohistochemical staining for CD31 (n=128) and CD105 (n=130) was performed to assess vascular surface area (VSA) separately for both factors using the Chalkley count method. Expression of estrogen (ER) and progesterone receptor (PR), HER2, Ki67, and epidermal growth factor receptor (EGFR) was analyzed immunohistochemically using tissue microarrays, allowing for stratification into molecular breast cancer subclasses (k-clustering k=5 by expression of 24 proteins). Correlation analysis between CD31 and CD105 expression and molecular subtypes was carried out using Pearson correlations. Univariate survival estimates were determined by Kaplan-Meier analysis and tested for significance by log rank statistics. Multivariate survival modeling was performed by a generalized Cox model, with linear proportional hazards terms in the first block and time-varying interactions in the second block.

**Results:** Both VSA/CD 31 and VSA/CD105 showed significantly higher expression among HER-2 and basal-like breast cancer subtypes (p=0.007), as well as among cases with increased Ki67 (p=0.005). Increased VSA/CD105 (above a median of 1.295) was associated with significantly decreases in both EFS (p=0.01) and OS (p=0.02). In multivariate analysis (including therapy, tumor size, age and grade as well as expression of ER, PR, HER-2 and Ki-67) increased VSA/CD 105 was an independent prognostic factor for decreased

EFS (HR=1.68, p=0.05). Dose intensification showed significantly increased efficacy (for OS and EFS) only among patients with low VSA/CD105 tumors. **Discussion:** Expression of angiogenic markers may mirror/confer resistance to chemotherapy among patients with breast cancer, particularly in the context of dose intensified chemotherapy and may explain differences observed among distinct molecular breast cancer phenotypes. The correlation observed between angiogenic markers and distinct molecular subtypes may serve as a rationale for exploring antiangiogenic treatment options in patients carrying these tumors.

#### So-017

##### **Differential expression of miRNAs and its target mRNAs between DCIS and invasive ductal breast cancer**

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**Aims:** The potential identification of miRNAs and its target mRNAs which might serve as progression markers between DCIS- and invasive ductal breast cancers (IDC).

**Material and Methods:** Prior analyses on microdissected native breast cancer tissues identified 548 differentially transcribed genes. The gene set was optimized by biostatistical analyses to 9 possible marker genes for progression. Micro-dissected FFPE tissues of the same tumors was used for the validation by qRT-PCR and for miRNA array analyses. The latter results were again validated by qRT-PCR.

**Results:** The 9-gene progression set correctly classified DCIS and IDC components in 23/24 cases. Eight of the 9 possible marker genes investigated so far were correctly validated in FFPE tissues. In addition, 20 miRNAs differentially expressed between DCIS and IDC were identified, 4 of which had already been described in tumors. Interestingly, these 4 miRNAs have binding sites on 8 of the 9 possible marker genes.

**Conclusions:** A small gene set of potential progression markers obtained by microarray analysis of native breast carcinomas could successfully be validated on FFPE tissues. In addition, a set of targeting miRNAs was identified that might thus also serve as prognostic markers. Further functional analyses are still ongoing.

#### So-018

##### **Gene amplification in ductal carcinoma in situ of the breast**

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**Aims:** Multiple different biologically and clinically relevant genes are often amplified in invasive breast cancer, including HER2, ESR1, CCND1 and MYC. So far, little is known about their role in tumor progression. To investigate their significance for tumor invasion, we compared pure ductal carcinoma in situ (DCIS) and DCIS associated with invasive cancer with regard to the amplification of these genes.

**Methods:** Fluorescence in situ hybridization (FISH) was performed on a tissue microarray containing samples from 130 pure DCIS and 159 DCIS associated with invasive breast cancer. Of the latter patients, we analyzed the intraductal and invasive components separately. Lymph node metastases of 23 patients with invasive carcinoma were also analyzed.

**Results:** Amplification rates of pure DCIS and DCIS associated with invasive cancer did not differ significantly (pure DCIS vs. DCIS associated with invasive cancer: HER2 22.7% vs. 24.2%, ESR1 19.0% vs. 24.1%, CCND1 10.0% vs. 14.8%, MYC 11.8% vs. 6.5%; p>0.05). Furthermore, we observed a high con-

cordance of the amplification status for all genes if in situ and invasive carcinoma of individual patients were compared. This applied also to the corresponding lymph node metastases.

**Conclusions:** Our results indicate no significant differences between the gene amplification status of DCIS and invasive breast cancer concerning HER2, ESR1, CCND1 and MYC. Therefore our data suggest an early role of all analyzed gene amplifications in breast cancer development.

#### So-019

##### **Evaluation of novel DNA methylation markers for early breast cancer detection from blood serum**

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**Aims:** For the early detection of breast cancer, the development of robust blood-based biomarkers that accurately reflect the host tumour is an emerging field. In this study we analysed three novel putative biomarkers, ITIH5, DKK3 and WIF1, with regard to their methylation frequencies in breast cancer serum. Thereby, this is first study proving the methylation status of the mentioned genes in blood serum from breast cancer patients. It is our long term goal to define a methylation marker panel that allows early and unequivocal detection of breast cancer from blood samples.

**Methods:** A total of 127 serum samples from patients with histological confirmed breast cancer were analysed. Serum was collected before primary surgery of patients. Twenty serum samples from age- and sex-matched normal healthy volunteers were included as controls. Circulating cell-free DNA was extracted from 1 ml serum using the ZR Serum DNA Kit (Zymo Research). The methylation status of candidate genes in serum DNA was analysed by MSP after bisulphite-conversion and evaluated in a qualitative manner.

**Results:** Hypermethylation of the mentioned candidate genes could be detected in DNA from blood serum at the following frequencies: DKK3: 38% (48/127); WIF1: 40% (51/127); ITIH5: 25% (32/127). Aberrant DNA methylation was detectable in blood serum from patients with small (pT1) and node-negative (pN0) breast cancers and even in DCIS cases. In general, serum from control patients was unmethylated. Combining these three potential serum methylation markers increased the breast cancer detection frequency to 58% (74/127), a sensitivity level still not sufficient.

**Conclusions:** Our data show that tumour-specific methylated DNA of putative tumour suppressor genes can readily be detected in the serum of patients affected with breast cancer. Carefully selected marker panels of 5–6 genes may be sufficient to detect >90% of breast cancers in a blood-borne test, a goal we would like to achieve in the second phase of this project.

#### So-020

##### **Intratumoral lymphocytes in breast cancer and its clinical significance**

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**Aims:** Intratumoral lymphocytes (ITL) play a role in cancer biology. In several tumor types it could be shown that increased levels of ITL are better for prognosis. However, in breast cancer the significance of ITL is controversial. Therefore, we investigated 750 breast cancer samples with clinical follow up to determine the clinico-pathological impact of ITL.

**Methods:** A tissue microarray with 530 ductal (70.7%), 107 lobular (14.2%) and 113 other breast cancer types (15.1%) were evaluated for expression of CD4+, CD8+, FOXP3 and IL-17. All lymphocytes were counted per tissue spots. ROC curves were used to determine cut-offs for each lymphocyte marker.

**Results:** Increased total number of CD4+ and FOXP3 ITL were significantly (p<0.001) associated with higher tumor grade (B.R.E.) and negativity for es-

trogen receptor ( $p < 0.001$ ;  $p = 0.003$ ). Total number of CD8+ and IL-17 ITL were not associated with any clinico-pathological parameters. However, increased numbers of intra-epithelial CD8+ ITL showed the same association as total number of CD4+ and FOXP3 ( $p < 0.001$ ). Increased total number of CD4+ ( $p = 0.019$ ) as well as increased intra-epithelial CD8+ ( $p = 0.032$ ) ITL were significant associated with poorer survival.

**Conclusions:** Type, localization and amount of ITL seemed to play a role in breast cancer. Increased number of CD4+, FOXP3 and CD8+ were associated with unfavorable tumor features (high grade, estrogen negativity). Increased CD4+ ITL was associated with poorer survival.

#### So-021

##### Analysis of biomarkers in FFPE tissues – what about uPA and PAI-1?

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**Aims:** Node-negative breast cancer patients presenting with low levels of Urokinase-Typ Plasminogen Activator (uPA) and its inhibitor PAI-1 (Plasminogen Aktivator Inhibitor-1) have a very low risk of disease recurrence. The aim of our study is to establish a method for quantitative analysis of both markers in formalin-fixed, paraffin embedded (FFPE) tissues.

**Methods:** Antibodies specific for uPA and PAI-1 were validated using Western blot. We adapted our recently established methodology for protein extraction from FFPE tissues for optimal extraction of uPA and PAI-1. A pilot study for quantitative measurements using protein extracts from FFPE breast cancer tissues and protein microarray technology was performed.

**Results:** We selected three antibodies to uPA and two to PAI-1 that showed specific signals in Western blots. We successfully established a protocol for isolation of uPA and PAI-1 from FFPE breast cancer samples. For protein microarray-based quantitative analysis two of the antibodies to uPA and one to PAI-1 were found to be specific and sensitive to detect expression differences in patient samples.

**Conclusions:** The results of our study fortify the use of FFPE breast cancer tissues for quantitative analysis of uPA and PAI-1 antigen in the routine clinical setting. The next step is to compare protein microarray data with ELISA data in order to define standards for the analysis of uPA and PAI-1 from FFPE tissues.

#### So-022

##### Evaluation of incomplete tumor regression scores for neoadjuvant chemotherapy of breast cancer and predictive value of immunohistology

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**Aims:** Neoadjuvant chemotherapy is an important modality for locally advanced breast cancers. However, because of the difficulties to evaluate partial regression, there is no agreement how to evaluate the partial tumor response.

**Methods:** A total of 220 patients cases who had received neoadjuvant chemotherapy in two clinical trials were evaluated for histological regression scores using four different systems (Miller-Payne, Sataloff, residual cancer burden [RCB] score, and Sinn). Regression scores were compared with each other and with predictability by immunohistology (ER, PR, HER2, bcl2, Ki67, p53) before chemotherapy. Follow-up was available for one of both clinical trials (133 patients).

**Results:** Regression scores were very similar with the Miller-Payne system and RCB system, but did not well correlate with the Sataloff scores with 57% of RCB incomplete regression (classes 1,2,3) being in Sataloff categories TA or TB (good responders). Immunohistology before chemotherapy was predictive for complete and near complete remission (HER2 overexpression, proliferative index, ER negativity).

**Conclusions:** With the use of a regression grading system it is possible to better describe the effect of neoadjuvant chemotherapy, but there is only limited

correlation between the four most commonly used scoring systems. The most important difference is the definition of complete tumor regression (pCR). For statistical purposes tumor regression can best be evaluated with the continuous residual cancer burden (RCB) score.

