of VHL function increases the expression of several growth factors like VEGF, PDGF and TGF-a. The activity of these factors is associated with tumour angiogenesis, growth and progression. Multitarget kinase inhibitors such as Sunitinib and Sorafenib, focusing on the inhibition of the involved pathways, constitute the current gold standard in therapy. However, non-responders and side effects suggest that our knowledge about the affected signal networks in renal cancer is still incomplete.
The aim of this proteome project is to analyze human kinases (kinome) as major and druggable signaling components in renal cancer patients systematically. Both protein regulation and site-specific phosphorylation, signifying their activity status, were comparatively examined by quantitative peptide sequencing (LC-MS/MS): Tumour samples and "healthy" counterparts dissected from nephrectomies were used as starting material to affinity purify more then 150 kinases by chemical proteomics. iTRAQ^{TM} peptide labelling of the kinase-enriched fractions in combination with a novel statistical validation method allowed the detection of RCC-associated alterations. In addition to already known cancer-related proteins this approach suggests novel kinases that have to be considered for diagnosis and as potential drug targets.

178 Development of a novel PEG-DOX-E-[c(RGDfK)2] conjugate for avb3 integrin-targeted cancer therapy

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Background: Targeting drugs that will affect selectively the tumour site is of great challenge and thus has become a critical issue while designing an anticancer drug. Doxorubicin (DOX) is extensively used in cancer therapy; however, it is cardiotoxic in cumulative doses and chemoresistance can evolve with its prolonged use. Conjugation of a chemotherapeutic agent with a water-soluble polymeric carrier prolongs its circulation time, promotes its accumulation at the tumour site due to the enhanced permeability and retention (EPR) effect and prevents the drug from extravasating into healthy tissues. We synthesized a PEG-DOX-E-[c(RGDfK)$_2$] conjugate which actively and selectively targets endothelial and tumour cells overexpressing $\alpha_v\beta_3$ integrin.
Methods: a_vb_3 cell surface expression was determined by flow cytometry. The fluorescent properties of doxorubicin were utilized to follow the cellular uptake of PEG-DOX-E-[c(RGDfK)$_2$]. The cytotoxicity profile of the conjugate was assessed by MTT assay. The ability of PEG-DOX-E-[c(RGDfK)$_2$] conjugate to overcome DOX-resistance was determined by cytotoxicity assay on M109 sensitive and resistant murine lung carcinoma cells. The anti-angiogenic properties of our conjugate were evaluated on human umbilical vein endothelial cells using cytotoxicity and adhesion to fibrinogen assays. Tumour specific accumulation of PEG-E-[c(RGDfK)$_2$] in mCherry-labeled mammary adenocarcinoma inoculated in mice was followed by non-invasive fluorescence imaging.
Results: The PEGylation of DOX and E-[c(RGDfK)$_2$] had resulted in a conjugate of 15 kDa in size. PEG-DOX-E-[c(RGDfK)$_2$] conjugate binds to U87-MG glioblastoma cells overexpressing a_vb_3 integrin, internalizes and demonstrates a similar cytotoxic effect as free DOX following incubation. PEG-DOX-E-[c(RGDfK)$_2$] conjugate overcomes resistance to DOX of M109R murine lung carcinoma cells. PEG-DOX-E-[c(RGDfK)$_2$] had an inhibitory effect of ~75% on HUVEC attachment to fibrinogen. Preliminary *in vivo* near-infrared studies revealed that a PEG-E-[c(RGDfK)$_2$]-cyanine conjugate preferentially accumulated in mCherry-labeled-DA3 murine mammary tumours.
Conclusions: Our results show a proof of principle for a selective delivery of DOX to endothelial and cancer cells overexpressing a_vb_3 integrin. By showing the advantages of our conjugate which accumulates selectively at the tumour site, we hope to warrant it as a novel targeted, anti-angiogenic and anticancer therapy.