#### So-023

##### Reduction of CD44+/CD24- breast cancer cells by conventional cytotoxic chemotherapy

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**Aims:** Breast cancer cells with the CD44+/CD24- phenotype have been associated with stem cell properties. To analyze effects of cytotoxic chemotherapy on these cells we examined CD44+/CD24- cells in primary breast carcinomas before and after neoadjuvant chemotherapy.

**Methods:** Core biopsies before chemotherapy and final resection specimen of 50 breast carcinomas treated preoperatively with epirubicin/cyclophosphamide were analyzed for CD44+/CD24- cells using double immuno-fluorescence. Findings were validated in a second series of 16 breast cancer patients that preoperatively had received either 4 cycles of doxorubicin/pemetrexed or 4 cycles of doxorubicin/ cyclophosphamide, each followed by 4 cycles of docetaxel.

**Results:** Before treatment, an average of 4.4% of the tumor cells in the initial 50 cases displayed a CD44+/CD24- phenotype. Following chemotherapy with EC, the frequency of CD44+/CD24- cells dropped to 2% ( $p = 0.008$ ). Similar results were observed in the patients treated with AP/AC-Doc (8.7% CD44+/CD24- cells before and 1.1% after chemotherapy). In addition, no association was seen between the frequency of CD44+/CD24- cells and the response to chemotherapy or patient survival. However, patients with tumors containing high numbers of CD44+/CD24- cells more frequently developed bone metastases in the course of disease.

**Conclusions:** Our findings challenge the proposed role of CD44+/CD24- cells as cancer stem cells in tumor resistance to chemotherapy as they apparently are not selected by conventional cytotoxic agents.

#### So-024

##### CCL22 and FoxP3 are strongly expressed in human medullary breast cancer

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**Aims:** Medullary subtype of breast carcinoma (MC) is characterized by prominent lymphocytic infiltrates and favorable clinical outcome. It is thought that the high number of tumor-infiltrating lymphocytes in MC may help to control tumor growth and thus contribute to good prognosis. Specific subtypes of tumor-infiltrating lymphocytes, such as FoxP3+ regulatory T cells (Treg), can however also suppress tumor immune responses and this particular issue has so far not been addressed for MC.

**Methods:** To determine MC infiltration by Treg, we stained FoxP3+ cells in paraffin-embedded material of MC (n=13) and of ductal (DC) or lobular breast carcinoma (n=24) as reference. Further the relation of FoxP3/CD8 T cells was determined and all tumors were stained for Treg-attracting chemokine CCL22.

**Results:** In contrast to DC, all MC were strongly infiltrated by FoxP3+ cells with focal clusters of more than 200 FoxP3+ Treg (high power field). The presence of FoxP3+ was associated with high numbers of tumor infiltrating CD8+ cells. The FoxP3/CD8 ratio (0.45) in MC showed that CD8+ cells clearly exceeded the amount of infiltrating Treg. Both, MC and DC further contained myeloid-shaped cells expressing the chemokine CCL22 and in some cases CCL22 was expressed by the cancer cells themselves.

**Conclusions:** In summary, we show that MCs are strongly infiltrated by FoxP3+ cells and the chemokine CCL22 expressed by both immune cells and cancer cells may be involved in this process.

#### So-025

##### **Androgen-receptor expression in triple negative breast cancer – results from the neoadjuvant GeparTrio trial**

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**Aims:** Triple negative (TN) breast cancer is characterized by lack of expression of estrogen- (ER), progesterone- (PgR) and Her2-receptor and is associated with aggressive behaviour and poor prognosis. Treatment options for those patients are limited to chemotherapy only. There is growing evidence that androgen receptor (AR) might play a role in triple negative breast cancer. Aim of this study was to investigate if there is a different expression of AR in the GeparTrio study cohort and to elucidate whether AR might be used as target for treatment of TN breast cancer.

**Methods:** Tissue microarrays were constructed from a subgroup of at the whole 1711 patients (pts) from the GeparTrio trial. We analyzed AR expression in a cohort of 682 patients by immunohistochemistry. Expression levels were categorized from 0, to 3+-. We evaluated the associations between AR expression and clinic-pathological parameters and pathological complete response (pCR; ypTo/ ypTis, ypNo).

**Results:** 409 pts (47.8%) out of 682 were AR positive. We found significant correlations between AR and tumor grading ( $p < .001$ ), age ( $p = .006$ ), ER/PgR Status ( $p < .001$ ), pCR ( $p < .001$ ). 86 TN tumors were evaluable for further analysis. Of these 86 tumors 35 (40.7%) had a pCR. 24 (27.9%) expressed AR whereas 62 (72.1%) did not. Significant correlations could be confirmed for tumor grading ( $p = .007$ ) and age ( $p = .020$ ). The pCR rate was 43.5% for the TN/AR negative tumors versus 33.3% for TN/AR positive tumors ( $p = 0.3872$ ).

**Conclusions:** AR is expressed in primary breast cancer. AR negative tumors showed a statistically significant higher pCR to TAC than AR positive tumors. The highest pCR rate was found in triple negative, AR negative tumors. Further studies should investigate the correlation with long term follow up as well as the benefit of AR inhibition for TN tumors.

#### So-026

##### **Prognostic significance of basal phenotype in triple-negative breast cancers**

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**Aims:** Triple negative breast cancers are frequently associated with a basal phenotype, but there is a distinct subgroup of cancers which not basal-like, and are yet ill defined yet. Therefore, in the present study, we have analyzed the expression of basal and other markers the triple negative breast cancers and correlated the results with follow-up.

**Methods:** 158 triple-negative invasive breast carcinomas were used for the construction of tissue microarrays. The tumors were further characterized by immunohistology of CK5/6, CK14, CK18, EGFR, p53, c-KIT, KI-67, bcl-2, and p16. Follow-up was obtained for all patients.

**Results:** 102 tumours (66%) showed a basal phenotype by being positive for either CK5/6 or CK14. The non-basal triple-negative cancers differed from the carcinomas with a basal phenotype by having a lower proliferative rate ( $p = 0.04$ ), and were less frequently CD117 positive (14% vs. 32%,  $p = 0.01$ ) and less frequently overexpressed p16 (31% vs. 52%,  $p = 0.01$ ). Significant differences were observed for overall 5-year-survival rates even when using other definition of basal phenotypes ( $p = 0.03$ , Log-Rank test).

**Conclusions:** Non basal-like triple-negative breast cancers differ from basal-like triple-negative breast cancers in several aspects clinicopathological as-

pects having a higher malignant phenotype, and also by 5-year survival rates. Therefore, it seems to be important to recognize the basal phenotype in triple-negative breast cancers.

#### So-027

##### **Basal and luminal mammary epithelial cells display distinct microRNA expression profiles**

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**Aims:** Although microRNAs have been investigated extensively in breast cancer research, little is known about the microRNA signature of normal breast epithelial cells.

**Methods:** In order to study whether normal luminal and basal breast epithelial cells display different profiles, the expression of 664 microRNAs was assessed in microdissected cells from normal breast tissue samples ( $n = 5$ ) by real time PCR.

**Results:** From 116 microRNAs expressed in breast epithelial cells 69 revealed different expression levels between luminal and basal cells enabling unequivocal discrimination by cluster analysis. Fourteen microRNA species were individually validated and quantified in the test material and additional four samples of normal breast tissue. Eight microRNAs (let7c, miR-125b, miR-126, miR-127-3p, miR-143, miR-145, miR-146b-5p, miR-199a-3p) were significantly up-regulated in basal relative to luminal cells, ranging from 4-fold (let7c) to 1000-fold over expression (miR-199a-3p). miR-200c and miR-429 were expressed at significantly higher levels in luminal as compared to basal cells.

**Conclusions:** We conclude that different microRNA expression profiles exist for luminal and basal mammary epithelial cells. These data harbour the potential for differentiation studies of breast cancer.

#### So-028

##### **Definition of breast cancer subtypes with predictive implications by the reliable RT-PCR quantification of 7 markers**

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**Aims:** To validate a robust, accurate and cost effective screening test for routine management of primary breast cancer treatments also applicable in core biopsies.

**Methods:** Gene expression of ER, PR, HER2, E2F1, uPA, MMP7 and STAT1 was assessed by quantitative RT-PCR in 1100 samples with known ER, PR and HER2 status defined by IHC and FISH. The 7 genes were correlated and validated with clinical outcome within the biological defined subsets.

**Results:** ER, PR and HER2 status as defined by RT-PCR successfully identified breast cancer subtypes, with a 95% sensitivity and specificity as compared with reference methods. E2F1 was shown to resume the proliferative rate of cancer cells and to represent the main component of several multigene signatures including the gene-expression grade index (GGI). High levels of E2F1 were significantly indicative of increased risk of metastases in ER positive patients treated with tamoxifen, but correlated with improved survival rates in ER negative patients receiving chemotherapy. uPA and MMP7 correlated with increased aggressiveness in the HER2 positive subset, but also with better response to chemotherapy. STAT1 added information exclusively in the triple-negative subset.

**Conclusions:** This proposed testing system will be helpful in routine analyses of primary breast cancer samples.

## So-029

**Classification of HER2 receptor status in breast cancer tissues by MALDI imaging mass spectrometry**

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**Aims:** Clinical laboratory testing for HER2 status in newly diagnosed, primary breast cancer tissues is critically important for therapeutic decision making. Matrix-assisted laser desorption/ionization (MALDI) imaging mass spectrometry (IMS) is a powerful tool for investigating proteins by the direct and morphology-driven analysis of tissue sections. Unlike immunohistochemistry (IHC), MALDI-IMS enables the acquisition of complex protein expression profiles without any labelling. We hypothesized that MALDI-IMS may determine HER2 status directly from breast cancer tissues.

**Methods:** Breast cancer tissues (n=48) predefined for HER2 status by IHC and fluorescence-in-situ-hybridization were subjected to MALDI-IMS and protein profiles were obtained by direct analysis of tissue sections. Protein identification was performed by tissue micro-extraction and fractionation followed by top-down tandem mass spectrometry. A discovery and an independent validation set were used to predict HER2 status by applying proteomic classification algorithms.

**Results:** We found that specific protein/peptide expression changes strongly correlated with the HER2 over-expression. Among these, we identified m/z 8404 as Cysteine-rich intestinal protein 1. Of particular note, the proteomic signature was able to accurately define HER2-positive from HER2-negative tissues achieving high values for sensitivity of 83%, for specificity of 92% and an overall accuracy of 89% (95% CI: 65–99%).

**Conclusions:** Our results underscore the potential of MALDI-IMS proteomic algorithms for morphology-driven tissue diagnostics such as HER2 testing and show that MALDI-IMS can reveal biologically significant molecular details from tissues which are not limited to traditional high-abundance proteins.

## So-030

**Human epidermal growth factor receptor 2 (HER-2) expression in breast cancer patients: a swiss cost-effectiveness analysis of different predictive assay strategies**

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**Aims:** Trastuzumab has conferred significant clinical benefits in HER-2-positive breast carcinomas. HER-2 status is determined by immunohistochemistry (IHC) and/or fluorescence in situ hybridization (FISH), but appropriate assessment of HER2 status remains subject to considerable debate. Data on the health economic impact of HER2 test strategies are limited.

**Methods:** A life-long Markov state transition model was used to assess costs and effectiveness of HER2 assay strategies (based on IHC, FISH, both combined or FISH confirmation of IHC2+) for a hypothetical cohort of breast cancer patients from the perspective of the Swiss health system. We compared clinically relevant strategies of predictive testing and subsequent trastuzumab treatment of HER-2-positive patients only.

**Results:** FISH testing was the most cost-effective strategy with an incremental cost-effectiveness ratio of €12'245 per additional quality adjusted life year (QALY) gained, compared to no trastuzumab treatment. The next best strategy was parallel IHC and FISH, with costs of €400'154/QALY gained com-

pared to FISH alone. FISH as primary HER-2 testing modality remained the preferred option in deterministic and probabilistic sensitivity analysis.

**Conclusions:** Predictive testing to identify adjuvant breast cancer patients who benefit from trastuzumab treatment is a clinical and economic necessity. Our model identifies FISH as the most cost-effective approach. It is demonstrated that analysis of total costs and benefits by life-long cost-effectiveness models can support decision making on predictive testing strategies.

## So-031

**Predictive and prognostic value of hormone receptor and HER2 expression in patients treated with neoadjuvant chemotherapy for operable breast cancer**

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**Aims:** We classified breast carcinomas based on ER, PgR, and HER2 expression in a cohort of 116 participants of the GeparDuo study on anthracycline/taxane-based neoadjuvant chemotherapy for operable breast cancer, and tested for associations with pathological complete response (pCR) and disease-free survival (DFS).

**Methods:** We used immunohistochemistry and silver-enhanced in situ hybridization on tissue microarrays (TMAs) of pretherapeutic core biopsies.

**Results:** pCR rates were significantly different between the biology-based tumor types with HR+/HER2+ coexpressing and HR-/HER- (triple negative) tumors having higher pCR rates than HR+/HER2- (luminal) carcinomas (p<0.05). DFS was different in the biology-based tumor types (p<0.0001) with HR+ tumors having the best prognosis irrespective of HER2 expression, and HR- (triple negative and HR-/HER2+) tumors showing the worst outcome. Biology-based tumor type was an independent prognostic factor for DFS in multivariate analysis (p<0.0001).

**Conclusions:** A biology-based breast cancer classification using ER, PgR, and HER2 bears independent predictive and prognostic potential. Patients with HR-/HER- and HR-/HER+ tumors, have an unfavorable prognosis and are in need for additional treatment options.

## So-032

**HER2 gene and chromosome 17 centromere co-amplification in breast cancer**

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**Aims:** The HER2 status of breast carcinoma is usually determined by HER2 immunohistochemistry and/or HER2 dual color fluorescence in situ hybridization (FISH) or silver in situ hybridization (SISH). Evaluation of the centromeric region of chromosome 17 is a necessary component to distinguish chromosomal polysomy from true HER2 gene amplification. The centromeric region can be co-amplified along with the HER2 gene. The relevance of this finding for prognosis of breast cancer patients is unclear and the mechanism of co-amplification is not fully understood.

**Methods:** We performed a retrospective analysis of HER2/centromere 17 co-amplification in a large set of routinely tested breast cancer samples. The frequency of HER2/centromere 17 co-amplification was determined using situ hybridization (FISH or SISH) with HER2 and chromosome 17 centromeric probes (CEP17). The amplicon size was determined by FISH with different probes.

**Results:** HER2/CEP17 co-amplification was observed in 17 of 5000 breast carcinomas (0.3%). The tumors were mainly poorly differentiated invasive ductal carcinomas, half of them being hormone receptor positive. All cases were considered clinically as HER2 positive.

**Conclusions:** HER2/CEP17 co-amplification is a rare event, occurring in less than 1% of invasive breast carcinomas. The formation of large HER2 amplicons involving the centromeric regions is a potential mechanism for this co-amplification. The potential relevance of the amplification of genes included in this amplicon has to be determined.

#### So-033

##### **Dual colour in situ hybridisation is a reliable technique for the assessment of Her2/neu status in invasive breast carcinoma but seems to detect a different subset of amplified score 2+ tumours**

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**Aims:** Fluorescence in situ hybridization (FISH) is considered the gold standard for the assessment of Her2/neu amplification. The aim of this study was to investigate whether a recently developed chromogenic in situ hybridization (CISH) technique is comparable to a well-established FISH method.

**Methods:** 106 cases of invasive ductal carcinomas of the breast with established Her2/neu status by HercepTest Dako and Vysis FISH were retrospectively analyzed using DAKO DuoCISH. 82 cases revealed Hercep Test score 2+, 16 cases score 3+ and 6 cases score 1+. Formalin fixed, paraffin embedded tissue both from core needle biopsy and from surgical specimens was used (fixation time 16–48 hours). The Dako DuoCISH uses both a centromere probe for chromosome 17 and a probe for the Her2 gene coupled with 2 different chromogens. The reaction was carried out on an autostainer. The analysis was performed according to the CAP guidelines by a single investigator using a 100x objective. All discrepant cases were reassessed.

**Results:** The Her2/CEP17 coefficient did not differ significantly between FISH and CISH ( $p=0.9$ , paired t test). All score 3+ and 1+ cases were concordant in CISH and FISH (100% amplified and 100% not amplified, respectively). 23 (28%) of the score 2+ cases were amplified by FISH compared to 28 (34%) by CISH ( $p=0.5$ ; Fisher's exact test: not significant). Amplification was discrepant between FISH and CISH for 11 (13%) score 2+ cases (3 FISH amplified/CISH non-amplified cases, 8 CISH amplified/FISH non-amplified cases; Her2/CEP17 coefficient 1.2–4.75). 7 of the CISH amplified/FISH non-amplified cases had a coefficient between 2.09 and 2.75 and 2 showed a heterogeneous amplification.

**Conclusions:** DAKO DuoCISH seems to be a reliable technique for the assessment of Her2 amplification and allows long-term storage of the slides. Morphology is better preserved and the detection of amplified foci in heterogeneous tumours is easier. DAKO DuoCISH seems to detect different Her2-amplified breast carcinomas in the score 2+ subgroup compared to Vysis FISH. The impact on therapeutic response and outcome needs to be further evaluated.

#### So-034

##### **Quantitative analysis of centrosomes in breast cancer in correlation to DNA-ploidy and clinicopathological markers**

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**Aims:** The purpose of the study was to analyse centrosome morphology in breast cancers with different DNA ploidy and clinicopathological markers.

**Methods:** The centrosomes of 45 invasive breast cancers were stained immunohistochemically with an antibody against gamma-tubulin (GTU-88; SIGMA) and quantitatively analysed for number, size and shape with Spectracube SD-200H (Applied Spectral Imaging). The DNA ploidy was estimated with an OPTIMAS<sup>®</sup> based image cytometry workstation. The statistical analysis was done by t-Test according to Student ( $p<0.05$ ).

**Results:** Centrosomes were larger in non-diploid tumors than in peridiploid tumors. Centrosomes of breast cancers larger than 2 cm differed in number and shape from those of smaller breast cancers. Lymph node positive breast cancers showed larger centrosomes than lymph node negative tumors. Centrosomes in grade 1 tumors differed in their shape from centrosomes in tumors with a histopathological grade 2. Further differences in size and shape of

centrosomes were detected in breast cancers with different expression status of estrogen receptor and p53.

**Conclusions:** The number and morphology of centrosomes in breast cancer is not only associated with DNA-ploidy, but also with different expression of clinicopathological markers. Thus, the analysis of centrosomes may contribute to improvement of prognostication in breast cancer. However, this requires the analysis of a considerably higher number of cases under standardised conditions.

## Sitzung: AG Oralpathologie

#### So-035

##### **Sialolipoma of the parotid gland**

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**Case report:** Sialolipoma is a relatively new and rare variant of lipoma of the salivary glands. It is characterized by the combination of classical lipoma morphology with non-neoplastic ductulo-acinary salivary tissue components. We describe a sialolipoma in a 43 year-old man presenting with a parotid mass. The clinical, radiological and pathomorphological characteristics of this parotid sialolipoma are discussed. Less than 30 sialolipomas in different locations have been published, about half of them in the parotid gland. Although conventional lipomas remain the vast majority of lipomatous salivary gland lesions, sialolipoma should be recognized as a distinct entity among the benign salivary gland tumours (Der Pathologe, November 2009).

#### So-036

##### **Expression of p-AKT is higher in relapsing adenoid cystic carcinomas of head and neck**

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**Aims:** Adenoid cystic carcinomas are rare tumors with an indolent clinical course, but frequent local relapses. The identification of tumors with a higher relapse risk seems to be interesting. Hence we investigated parameters of glucose metabolism, which were found associated with poor prognosis in other malignancies.

**Methods:** Specimen of 29 patients were investigated immunohistochemically with antibodies against p-AKT, TKTL-1 (transketolase-like 1), M2PK (M2 pyruvate kinase), and GLUT-1. Proliferation was investigated by staining with Ki67. The tumors were located at the major or minor salivary glands. Only the typical cribriform subtype was investigated. The initial tumor stage was pT1 or pT2.

**Results:** Expression of p-AKT was significantly ( $P=0.036$ ) associated with a higher relapse risk in multivariate analysis. Low expression of M2PK was non-significantly ( $P=0.065$ ) predictive for a higher risk. TKTL-1 and GLUT-1 were expressed in the majority of cases, albeit not associated with relapse risk.

**Conclusions:** Adenoid cystic carcinomas positive for p-AKT show a higher relapse risk. However, other parameters of glucose metabolism investigated here did not show a predictive value in this study.

**So-037****Placebo-controlled Rituximab treatment in Sjögren's syndrome: histological proof of glandular restoration**

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**Aims:** In Sjögren's syndrome (SS) (immuno-)histological changes in repeated parotid gland biopsies were compared before and after Rituximab treatment versus placebo treatment. Findings were correlated to salivary flow rate.