179 Elevated expression of cyclooxygenase-2 is a negative prognostic factor for disease free survival and overall survival in patients with gastric carcinoma

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Background: Cyclooxygenases regulate the production of prostaglandins and play a role in tumour development and progression. We investigated the prognostic impact of expression of the cyclooxygenase (COX) isoform, COX-2, on disease-free survival and progression-free survival in patients with primary gastric adenocarcinoma (any pN any pT) without distant metastasis as well as the association between COX expression and other clinicopathologic parameters.
Patients and Methods: A cohort of 194 patients with gastric cancer (123 males, 87 women) without distant metastasis who underwent R0 gastric resection were enrolled in this study. Immunoistochemical immunoreactivity was assessed by the intensity of staining and percentage of positivity areas. Association between factors including clinico-pathological variables and COX-2 scores, were assessed by χ^2 and Student t test. Survival rates were calculated using Kaplan–Meier method and the difference between the groups were analyzed by log-rank test.
Results: A correlation between COX-2 expression, grading and advanced penetration dept (mean COX-2 expression 74% in early gastric cancer (EGC) versus 52% in non-EGC, $p = 0.0017$). There was an association between COX-2 expression and the presence of lymph-node metastasis ($p < 0.0001$, χ^2). We also observed a significant association between COX-2 expression and relapse of disease ($p = 0.05$ KM) but not with poor survival.
High COX-2 protein expression, serosal invasion (pT3-pT4), and presence of lymph-node metastasis are poor prognostic factors in patients with gastric carcinoma without distant metastasis. COX-2 expression in any percentage strongly correlates with lymph-node invasion and penetration dept, so it may indicate tumour aggressiveness.
Conclusions: The current data suggest that increased expression of COX-2 may play a role in the progression of primary gastric carcinoma. It remains to be investigated whether treatment with selective inhibitors of COX-2 may be an additional therapeutic option for patients with gastric carcinoma.

180 Biomarker discovery in plasma of breast cancer patients using microspot immunoassays

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The potential use of biomarkers in breast cancer includes aiding early diagnosis, determining prognosis and predicting response or resistance to different therapies. The ease with which blood can be sampled makes it a logical choice for biomarker applications.
Over the past few years different protein microarray platforms emerged as experimental tools for biomarker discovery. The microarray format allows for simultaneous determination of various parameters from a minute amount of sample within a single experiment. The experimental design of microspot immunoassays is based on antibody pairs specifically recognizing different epitopes of the analytes. One antibody is used to capture the analyte from the complex sample and the second antibody is used for detection.
Various transmembrane proteins are proteolytically released from the cell surface by a process known as ectodomain shedding both under normal and pathophysiological conditions. We have developed a microspot immunoassay for the evaluation of biomarker signatures focusing on the ectodomain shedding products of the ERBB1, ERBB2, and ERBB3 receptor. In addition, the ectodomain shedding product of the MET receptor is quantified as well. This 4-plex microspot immunoassay has been used to determine target protein concentrations in 100 plasma samples from breast cancer patients taken at primary diagnosis. The resulting quantitative data was compared with clinical data, e.g. lymph node status. This study gives an overview of baseline ectodomain shedding product levels in breast cancer patients at primary diagnosis and serves as a basis for a long term follow up study in these patients. Approval of the study was obtained from the local ethics committee at the University of Heidelberg.

181 Accuracy of castPCR-based KRAS testing on paraffin embedded tissues

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Background: Predicting targeted therapy sensitivity has become a part of the standard care of patients with solid tumours to optimize treatment options. Molecular marker testing for patient care implies that technologies move from the bench to the clinics. Testing methods have therefore to be properly validated and quality control procedures have to be established. Care samples can be heterogeneous, possibly due to poor quality and quantity. Using KRAS testing as an example, we report here the analytical performances of the competitive allele specific TaqMan PCR (castPCR) test developed by Applied Biosystems.
Material and Method: CastPCR assays were designed and manufactured by Applied Biosystems. CastPCR assays for seven KRAS mutations were tested on an ABI7900HT using Universal Genotyping Master Mix (Applied Biosystems, Foster City, USA). Eight mutated cell lines were initially used to validate the assays (H1573:p.G12A; H358:p.G12C; A427:p.G12D; LS123:p.G12S; SW620:p.G12V; Lovo:p.G13D; SW48:Wild Type; Tours: p.G12R). Tours is a cell line obtained after directed mutagenesis for G12R mutation. DNAs were extracted using QIAamp DNA Mini Kit (Qiagen, Courtaboeuf, France). Mutated DNAs were titrated in the wild type DNAs from 100% to 0.5%. Twenty-four anonymous tumours and 12 non-tumour tissues