**Methods:** 20 patients were treated with rituximab, 10 with placebo. Immunohistological studies in pre- and post parotid biopsies allowed analysis of the infiltrate, B-/T-cell ratio (CD20,CD79a,CD3), presence of lymphoid follicles (LF) and lymphoepithelial duct-lesions (LEL), and parenchymal regeneration (Ki67).

**Results:** Comparing pre- and post-biopsies, in patients treated with Rituximab, the amount of overall lymphoid infiltration (focus score), of LF and of LEL decreased significantly, while these parameters were stable or slightly increased in placebo patients. In more than half of the cases treated by Rituximab LF and LEL completely had disappeared, as well that in most cases the initially reduced parotid flow rate had increased, and the sodium concentration in saliva significantly had decreased.

**Conclusions:** The presented findings in this double blinded, placebo-controlled study substantiate own previous casuistic observations of a major glandular restoration in SS after treatment with Rituximab. Especially, the reduction of inflammation and major loss of LF might correlate to observed increase of salivary flow, and the major or complete redifferentiation of LEL to regular striated ducts might correlate to the observed normalization of sodium. These histological findings support the efficacy of Rituximab treatment in SS.

**So-038****Laryngeal inflammatory myofibroblastic tumors – a heterogeneous entity**

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**Aims:** Inflammatory myofibroblastic tumors (IMFTs) of the larynx are rare. We report the clinical presentation, histomorphology, and new molecular findings of 2 cases.

**Methods:** Paraffin-embedded specimens were stained immunohistochemically (eg, vimentin, AE1/3, Alk-1, smooth muscle [sm-]actin, p53, Rb1, immunoglobulin G4 [IgG4]). Epstein-Barr virus-encoded RNA (EBER) in situ hybridization and HHV8-polymerase chain reaction (PCR) were done. Comparative genomic hybridization (CGH) was performed.

**Results:** Case 1 was that of a 56-year-old man with an infiltrating plasma-cell-rich tumor (Alk-1-, sm-actin+). Plasma cells were strongly positive for IgG4. CGH was unsuspecting. Case 2 was that of a 34-year-old woman with an exophytic tumor (Alk-1+). CGH revealed losses on 13q14-22. The few plasma cells were negative for IgG4. The proliferation (Ki67) was low in both cases.

**Conclusions:** Different types of IMFTs may exist and could indicate different therapeutic strategies. Alk-1-positive cases with only scattered inflammatory cells could represent the neoplastic variant, whereas cases rich in plasma cells could be associated with IgG4 sclerosing diseases.

**So-039****Laminin matrix remodelling and stroma formation in oral squamous cell carcinoma (OSCC)**

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**Aims:** Laminins (Ln 's) are generated by alternative assembly of different  $\alpha$ ,  $\beta$  and  $\gamma$  chains. Changes in tumour cell expression and in basement membrane (BM) distribution are associated with OSCC progression. The contribution of Ln 's to stroma and vessel formation is not fully understood but might be crucial for invasive phenotype development.

**Methods:** The immunohistochemical expression of the Ln chains  $\alpha 2$ ,  $\alpha 3$ ,  $\alpha 4$ ,  $\alpha 5$ ,  $\beta 2$ ,  $\beta 3$  and  $\gamma 2$  was qualitatively and semiquantitatively analysed in the stroma in OSCC of different tumour grade in comparison to normal / hyperplastic mucosa. By CD31 or  $\alpha$ -smooth muscle actin (ASMA) double labeling, the relation to myofibroblasts or vessels was assessed. Stromal mRNA expression was evaluated by RT-PCR after microdissection.

**Results:** Ln  $\alpha 2$  significantly decreases and stromal Ln  $\alpha 3$ ,  $\alpha 4$ ,  $\alpha 5$ ,  $\beta 2$ ,  $\beta 3$ , and  $\gamma 2$  as well as ASMA significantly increase with raising grade in OSCC stroma. Ln  $\alpha 3$ ,  $\alpha 4$ ,  $\beta 2$ ,  $\beta 3$ , and  $\gamma 2$  significantly correlate to ASMA.  $\alpha 3$ ,  $\alpha 4$ ,  $\alpha 5$ , and  $\gamma 2$  mRNA is found in OSCC stroma.  $\alpha 3$  is significantly decreased and  $\gamma 2$  significantly increased in vessels with neoplastic transformation.

**Conclusions:** OSCC progression is associated with a stromal up-regulation of laminins possibly contributing to invasive front reorganization. This process is accompanied or mediated by myofibroblast development. The reorganization of vascular BM concerning  $\alpha 3$  and  $\gamma 2$  Ln 's seems to reflect a special tumour endothelial cell interaction during OSCC progression.

**So-040****Is the improved prognosis of p16 positive oropharyngeal cancer dependent of the treatment modality?**

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**Aims:** The incidence of HPV induced oropharyngeal squamous cell carcinoma (OPSCC) increases in the western countries. Overexpression of p16 is considered a surrogate marker for HPV infection. Compared to patients with p16 negative OPSCC, these carcinomas show a significantly better prognosis, reported to be due to increased radiosensitivity. The objective of the present study was to analyze the impact of p16 expression status on the prognosis of OPSCC and to compare its relevance in OPSCC treated by either radiotherapy (RT) or primary surgery.

**Methods:** Results are based upon a tissue microarray of 288 head neck squamous cell carcinomas including 85 OPSCC with complete clinico-pathological and follow-up data.

**Results:** p16 positivity correlated significantly with oropharyngeal tumor localization ( $p < 0.001$ ). Analyzing OPSCC alone, p16 expression did not correlate with clinico-pathological features such as T and N stage, tumor grading, or tumor localization within the oropharynx. Patients with p16 positive OPSCC showed a significantly longer overall survival than those with p16 negative tumors ( $p = 0.007$ ). This result was confirmed in the 26 patients with OPSCC treated by RT ( $p = 0.096$ ) as well as in those 59 OPSCC patients treated by primary surgery ( $p = 0.067$ ).

**Conclusions:** p16 is a most relevant prognostic parameter in OPSCC, regardless of the treatment modality chosen and independent of T & N stage and grading.

## So-041

### HPV status in head and neck tumors

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**Aims:** Previous studies indicated a high prevalence of high risk (HR) HPV in tonsillar carcinoma. The aim of the present study was to confirm this association by applying two different detection methods and to evaluate morphology in the prediction of HPV positive head and neck carcinomas (HNSCC).

**Methods:** DNA was isolated from 49 HNSCC primaries and 27 lymph node metastases. Amplification of the L1 gene was performed by PCR. Evidence for HPV was derived from agarose gel electrophoresis and HPV typing by microarray chip hybridization. "Typical" morphology and presence of lymphatic tissue were correlated with the HPV status.

**Results:** The highest rate of HPV positive tumors were observed in the tonsils with 76% of the patients and 81% of tumor samples being positive for high risk HPV. DNA chip analysis detected significantly more often HPV positive cases than agarose gel electrophoresis. HPV 16 was the most frequent type being present in 98% of cases, once HPV 33 was detected. There was a good concordance (21/23 cases) between the HPV status of the primary tumor and its corresponding metastasis. In 2 cases the metastases were HPV negative and primaries positive. Morphology was weakly correlated with a positive HPV status.

**Conclusions:** Our results confirmed the high prevalence of HR-HPV in tonsillar carcinomas with a rate that was even 20% higher than those reported in the literature. HPV typing is useful to identify metastases and corresponding primary tumors. Morphology may suggest a positive HPV status that needs to be confirmed by molecular tests.

## Sitzung: AG Paidopathologie

## So-042

### Detecting the cutting-edge. A tool for high sensitive and absolute specific detection of tumor cells

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**Aims:** To detect tumor cells as sensitively and specifically as possible in different tissue types is one major goal of cancer diagnostics.

**Methods:** We mapped the amplified genomic regions (ampGR) around the proto-oncogene MYCN from about 40 primary human neuroblastomas and 3 neuroblastoma cell lines, using a high resolution Tiling Array. Based on the array data we were able to build virtual fusion sites of the ampGRs (amplicon-fusion-sites (AFS)) that served as blueprint for further AFS-PCR design. Specific AFS-PCR fragments were then sequenced. Based on the exact AFS sequences we established quantitative real time PCR assays.

**Results:** All AFS and AFS-PCR fragments identified were absolute tumor cell specific and unique for each patient. AFS-PCR was highly sensitive and uncovered one tumor cell out of 10<sup>6</sup>–10<sup>7</sup> control cells, dependent on the AFS base composition.

**Conclusions:** As AFS-PCR is not limited to a specific tumor type but rather transferable to every entity of malignancy, provided that the individual tumor cells harbour ampGR, our findings supply a manual for a feasible diagnostic tool for approximately 20–30% of all individual malignancies. AFS-PCR is suitable to sensitively detect tumor cells in different tissue types like bone marrow, peripheral blood or cerebral fluid at the time point of diagnosis and during therapy, contributing to established MRD diagnostics.

## So-043

### Lim1 an embryonal transcription factor with oncogenic potential?

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**Aims:** Renal organogenesis is a very complicated process with many and complex mesenchymal-epithelial interactions. Lim1, a member of the homeobox gene family, induces kidney formation in the intermediate mesoderm. In our study we investigated the role of Lim1 in human renal development, cystic malformation of the kidney and several renal neoplasms.

**Methods:** Different stages of renal development and multicystic dysplastic kidney were investigated by immunohistochemistry. Additionally we evaluated renal malignant neoplasms.

**Results:** Expression of Lim1 was first noted at 10 weeks of gestation, and lasted during the second and into the third trimester. In none of the multicystic dysplastic kidneys expression of Lim1 could be detected. The renal neoplasms investigated so far showed reactivation in part of embryonal renal neoplasms, but not in renal cell carcinomas.

**Conclusions:** Our results demonstrate that Lim1 plays a role in human renal development but is not expressed in dysplastic kidneys. In paediatric renal neoplasms Lim1 appears to be reactivated. In contrast, Lim1 does not appear to play a role in the pathogenesis of adult renal cell carcinomas.

## So-044

### Rare case of a diffuse neonatal hemangiomas combined with diffuse multifocal chorangiomas and placental mesenchymal dysplasia

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**Aims:** Diffuse neonatal hemangiomas (DNH) is characterised by multiple hemangiomas of the skin and visceral organs. Whereas solid or circumscribed hemangiomas are a common finding, DNH is often associated with a poor prognosis. We present the rare case of DNH associated with a diffuse chorangiomas and mesenchymal dysplasia. Administration of corticosteroids and VEGF-receptor antibodies could not circumvent the fatal outcome.

**Methods and Results:** Autopsy revealed a diffuse infiltration of the visceral organs, most pronounced in liver and lungs, together with shock organs. Immunohistochemical stainings with VEGFR-antibodies were negative. In the placenta a diffuse multifocal chorangiomas plus focal aspects of a mesenchymal dysplasia were diagnosed.

**Conclusions:** The association of DNH and diffuse multifocal chorangiomas plus mesenchymal dysplasia has to our knowledge not yet been described. We discuss the major clinicopathological findings of this very rare case and discuss possible pathomechanisms such as a placental origin of DNH. The negative immunohistochemical staining with VEGFR-antibodies could be explained by a) a blocking of the VEGF-receptor by the therapeutically applied VEGFR antagonists effacing a positive VEGFR immunohistochemistry, b) a missing or reduced VEGFR expression.

**So-045****On the diagnostic approach of pediatric malignant renal tumours**

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**Aims:** Most pediatric renal tumors are nephroblastomas. Other renal neoplasms like renal cell carcinomas (RCC) e.g. are rare in children. The preoperative diagnosis and therapeutic strategy is generally based on imaging findings, often favouring the diagnosis of nephroblastoma. Fine needle aspiration biopsy (FNA) is recommended only in exceptional cases with ambiguous radiological results.

**Methods:** We report on a 7 year old boy with a kidney tumor. Radiological results could not assess dignity and tumor entity reliably. Taking into consideration clinical presentation and data, nephroblastoma was diagnosed. FNA was not performed. Neoadjuvant therapy was given according to the guidelines for nephroblastoma (SIOP 2001/GPOH) followed by tumor nephrectomy.

**Results:** Histologically an RCC was diagnosed with hardly any tumor regression. Immunohistochemistry proved an Xp11.2 translocation.

**Conclusions:** Based on this case the diagnostic approach in cases of ambiguous imaging results in pediatric renal tumors are discussed with special emphasis on FNA. Latter can easily substantiate pretherapeutic and preoperative diagnosis and hence influence further therapy. Furthermore, in the event of RCC, Xp11.2 translocation should be screened immunohistochemically as this tumour subtype differs in prognosis and therapy from RCC without translocation.

**So-046****Array-CGH and quantitative PCR genetic analysis in a case with bilateral hypoplasia of pulmonary arteries and lungs and simultaneous unilateral renal agenesis**

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**Aims:** Aberrant embryologic development of the lungs is a potentially lethal malformation which can be associated to other malformations, particularly of the kidneys. Bilateral hypoplasia of the lungs is very uncommon and in this study we characterise associated anatomic and genetic findings of a unique case.

**Methods:** Routine post-mortem examination, re-evaluation of clinical data, genetic analysis: array-based comparative genomic hybridisation (array-CGH) and quantitative PCR.

**Results:** Case report: 20-year nullipara mother; male child; ultrasonographic hypoplasia of the lungs and unilateral renal agenesis (dexter); 41st week of gestation: lethal acute respiratory failure 20 minutes after birth. Pathological anatomy: extremely hypoplastic pulmonary arteries associated with unilobular lung rudiment and agenesis of pulmonary veins; unilateral renal agenesis. Array-CGH derived karyotype: 46,XY,dup(1)(p21p21).

**Conclusions:** The lethal bilateral hypoplastic lung with associated unilateral renal agenesis might be an extreme variant of previously described simultaneous dysmorphogenesis of the pulmonary and renal system.

**So-047****Intrauterine segmental midgut atresia in a gastroschisis with closure of the abdominal wall defect around the prolapsed intestinal remnant**

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**Aims:** The association of gastroschisis and intestinal atresia is well known. We describe such a patient with a mummified right paraumbilical intestinal remnant.

**Methods:** A male baby, born at the 34th WoG, presented with gastroschisis, which was detected by sonography during pregnancy. At birth only the carnified terminal ileum and caecum were prolapsed and the abdominal wall was closed around. Surgical exploration showed atresia of the ileum (type III A) including a microcolon and an intestinal atretic remnant on both sides blind ending, provided by the ileocolic artery. This segment was resected and ileostomy with ascendostomy was carried out. A second surgical look was also carried out, because the ileum did not recover, and such segment was resected as well. Finally a jejunum-ascending-stomy was applied six weeks later. The results matched with a partial short bowel syndrome.

**Results:** Histological examination of the intestinal remnant showed necrotic intestine parts with inflammatory reaction and calcified meconium as well as almost normal configured segments with vascular ectasia.

**Conclusions:** In a gastroschisis the herniated intestine can become, due to loss of circulation (volvulus, incarceration), atretic identifying several possible abnormal circulatory pathways.

**So-048****Acardius acormus with exceptionally advanced brain development and ectopic intracerebral (ocular?) pigmented epithelium**

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**Aims:** Acardius represents the most severe fetal malformation that occurs in one twin of a monogygotic gestation. Malformations are due to placental arterio-arterio and veno-venous shunts between the fetoplacental circulation of both twins whereby the acardius is perfused with blood directly from the pumping twin. Hence the acronym TRAP (twin-reversed arterial perfusion) sequence is used as well. We report the case of an acardius of the 31th gestational week.

**Methods and Results:** Post mortem examination of the heterogenous trunk-resembling mass without extremities displayed a fairly well developed oral cavity and pharynx, a rudimental heart and adrenal glands, an ovary and an exceptionally advanced (nearly appropriate for age) stage of brain development with agenesis of olfactory and optic nerves, glio-neuronal leptomeningeal heterotopias and (probably ocular) pigmented epithelium in the frontobasal cortex. Though there were two eyelids with eyelashes there was no evidence of an ocular development in either socket.

**Conclusions:** To our knowledge an acardius acormus with such an advanced stage of brain development has not been described. The ectopic, probably ocular intracerebral pigmented epithelium seems to be a unique finding as well.

## So-049

### The fetal lens still – a mystery?!

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**Aims:** On histology, the crystalline lens is especially in fetal and infant eyes – an organ susceptible to numerous artifacts. Nevertheless, evaluation of the lens is often of paramount importance in the classification of fetal syndromes or forensic questions. Thus, the aim of our study is to evaluate various factors that might have an impact on lens histomorphology.

**Methods:** Together with the Division of Paidopathology, we have by now collected more than 400 eyes from 230 fetuses. In most instances, there was induced fetocid while spontaneous abortion or post-natal death were rather infrequent. Specimens were fixed in formalin, and in selected cases one eye was put into Glutaraldehyde for comparison. All systemic findings as well as data related to the termination procedure were recorded. - Some individual cases shall be presented to demonstrate the variability of histopathologic findings.

**Results:** Among our cohort of more than 350 fetal eyes with a gestational age between 9 and 38 weeks, most eyes revealed morphologic features different from the descriptions available in textbooks. Numerous specimens showed the well-known phenomenon of an indentation at the posterior pole, accompanied by various morphologic changes of the subcapsular cortex. No association with syndromes potentially involving the lens was found. In addition, one unusual case of a pre-term born child with unilateral aphakia will be discussed.

**Conclusions:** Alterations in fetal lens morphology are extremely frequent and variable. These have to be carefully taken into account when interpreting post-mortem findings.

## So-050

### Does smoking influence placental VE-cadherin expression?

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**Aims:** The effects of nicotine are seen in every trimester of pregnancy, ranging from increased spontaneous abortions in the first trimester to increased premature delivery rates and decreased birth weights in the final trimester. Vascular endothelial cadherin (VEC), a calcium-dependent homotypic adhesion molecule, contributes to endothelial assembly and VEGF-mediated survival during angiogenesis. In human term placentas, it is expressed by villous vessels endothelium and extravillous cytotrophoblasts. To evaluate the influence of tobacco on placental VEC expression and vessel density we compared VEC expression in human placental vasculature and cytotrophoblasts of smoking and healthy patients of various gestational age.

**Methods:** In 50 placentas (smokers: 26, control: 34) endothelial VEC expression as well as that by trophoblastic epithelial cells was studied by immunolocalisation using an antibody against VEC. Endothelial and trophoblastic antigen-expression as well as vessel density was evaluated by computer-assisted image analysis (Adobe Photoshop 7.0).

**Results:** In comparison to the control group, in placentas of smokers endothelial as well as trophoblastic VEC expression was unequivocally increased while number of vessels was reduced.

**Conclusion:** Considering the fact that VEC plays a vital role in placental angiogenesis, increased endothelial VEC expression concomitant with a reduced vascularisation is astonishing. Nevertheless, these findings could support the theory that – by altering localisation and function of VEC – tobacco (and here especially Cadmium) can act angiostatically. Furthermore it has to be discussed whether this enhanced VEC expression plays a vital role in the prevention of preeclampsia as pregnant smokers are at a 33% reduced risk of developing preeclampsia. The augmented VEC expression by trophoblast cells has to be discussed with view to the fact, that yet unknown trophoblast-

derived substances increase vascular permeability; an increased trophoblastic VEC expression could circumvent this mechanism.

## So-051

### Gaucher's disease with a hydropic phenotype – differential diagnosis and mutation analysis

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<sup>3</sup>Centrum für Humangenetik, Dr. H. Haas-Andela, Dr. G. Schmidt, H. Schudt, Linden

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**Aims:** Gaucher's disease is an autosomal recessive lysosomal storage disorder caused by a deficiency in the enzyme glucocerebrosidase. This deficiency results in accumulation of monocytes and macrophages and thus produces multiple organ and skeletal complications. Presentation and age at symptom onset are highly variable. Three disease states have been classified based on presence and severity of neurological involvement. We present an extreme phenotype in a perinatal lethal hydrops and give an overview of differential diagnosis concerning nonimmune hydrops fetalis (NIHF).

**Methods:** Fetal sonography revealed severe NIHF of unknown origin in a male fetus of consanguineous Turkish parents. Prenatal serological analysis did not confirm suspected CDG syndrome. Because of developing pre-eclampsia the pregnancy was terminated at 33 gestational weeks and autopsy was performed.

**Results:** Postmortem examination revealed a thick, collodion like skin and pronounced hepatosplenomegaly. Histology evidenced abundant PAS-positive storage cells of wrinkled tissue paper appearance in multiple organs, compatible with Gaucher cells. Thus we suspected severe Type 2 Gaucher disease resulting in a collodion baby phenotype. Mutation analysis of the glucocerebrosidase gene in 1q21 (GBA, 606463) confirmed our diagnosis.

**Conclusions:** Acute neuronopathic Gaucher's disease (type 2) is classically considered to be a disease of late infancy, but also includes a spectrum of variant phenotypes such as perinatal lethal hydrops, or the collodion baby phenotype in the newborn period. Post mortem examination including goal directed application of molecular techniques are required for the final diagnosis. This case exemplifies the manifestation of a storage disease in early fetal period due to lack of maternal compensation of the fetal enzyme deficiency. On the other hand it underlines the need for an interdisciplinary cooperation and thereby the interdisciplinary status of the fetal pathologist.