from paraffin embedded specimens were also examined for possible mutations. All PCR reactions were run in triplicates.
Results: In cell lines, mutations were identified without ambiguity up to the 0.5% dilution. No positive samples were found in non-tumour tissues. This first analytic part allowed us to define for each mutation proper Cq cut-off to analyse KRAS mutation in specimens. The results obtained by castPCR for the 24 tumours were concordant to those previously reported by three different methods (HRM-sequencing, snapshot and TaqMan probes).
Conclusion: This work shows different validation steps of a new KRAS genotyping technology, suitable for KRAS determination in the clinics on paraffin embedded specimens with a very good sensibility and specificity. It is a quick, one-step technology compatible with diagnosis demand. Testing is on going using the 7500 Dx Fast that is to be CE IVD marked. The assessment of this highly sensitive technology will be carried out on body fluids to analyse its accuracy for KRAS testing when tumour tissues are not available.

182 Clinical significance of target genes of Wnt/beta-catenin pathway in hepatocellular carcinoma

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Background: Hepatocellular carcinoma (HCC) is the sixth most common cancer worldwide and is particularly prevalent in Southeast Asia and China including Hong Kong. Wnt/beta-catenin pathway is one of the important signaling pathways and therapeutic target in liver cancer. In this study, we aimed to reveal the expression profile of these target genes and to investigate their clinicopathological significance in human HCC.
Material and Methods: To elucidate the molecular mechanism of the deregulation of Wnt/beta-catenin pathway in HCC, we performed high-throughput quantitative RT-PCR analysis (Low Density Microarray, LDA) to study the gene expression patterns of Wnt-signaling molecules, include Wnt ligands, Fizzled receptor proteins, Wnt-related genes, on 38 pairs of human HCC samples and their corresponding non-tumourous livers. Ten target genes of Wnt/beta-catenin signaling were also evaluated, include c-myc, cyclinD1, LEF1, c-jun, Axin-2, VEGFA, DKK1, Frizzled 7, Twist1 and EGFR.
Results: LEF1 and DKK1 were found to be significantly overexpressed in human HCC (63% and 66%, respectively) when compared with their corresponding non-tumourous livers ($P < 0.001$ and $P = 0.005$, respectively). In addition, the expression level of LEF1 and DKK1 positively correlated with one another ($P = 0.037$). LEF1 was found to significantly correlate with venous invasion, absence of encapsulation and advanced tumour stage ($P = 0.043$, 0.008 and 0.045, respectively). The combination of LEF1 and DKK1 further increased their correlation with clinicopathological parameters in human HCC, like venous invasion and poorer cellular differentiation (Edmondson grading) ($P = 0.021$ and 0.047, respectively).
Conclusions: Both LEF1 and DKK1 are upregulated in human HCC and associated with more aggressive tumour behaviour.
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183 Correlated expression analysis of VEGF family members and lipid inflammatory mediators in human colon polyps and carcinomas and liver metastases