## So-052

### Association of rare chronic histiocytic intervillitis of the placenta with assisted reproduction-induced pregnancies

see abstract no. Sa-032

## So-053

### Monophasic epithelial nephroblastoma as unusual cause of recurrent macrohematuria in a 12 year old girl

see abstract no. Sa-033

## So-054

### Histomorphological tumor progression in pediatric/ syndromic GISTs seems to be comparable to that seen in sporadic GISTs in adults: presentation of a pediatric GIST and of a new case of Carney triad

see abstract no. Sa-034

## Sitzung: AG Pneumopathologie

## So-055

**EGFR-Mutations in non-small cell lung cancer prevalence, correlation with other biomarkers and intratumoral heterogeneity**F. Länger<sup>1</sup>, D. Jonigk<sup>1</sup>, N. Dickgreber<sup>2</sup>, S. Fischer<sup>3</sup>, H.H. Kreipe<sup>1</sup>, U. Lehmann<sup>1</sup><sup>1</sup> Institut für Pathologie der Medizinischen Hochschule Hannover<sup>2</sup> Klinik für Pneumologie der Medizinischen Hochschule Hannover<sup>3</sup> Klinik für Herz-Thorax und Gefäßchirurgie der Medizinischen Hochschule Hannover

**Aims:** EGFR-mutations are predictive for the therapy with tyrosinkinase-inhibitors (TKI) in non-small cell lung cancer (NSCLC). As testing for EGFR mutations is time consuming and expensive, data regarding the prevalence and intratumoral heterogeneity as well as the association with other biomarkers would be helpful in clinical practice.

**Methods:** 200 consecutive specimen of NSCLC were tested for EGFR mutations either by direct sequencing (exons 19, 21) or pyrosequencing methods (exon 20). In a subset of cases (n=50) multiple tumor blocks were analyzed. Multi-tissue arrays were constructed and immunohistochemistry with a broad panel of antibodies against adhesion (CD56), receptor (EGFR, ER, PR) and differentiation molecules (TTF1, CK7, p63) was done.

**Results:** Overall 9.6% of all NSCLC revealed mutations of the EGFR gene, preferably in exons 19 and 21. 25% of mutated cases revealed intratumoral heterogeneity of the genotype. Mutations were associated with a non-mucinous type of papillary or bronchioalveolar adenocarcinoma, TTF1 and ER expression.

**Conclusions:** Using histological typing and a small set of immunohistological biomarkers EGFR mutated NSCLC can be predicted with high sensitivity (>90%) though lower specificity (60%), thus making a reliable preselection of tumors to be tested for EGFR mutations possible. Testing multiple tumor blocks has to be considered.

## So-056

**Fatty acid synthase is inversely correlated with epidermal growth factor receptor in non-small cell lung cancer**V. Tischler, L. Morra, I. Schmitt-Opitz<sup>1</sup>, G. Kristiansen, H. Moch, A. Soltermann

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**Aims:** Fatty acid synthase (FASN), the key enzyme for de novo synthesis of fatty acids, is overexpressed in many tumours and associated with more aggressive behaviour. Epidermal growth factor receptor (EGFR) regulates FASN expression via MAPK and PI3 K pathways. Conversely, inhibition of FASN enforces EGFR tyrosine kinase inhibition via disruption of microdomains. We evaluated FASN and EGFR in non-small cell lung cancer (NSCLC).

**Methods:** Cytoplasmic FASN and membranous EGFR protein expression were analysed immunohistochemically on a tissue microarray of 526 resected NSCLC specimens and correlated with clinicopathologic parameters. A549 cells were treated with the FASN inhibitor C75.

**Results:** High expression (semiquantitative score 2–3) of FASN was found in 52% (272/526) and of EGFR in 34% (179/526) of the tumours. FASN and EGFR expression were inversely correlated ( $p < 0.001$ ;  $cc = -0.157$ ). High FASN was associated with adenocarcinoma, younger age and higher grade, whereas high EGFR was associated with squamous cell carcinoma, higher pT stage and female gender. Prognostic significance was not observed. A549 cells showed high FASN but low EGFR expression. Treatment with C75 for 24 h decreased cell migration and proliferation.

**Conclusions:** The association of high FASN expression with the adenocarcinoma histotype of NSCLC could convey a new therapeutical option by means of addition of FASN inhibitors to regimens based on EGFR TKI such as erlotinib.

## So-057

**KRAS amplification in non-small cell lung carcinoma**Ann-Cathrin Stiedl<sup>1</sup>, Patrick L. Wagner<sup>2</sup>, Theresia Wilbertz<sup>1</sup>, Nasser K.Altorki<sup>2</sup>, Falko Fend<sup>1</sup>, Holger Moch<sup>3</sup>, Alex Soltermann<sup>3</sup>, Sven Perner<sup>1</sup><sup>1</sup> Institute of Pathology, Eberhard-Karls University Hospital of Tübingen, Tübingen, Germany<sup>2</sup> Cardiothoracic Surgery, New York Hospital/Weill Cornell Medical Center, New York, NY, USA<sup>3</sup> Institute of Pathology, Universitätsspital Zürich, Switzerland

**Aims:** Amplification of 12p12.1, containing the KRAS gene, is one of the commonest amplification events in lung adenocarcinoma. Although activating KRAS mutations are well characterized, KRAS amplification as an oncogenic mechanism is relatively unexplored. We have previously demonstrated that KRAS amplification is associated with increased p21 expression in NSCLCs, and that amplification is often associated with an activating KRAS mutation. In the current study, we sought to determine the frequency of KRAS amplification in NSCLC and to identify associated clinicopathologic features.

**Methods:** Fluorescence in situ hybridization, utilizing a probe for the KRAS gene, was applied to a series of 385 NSCLCs, including 300 tumors consecutively resected with curative intent. KRAS amplification was compared with clinicopathologic features derived from a prospectively collected patient database. Statistics: categorical variables, Fisher's exact test; continuous variables, student's t test; overall survival, Kaplan-Meier method and log-rank test;  $p < 0.05$ .

**Results:** Among 385 NSCLCs, 58 (15%) exhibited KRAS amplification. Amplification was significantly associated with larger mean tumor size ( $p = 0.003$ ), poor differentiation ( $p = 0.004$ ) and pleural invasion ( $p = 0.046$ ). KRAS amplification was not associated with patient age, gender, race, or smoking history; angiolymphatic invasion; or node status. Although the rate of KRAS amplification did not differ between squamous cell and adenocarcinomas, among adenocarcinomas KRAS amplification was far less common in tumors consisting of pure bronchioalveolar subtype versus those of mixed or invasive subtypes ( $p = 0.004$ ). At a median followup of 2.3 years, no difference in overall survival based on KRAS amplification status was noted.

**Conclusion:** KRAS amplification is seen in a substantial minority of NSCLCs, and is often but not always associated with an activating KRAS mutation. KRAS amplification is associated with increased p21 protein levels, as well as poor prognostic indicators including larger tumor size, poor differentiation and pleural invasion. Further studies will be necessary to characterize the oncogenic mechanisms of KRAS amplification, its relationship with activating KRAS mutation, and its prognostic significance.

## So-058

**Proof of principle: expression of thymidylate synthase, a putative biomarker for pemetrexed, in biopsies and corresponding resection specimens of non-small cell lung cancer (NSCLC)**Arne Warth<sup>1</sup>, Martin Steins<sup>2</sup>, Hendrik Dienemann<sup>3</sup>, Michael Thomas<sup>2</sup>, Peter Schirmacher<sup>1</sup>, Philipp Schnabel<sup>1</sup>, Esther Herpel<sup>1</sup><sup>1</sup> University Hospital Heidelberg, Institute for Pathology<sup>2</sup> Thoraxklinik Heidelberg, Department of Thoracic Oncology<sup>3</sup> Thoraxklinik Heidelberg, Department of Thoracic Surgery

**Aims:** Pemetrexed (Ptx), an inhibitor of thymidylate synthase (TS) has been successfully used in the second-line therapy of NSCLC. TS expression in NSCLC is supposed to have a predictive value, at least for carcinomas with a non-squamous histology. Since most patients eligible for Ptx are not operable, only biopsies are available to test for TS expression. We aimed to determine if TS expression in biopsies corresponds to the expression in the resection specimens.

**Methods:** We analyzed TS expression by immunohistochemistry in 84 consecutive NSCLC biopsies and their corresponding resection specimens. We applied a scoring system according to the predominant immunohistochemical reaction pattern.

**Results:** TS is frequently but heterogeneously expressed in NSCLCs both in nuclei and in the cytoplasm. 2 of the matched pairs were scored lower in bi-

opsies, 46 pairs matched (55%), and 35 pairs (~42%) were scored higher in biopsies compared to the resection specimens. The higher scores in biopsies, especially found in adenocarcinomas, may be a consequence of the higher TS expression in the tumor margins.

**Conclusions:** Immunohistochemical assessment of TS expression in NSCLCs is suitable and the applied scoring system would be appropriate for the majority of the patients. Now, cut-off values for the histological subtypes of NSCLC reflecting the response to Ptx need to be determined.

#### So-059

##### Expression of haptoglobin in different benign and malignant cell-types of the human lung

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**Aims:** The acute phase protein Haptoglobin (Hp) consists of two different polypeptide chains. Originating from the liver; the biological function is binding free hemoglobin (Hb) and preventing oxidative stress. Elevated amounts of Hp in plasma were observed in the course of infection, inflammation and malignant diseases. Purpose of this study was to investigate protein and mRNA expression of Hp within human lung tissues and tumors.

**Methods:** Immunohistochemistry, in situ hybridization and RT-PCR analyzing 115 tissue samples. 47 adenocarcinomas, 42 squamous cell carcinomas, 13 small cell lung cancers and 13 tumor-free lungs were investigated.

**Results:** Immunohistochemistry and in situ hybridization revealed a high level of Hp expression in adenocarcinomas (40.4%) in contrast to squamous cell carcinomas (4.8%, expression in alveolar epithelial cells type II surrounding the tumor). One small cell carcinoma showed Hp expression. In tumor-free lungs we located Hp in alveolar macrophages; alveolar epithelial cells type II and bronchi. RT-PCR results confirmed elevated expression.

**Conclusions:** Due to the known immunosuppressive properties of Hp, its broad synthesis is strongly suggestive for a function as a fundamental pulmonary local defense element.

## Neues zur S3-Leitlinie Lungenkrebs

#### So-060

##### ... aus Sicht der Pathologie

R.M. Bohle

Homburg/Saar

#### So-061

##### ... aus Sicht der Pneumologie

G. Goeckenjan

Kassel

#### So-062

##### ... aus Sicht der Chirurgie

J. Schirren

Wiesbaden

#### So-063

##### Cytological diagnosis of lung cancer with FISH in morphologically equivocal cases. Establishment of new diagnostic criteria

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**Aims:** Cytological diagnosis of lung cancer represents a challenging task, since equivocal cytological diagnoses are not seldom. To elucidate equivocal diagnoses, the LAVYsion multicolour fluorescence in situ hybridization probe set (Vysis, Inc. Downer's Grove) was applied for detection of chromosomal aneuploidy. Published scoring criteria for detection of malignancy so far are sometimes leading to false positive results. In this validating study we applied new scoring criteria for the detection of malignancy.

**Methods:** Bronchial washings/brushings or transbronchial fine needle aspiration biopsies were obtained from a prospectively collected cohort of 210 patients with suspected lung cancer. Clinical and histological follow-up reference standard was met in 191 patients. A routine cytological diagnosis including the accepted diagnostic categories negative, doubtful, suspicious and positive was made and suspicious areas on the slides were re-stained with the LAVYsion multicolour FISH probe set. For analysis of malignancy, a new scoring algorithm was applied.

**Results:** In the whole cohort, FISH achieved a specificity of 98,3% (only 1 seemingly false positive case out of 103 FISH positive cases). In cytologically equivocal diagnoses (n=55), FISH definitely identified malignancy in 78%. In cytologically tumour-cell positive slides (n=68) only one was FISH negative. In 68 cytologically negative slides FISH correctly identified 3 malignant cases.

**Conclusions:** Thus adjuvant application of FISH in cytologically equivocal diagnoses improves diagnostic accuracy. The application of FISH in cytologically negative cases would even enhance sensitivity. The new scoring algorithm is sensitive and do not reduce specificity.

#### So-064

##### Characterization of desmocollins in human lung cancer

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**Aims:** Desmocollins(DSCs) are members of cadherin family involved in carcinogenesis. However their roles in human lung cancer have not yet been well elucidated. The aims of this study were 1) to analyse DSCs expression in lung cancer; 2) to explore the mechanism for downregulation of DSCs; 3) to investigate the regulation of DSC3 expression, and the role of DSCs in cell-cell adhesion mediated by EGFR inhibition.

**Methods:** Expression of DSC 1-3 was analyzed by RT-PCR and Western blotting in lung cancer cell lines and normal lung cells (HBEC and SAEC). In primary lung tissues, the protein expression of DSC1-3 was evaluated by immunohistochemistry (IHC) in tissue microarrays. Methylation status of DSC3 was examined by demethylation test, bisulfite sequencing (BS) and methylation-specific-PCR (MSP) in lung cancer cell lines and in 65 primary lung tumours. The status of histone acetylation was evaluated by treatment with trichostatin A (TSA). To investigate the effect of P53 on DSC3, a putative target gene of P53, transfection with P53 wild type expression vector was performed in lung cancer cell line H2170 and H1299. To determine whether EGFR inhibition could promote desmosome assembly, the lung cancer cell lines will be treated with EGFR inhibitor gefitinib.

**Results:** In a majority of lung cancer cell lines, mRNA expression of DSC1 and 3 was downregulated. In primary lung tumours, more than 50% samples exhibited no expression of DSCs. Low expression of DSC1 was significantly linked to high grading tumours (p = 0.032), while high expression of DSC2 and DSC3 was significantly correlated to squamous cell lung cancer (SCC) (P=0,037 and P=0,000, respectively). Expression of DSCs was restored in 9 out of 11 lung cancer cell lines by 5'-aza-2'-DC and TSA. BS and MSP showed DNA methylation of DSC3 in the region of promoter and exon 1. In primary lung tumours, methylation was found in 44,6% of samples by MSP, which was associated with poor prognosis. Transfection with P53-expression vector re-

sulted in an increased expression of DSC3 in H2170 (DSC3 unmethylated cell line) but not in H1299 (DSC3 methylated cell line).

**Conclusions:** DSC1 and 3 were downregulated in lung cancer. Gene silencing of DSC3 could be explained by DNA hypermethylation. DSC2 and 3 could be markers for patients with SCC, while the methylation status of DSC3 could be a prognostic marker for lung cancer. The regulatory role of P53 on DSC3 should be further investigated in lung cancer cells.

#### So-065

##### Cancer stem cell markers of malignant pleural mesothelioma

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**Background:** Epithelial-to-mesenchymal transition (EMT) have an integral role in the pathogenesis of malignant pleural mesothelioma (MPM). EMT confers stem cell traits to tumor cells. We aimed for investigating the protein expression of putative cancer stem cell (CSC) markers Sox10, podoplanin, nestin and beta-catenin, together with the recognized EMT marker periostin in MPM.

**Patients and Methods:** Tumor tissue of a retrospective cohort of 352 MPM patients was analysed by immunohistochemistry of a tissue microarray (TMA) with antibodies against CSC markers. Protein expression data was correlated with clinico-pathologic parameters including histotype, asbestos exposure and survival as well as with the diagnostic qualifier calretinin.

**Results:** Of the 352 MPM, 117 were of epithelioid, 45 of sarcomatoid and 190 of biphasic histotype; total quadruplicate core number n=1408. Expression of SOX10 was found in 58.3%, nestin in 62.7%, beta-catenin in 58.6%, podoplanin in 51.3% and calretinin in 86.9% of the tumor cells. The CSC marker SOX10 correlated with the sarcomatoid and biphasic HT and interestingly with beta-catenin (p-value <0.001) and the EMT-marker periostin (p-value 0.002). In comparison to previous studies, Calretinin was associated with a better overall survival, like podoplanin and beta-catenin with the epithelioid HT, associated significant with podoplanin and beta-catenin and denoted also like nestin an inverse correlation with periostin (p-value <0.001).

**Conclusions:** CSC markers are differentially expressed in the histotypes, and EMT play an important role in development of MPM.

#### So-066

##### The amount of peritumoral lymphocytes are an independent prognostic factor in non small cell lung carcinoma (NSCLC)

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**Aims:** Non small cell lung cancer inhabit the highest mortality of solid tumors. Tumor infiltrating lymphocytes have been investigated in a variety of solid tumors with diverging results. We investigated the amount of intra- and peritumoral lymphocytes in regard of their impact on clinico-pathologic parameters and patients' survival.

**Methods:** 241 patients suffering from NSCLC operated on with curative intent had been included in our study, 3 cores of each tumor with 2.5 mm diameter were taken to build tissue multi arrays. Immunohistochemical analysis was performed on stains for CD3, CD8 and CD4/CD25 doublestaining. The total number of intra- and peritumoral lymphocytes was counted and correlated with clinico-pathologic parameter as well as survival.

**Results:** Within statistically significant results for the amount of intra- and peritumoral lymphocytes the total number of peritumoral T-lymphocytes (p<0.001) as well as of T-helper cells (p=0.001) and cytotoxic lymphocytes (p=0.005) were of prognostic impact: patients with a high number of peritu-

moral lymphocytes here showed a better outcome. In the multivariate analysis the number of peritumoral lymphocytes not only proved as an independent factor for patients' survival but also showed a higher impact on survival as the UICC-stage.

**Conclusions:** The number of peritumoral lymphocytes proves as a highly significant and independent prognostic marker in NSCLC. Therefore, immunogenic therapies are to be most probably effective also in NSCLC.

#### So-067

##### Analysis of signaling pathways from microarray data provides insight in the development of lung fibrosis

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**Aims:** Lungs from IPF patients show distinct fibrotic patches besides histologically almost normal appearing regions. Gene expression profiles from septa taken from both such regions (N: normal, F: fibrotic) were compared to profiles obtained from septa from healthy donor lungs. It turned out that the profile of N is already considerably altered compared to that of the donors. More and other changes are observed between F and donors. Here we want to apply a modern approach for pathway analysis of microarray data to investigate the pathophysiological changes in the development of IPF.

**Methods:** Septa from 10 IPF and 10 donor lungs were laser-microdissected from lung sections. Isolated RNA was amplified by SMART technology, Cy-labelled and subjected to Agilent Human Whole Genome dual-color microarrays. The spot intensity values were background corrected using the rma algorithm, the intensity ratios were loess normalized. KEGG pathways were analyzed by over-representation analysis (ORA) using candidates selected based on a 5% false-discovery-rate, and by gene set enrichment analysis (GSEA) based on the absolute t-values (mixed analysis).

**Results:** ORA revealed mTOR, Wnt, and p53 pathways perturbed in both, N and F. N additionally showed Notch, TGF $\beta$ , and Insulin. F additionally showed ErbB and VEGF. The results from GSEA include those from the ORA but additionally identify the MAPK pathway in F, ErbB in N and the Ca-signalling pathway in both.

**Conclusions:** The unbiased GSEA is more sensitive than the ORA. Notch signalling seems to play an important role in development of fibrosis in human lungs.

#### So-068

##### SOX2 amplification is a frequent event in squamous cell lung cancer

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**Aims:** Transcription factor SOX2 (3q26.3-q27) is a key regulator of pluripotency in embryonic stem cells and cooperates in the generation of induced pluripotent stem cells. In foregut development, SOX2 plays a critical role by maintaining cells in a pluripotent state. Recently, we found that SOX2 is amplified in about 30% of squamous cell lung and esophageal cancers (Bass et al. Nat Genetics. 2009). These findings suggest that SOX2 is activated by amplification as a lineage-survival oncogene. Activated SOX2 might return adult cells into a stemness state and thus participate in the carcinogenesis and progression of squamous cell lung carcinomas. Aim of our study was to verify SOX2 amplification and protein overexpression in non-small cell lung cancers (NSCLC).

**Methods:** A total of 902 NSCLCs (preliminary adenocarcinomas of the lung (ACL) and squamous cell lung carcinomas (SCLC)) from two independent population-based cohorts (New York, NY: ACL: n=298, SCLC: n=48; and Zurich, Switzerland: ACL: n= 243, SCLC: n=273) were assessed by fluorescence in-situ hybridization and immunohistochemistry. Within the SCLC cohort from Zurich, we assessed for association between SOX2 amplification and clinicopathologic features.