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Background: Inflammatory mediators, such as prostaglandin E_2 (PGE_2), and responsive angiogenic factors, mainly vascular endothelial growth factor A (VEGF-A), have emerged as pathways driving neo-angiogenesis and supporting the progression and metastasis of solid tumours.
Materials and Methods: To understand the relation in human solid tumours between COX and LOX-derived eicosanoids and expression of VEGF family members ($VEGF^F$) (VEGF-A, -B, -C, -D and PlGF), we performed a RT-qPCR comparative expression analysis of colon carcinoma samples. Considering shifts in expression profiles during tumour progression, a similar analysis was done for colon polyps and liver metastases.
Results: Up to now, tumour samples and matched normal colon tissues from 52 patients were analyzed. The results showed a complex and diversified expression phenotype. 88% of the tumour samples showed increased expression of at least one VEGF family member. In a considerable proportion of samples multiple VEGF family members were overexpressed with a predominance of VEGF-A and especially PlGF. Correlating the $VEGF^F$ and eicosanoid enzymes gene expression profiles not only revealed a clear linkage between both signaling pathways but also a clear association of COX2 with VEGF-A, VEGF-C and PlGF.
A similar analysis was performed on 23 colon polyps and 30 liver metastases. Strikingly, already in polyps a pronounced inflammatory expression profile with increased expression of COX enzymes was apparent. This was accompanied by an increased expression of mainly VEGF-A and PlGF. Also in liver metastases, an inflammatory signature accompanied by $VEGF^F$ expression was apparent. Remarkably, the $VEGF^F$ profiles observed in liver metastases were near indistinguishable to those from the primary colon carcinomas. Yet, the eicosanoid enzymes showed differential expression profiles which may be due to the different tumour microenvironment in the liver.
Conclusions: The results from this correlated expression analysis of VEGF family members and genes involved in eicosanoid biosynthesis are promising for the diagnosis and prediction of treatment outcome of colon cancer patients. In addition, our results clearly indicate that the perception of a $COX2/PGE_2$-driven VEGF-A expression, sustaining neo-angiogenesis, is an oversimplification.

184 Diagnostic radiation exposures among children diagnosed with leukemia vs. solid tumours in US, UK and Germany, 1995–2005

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Background: Determining the role of medical diagnostic radiation exposures in children subsrequently diagnosed with a type of cancer is challenging and even controversial. Recent literature reviews point to these difficulties in case studies and sample size due to the rarity of childhood cancers in general. This study will start with a literature review of both diagnostic x-rays and CT scans over a ten year period in the US, UK and Germany to determine both national differences and differences in frequency among children diagnosed with leukemia versus solid tumours.
Material and Methods: The author will use published sources, archival materials from Children's Oncology Group and parental questionnaire.to determine pre-natal, in utero and childhood exposures.
Results: Preliminary results indicate a higher frequency among children diagnosed with any type of leukemia, followed secondly by children with central nervous system tumours. Further studies of each major tyoe of sarcoma would be useful to further study.
Conclusions: This study found that children with leukemias were more likely to have a history of prenatal exposure to diagnostic radiation in the western nations studied over this time period. Much further and detailed study is warranted by time period and by cancer type.

185 Imaging by confocal endomicroscopy: new insight for in vivo tissue diagnosis of head and neck cancer

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Background: Histological analysis of tissues is essential for cancer diagnosis and to guide therapeutic choices, but a biopsy is an invasive procedure that may delay treatment decisions. The biopsies required for the diagnostic may also cause changes in local tissue and alter the assessment of small tumour extension during the resection procedure. Recently, non-invasive optical technologies based on the miniaturization of imaging systems have been proposed to achieve in vivo "optical biopsies". Among them, fibered confocal endomicroscopy (CEM) provide dynamic images of the microarchitecture of tissues during a conventional endoscopy. In this study, we evaluated the potential of CEM to aid the detection of precancerous lesions and laryngeal cancer.
Material and Methods: Fluorescent agents clinically approved or in clinical trials were used to enhance image contrast. A staining protocol directly transferable to humans has been developed for a futur clinical study. 47 non cancerous and cancerous tissue samples were taken from human surgical head and neck specimens after total or partial surgery. After topical application of acriflavine hydrochloride and sodium fluorescein, samples were imaged with CEM (Cellvizio, MKT), and conventional confocal microscope (Leica SPE). An histomorphologic correlation study was subsequently performed on 140 CEM images, 140 confocal images and 47 conventional histological preparations of the same samples. The images were interpreted by two pathologists in a double-blind trial.
Results: The 2 fluorescent dyes allowed a morphological analysis based on the cellular distribution, detection of nuclear abnormalities and visualization of disorders of keratinization. The histological diagnostic such as dysplasia or invasive squamous cell carcinoma could be interpreted with the images from CEM, although the image quality was inferior to conventional confocal microscopy. Statistical correlation with conventional histology is being finalized.
Conlusions: This study demonstrated the CEM ability to provide "histological-like images" that can be interpreted by pathologists, even on complex tissues as in the case of head and neck cancers. The study conducted jointly with clinicians and pathologists also identified the conditions of transfer of this