**Results:** In the New York cohort, 5.9% of ACL and 60.4% of SCLC showed a low level amplification of SOX2. High level amplification was found in 8.3% of the SCLC samples. In the Zurich cohort, low level amplification was detected in 6.5% of ACL and in 63% of SCLC samples. 9.6% of SCLC and 0.5% of ACL exhibited a high level amplification of SOX2. SOX2 amplified cases had a significantly higher SOX2 expression compared to non-amplified samples. Within the Zurich cohort we found that high level SOX2 amplification was significantly associated with higher pN stage and an average gain of 20 cigarette packyears.

**Conclusions:** We could confirm frequent SOX2 amplification and overexpression in a large subset of NSCLCs. According to our findings, SOX2 amplification is highly specific for squamous differentiation. The clinical relevance of SOX2 amplification status needs to be further analyzed on independent cohorts.

#### **So-069**

##### **ING4 deletion in non-small cell lung cancer and its clinical-pathological correlation**

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**Aims:** ING4 (INhibitor of Growth family, member 4) located at 12p13 is a tumor suppressor gene playing a role in the cell cycle, cell contact and DNA modification. Deletion of the ING4 gene has been described in human lung cancer, but its clinical significance has not been completely determined yet. Therefore, we investigated a large number of non-small cell lung cancer (NSCLC) and correlated ING4 gene status with clinico-pathological features.

**Methods:** We evaluated 1464 paraffin embedded NSCLC for ING4 gene status on a tissue microarray. We developed a Cy3 labeled fluorescent in-situ hybridization probe using the BAC RPII-433J6 mapping to chromosome 12p13. ING4 deletion was determined as ratio of ING4 gene to centromere 12 reference probe  $\leq 0.8$ .

**Results:** Informative results were obtained from 765 (52.3%). Among those were the following histological subtypes: 384 squamous- (50.2%), 187 adenocarcinomas (24.4%), 160 large cell- (20.9%), 22 bronchiolo-alveolar- (2.9%) and 10 adenosquamous carcinomas (1.3%) as well as 2 with sarcomatoid differentiation (0.3%). We found in 18 (2.4%) an ING4 deletion among them 10 squamous- (2.6%), 7 large cell- (4.4%) and one adenocarcinoma (0.5%). ING4 deletion was significantly ( $p=0.013$ ) associated with higher N-category and showed a tendency towards higher tumor stage ( $p=0.055$ ). Patients with an ING4 deletion showed a worse survival but this difference was not significant.

**Conclusions:** ING4 deletion in NSCLC is rare (2.4%). It occurs mostly in large cell- and squamous cell carcinomas and it is associated with lymph node metastasis. These findings underline the function of ING4 as a tumor suppressor gene.

# Autorenregister

Alle Erstautoren mit Programmheftnummern  
(Geladene Referenten sind kursiv)

## A

Adam, A. Do-019  
Adam, P. Do-039  
Agaimy, A. Fr-012  
Agaimy, A. Fr-013  
Agaimy, A. Fr-059  
Ahrens, P. Sa-018  
Alzoughbi, W. Do-057  
Anagnostopoulos, I. Do-040  
Anagnostopoulos, I. Fr-071  
Andrulis, M. Do-025  
Antony, P. Sa-110  
Aretz, S. Do-011  
Assmann, G. Sa-117  
Aulmann, S. So-023  
Aust, D. Do-016

## B

Baba, H. A. Do-018  
Baretton, G. Do-008  
Barth, T. Do-037  
Barth, T. Fr-148  
Baumhoer, D. Do-058  
Becker, K. F. Fr-141  
Becker, L. E. Sa-067  
Beleut, M. Fr-098  
Berezowska, S. So-004  
Berg, D. Sa-087  
Bergmann, F. Fr-065  
Berndt, A. So-039  
Bernhardt, A. Fr-005  
Bertram, S. Fr-048  
Bertz, S. Do-068  
Bertz, S. Do-069  
Bertz, S. Fr-105  
Bihl, M. Fr-040  
Bläker, H. Fr-019  
Blank, P. So-030  
Bock, O. Fr-153  
Bockmeyer, C. L. Fr-089  
Bockmeyer, C. L. Sa-043  
Bockmeyer, C. L. So-027  
Bohle, R. M. So-060  
Böhme, R. Sa-078  
Böllner, C. So-021  
Bornemann, J. Sa-058  
Brand, K. Fr-046  
Bräsen, J. H. Do-063  
Braun, M. Do-073  
Braun, M. Sa-051  
Breinig, M. Fr-066  
Breuhahn, K. Sa-106  
Bröcker, V. Fr-090  
Bronsert, P. Sa-007  
Buckendahl, A.-C. Fr-038  
Buettner, M. Sa-033  
Buettner, M. So-053  
Burandt, E. Sa-086  
Burandt, E. So-018  
Burger, M. Do-079

*Büttner, R.* Sa-114

## C

Calvisi, D. F. Do-021  
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Calvisi, D. F. Fr-050  
Calvisi, D. F. Sa-122  
Calzada-Wack, J. Fr-121  
Chen, Y. Sa-040  
Christgen, M. Sa-088  
Cui, T. So-064

## D

Daniels, M. Fr-014  
Darb-Esfahani, S. So-031  
Denkert, C. Sa-091  
Diederichs, S. Fr-140  
Diederichs, S. Sa-064  
Dietmaier, W. Sa-100  
Dimitrova, L. Do-042  
Droeser, R. So-020  
Dürkop, H. Fr-083

## E

Egervári, G. Fr-067  
Egger, A. So-050  
Eiminkel, J. Sa-005  
Elfimova, N. Fr-061  
Elste, A. Sa-049  
Eltze, E. Fr-115  
Engelhardt, B. Do-031  
Engels, K. Sa-009  
Eppenberger-Castori, S. So-028  
Ergin, B. Sa-047

## F

Faber, C. Fr-037  
Fend, F. Fr-143  
Fichter, C. D. Fr-009  
Fischer, C. So-040  
Flucke, U. Sa-019  
Franz, M. Sa-055  
Friedrich, K. So-034  
Fritzsche, F. Fr-093  
Fritzsche, F. Fr-118  
Fritzsche, F. Sa-024  
Fritzsche, F. Sa-057  
Fritzsche, F. So-035  
Fronhoffs, F. So-048

## G

Gajda, M. Do-067  
Gajda, M. Fr-062  
Gajda, M. Sa-003  
Gajda, M. Sa-014  
Galler, K. Sa-056  
Gaßler, N. Fr-017  
Gattenlöhner, S. Do-035  
Gattenlöhner, S. Fr-120

Gattenlöhner, S. Fr-146  
Gehoff, A. Fr-043  
Georg, G. Sa-081  
Gerhardt, J. Do-076  
Giedl, J. Fr-106  
Giger, O. Fr-032  
Glatz, K. So-011  
Glombitza, F. So-041  
Gluz, O. So-016  
Goeckenjan, G. So-061  
Goepfert, B. Fr-057  
Grabellus, F. Fr-084  
Grabellus, F. Sa-027  
Grasse, K. Fr-138  
Grobholz, R. Fr-114  
Groß-Weißmann, M.-L. Sa-065  
Guertl, B. So-043

## H

Hafner, C. Sa-116  
Hager, M. Fr-099  
Hager, T. So-047  
Haller, F. Do-006  
Haller, F. Fr-010  
Haller, F. Sa-104  
Hammerschmied, Ch. Do-064  
Hann von Weyhern, C. Fr-116  
Hannig, H. Sa-079  
Hansmann, M. L. Fr-149  
Haroske, G. Do-050  
Haroske, G. Do-051  
Hartmann, A. Fr-134  
Hashani, M. Sa-066  
Haybaeck, J. Fr-056  
Heikaus, S. Fr-100  
Helpap, B. Do-080  
Henkel, C. Do-070  
Henopp, T. Fr-162  
Herpel, E. Sa-037  
Herpel, E. Sa-070  
Herpel, E. Sa-071  
Herpel, E. Sa-072  
Hessel, H. Sa-092  
Hirsch, B. Fr-160  
Hlubek, F. Sa-080  
Höhn, A. K. Fr-102  
Höllner, S. Fr-077  
Höllner, S. Fr-158  
Horn, L.-C. Fr-101  
Horn, L.-C. Fr-107  
Horn, L.-C. Sa-001  
Horn, L.-C. Sa-002  
Horn, L.-C. Sa-006  
Horn, L.-C. Sa-013  
Horn, L.-C. Sa-016  
Horvath, H. Fr-034  
Huss, S. Fr-023  
Hussein, K. Do-027  
Hussein, K. Do-032  
Hussein, K. Sa-032  
Hussein, K. So-046  
Hussein, K. So-052

## I

Ihrler, S. So-037  
Ikenberg, K. So-010

## J

Jäkel, J. So-006  
Jankowiak, F. Fr-027  
Jayasinghe, C. Fr-045  
Jayasinghe, C. So-045  
Jöhrens, K. Fr-021  
Jöhrens, K. Fr-072  
Jonigk, D. Sa-036  
Joosten, M. Do-036  
Jung, A. Sa-103  
Jungbluth, A. Sa-062

## K

Kähler, D. Sa-039  
Kalinski, T. Do-043  
Kalinski, T. Sa-107  
Kallenbach, A. Fr-028  
Kapp-Schwörer, S. Do-030  
Kayser, G. Do-046  
Kayser, G. So-066  
Kemmerling, R. Fr-076  
Kiesl, A. Do-015  
Kirchner, T. Fr-126  
Kiss, S. Fr-003  
Klaus, C. Fr-035  
Klauschen, F. Do-048  
Kloten, V. So-019  
Klotzsche-von Ameln, A. Sa-048  
Knösel, T. Do-053  
Knösel, T. Sa-038  
Knüchel-Clarke, A. Sa-108  
Kohler, I. Do-001  
Köhler, K. Fr-011  
Koitzsch, U. Sa-076  
Koleganova, N. Do-061  
Koleganova, N. Do-062  
Koleganova, N. Sa-063  
Kraft, S. So-009  
Krech, T. Fr-123  
Kriegel, L. Do-014  
Kriegsmann, J. Fr-088  
Kristiansen, G. Do-072  
Kristiansen, G. Sa-029  
Kristiansen, G. Sa-097  
Kristiansen, G. So-014  
Küffer, S. Fr-137  
Kurt, R. Sa-098  
Kurz, K. Fr-079  
Kwecinski, M. Sa-125

## L

Lang, D. Sa-025  
Länger, F. So-055  
Langer, R. Do-005  
Langer, R. Fr-007  
Langner, C. Do-024  
Lasitschka, F. Fr-020  
Lassmann, S. Do-012  
Lassmann, S. Fr-131  
Lax, S. So-033  
Lay, M. Fr-039  
Lehmann, U. Sa-101  
Lenze, D. Fr-030  
Lenze, D. Fr-147  
Lenze, D. Sa-031