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

technique into clinical routine. A stiffening of the tip of the optical probe seems needed to improve the live imaging of laryngeal structures.

186 Study of zinc-dependent aggregation of metallothionein from human prostatic cancer cell lines

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Background: Metallothioneins (MTs) are heavy-metal binding cysteine-rich proteins. MTs are thought to play a role in tumour disease. They can be used for studying of course, prognosis and metastating of a tumour. The enhanced MTs expression leads to the formation of cytostatics resistance in tumour cells. One the current accepted opinion is that these mechanisms are closely connected to ability of MT to bind heavy metal ions and form aggregates.
The aim of this work was to study the changes in aggregation of MT under various concentrations of zinc and different redox conditions in human prostatic cell lines.
Material and Methods: Reduced apo-MT was oxidized with 0.75 and 3.5 % H_2O_2, 0.5 and 1 μM $K_2Cr_2O_7$, and 0.5 and 1 μM $KMnO_4$ for 2 hours at 37ºC. To the reduced and oxidized apo-MT $ZnCl_2$ was added. Reaction was measured spectrometrically for 1 hour at 37ºC. MT oxidation and interaction with zinc was evaluated by measuring of spectra within the range from 200 to 300 nm. Human cancer prostatic cell line (PC-3) and control cell line (PNT1A) were used.
Results: In *in vitro* experiments we observed Zn binding and aggregation of both (reduced and oxidized) forms of MT. The oxidized observed a peak with maximum at 250 nm. The height of signals of both forms increased with time of the interaction and $ZnCl_2$ concentration. Comparing reduced and oxidized MT, the oxidized molecules had higher binding capacity. The increased formation of MT aggregates in molecular weight higher than 75 kDa was observed in dependence on $ZnCl_2$ concentration and degree of oxidation as a proportion of the most intense signal at 25 kDa measured by Experion. In cancer cell lines increased viability was observed compared to controls and the same MT aggregates were observed as at standards in dependence on $ZnCl_2$ concentration.
Conclusion: It can be concluded, that zinc supports MT expression and growth in human prostatic cell lines and that MT forms aggregates in dependence on redox conditions and zinc concentration both in *in vitro* and in *in vivo* conditions.
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187 Expression of multidrug resistance gene and DNA ploidy in locally advanced gastric and gastroesophageal junction adenocarcinoma

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Background: We intended to assess the expression of multidrug resistance (MDR-1) gene & DNA ploidy and correlate these with disease outcome in patients with locally advanced gastric/gastroesophageal junction adenocarcinoma (LAGC) receiving neoadjuvant chemotherapy (NACT), surgery and postoperative chemoradiation (POCRT).
Materials and Methods: We enrolled 14 patients (pts) of LAGC (stage II-IV, age $\leqslant$70 years, KPS $\geqslant$70) in a single arm phase-II trial. After 2 cycles of NACT (cisplatin 80 mg/m^2 D1, capecitabine 2 g/m^2/day D1−14 q3 weeks), response was assessed by upper GI endoscopy & CECT abdomen. Pts with resectable tumours underwent radical total/subtotal gastrectomy with D2 lymphadenectomy & POCRT (45 Gy/25#/5 weeks, concomitant capecitabine 1.5g/m^2/day). Inoperable pts received salvage chemoradiation (SCRT) or best supportive care (BSC). MDR-1 gene expression was evaluated by flowcytometric assay of P-glycoprotein positivity. DNA ploidy of tumour was assessed by DNA flowcytometry.
Results: Median age was 50 years. Male to female ratio was 9:5. Tumour locations included GE junction/proximal stomach (4), distal stomach (8) & diffuse (2). Radiology showed N+ & T4 disease in 11 & 6 pts respectively. In 50%, tumours were initially unresectable. At a mean follow-up of 7.36 months, only 3 pts completed assigned treatment & all 3 had complete response (CR). Among others, locoregional progression, distant metastasis & noncompliance were noted in 4 (28.6%), 3 (21.4%) & 4 (28.6%) pts, respectively. After NACT, response rate, disease control rate & symptomatic benefit were 28.6%, 57.2% & 78.6%, respectively. MDR-1 expression was monotonously low (mean 5.13%) & was not turned on after NACT (mean 3.2%). Difference in MDR-1 gene expression in-between tumour (mean 5.13%) & control tissue from normal gastroesophageal mucosa (mean 4.43%) at baseline was unremarkable. 66.6% of the analyzed patients had pure diploid tumours & 33.3% had mixed (diploid & aneuploid) tumours at baseline. Multiploidy (>1 aneuploid stemline) was noted in 1 patient (11.1%). All 3 patients who completed the assigned treatment & had CR, had diploid tumours at baseline. All 3 patients who had distant failure (2 peritoneal dissemination & 1 liver metastasis) had a sizable aneuploid cell population at baseline.
Conclusions: NACT followed by POCRT is a novel & safe approach in LAGC. Still, only 21.42% pts completed assigned treatment & had CR, possibly owing to high noncompliance (28.6%) & adverse patient characteristics. MDR-1 gene pathway is probably not the major mechanism of chemoresistance in LAGC in our patients. DNA ploidy might be a useful prognostic marker in LAGC with distant failures being associated with aneuploidy.

188 A novel mechanism for lung cancer migration and invasion through LPA-CARMA3-NF-kappaB signaling axis

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Lung cancer is one of the most common cancers in the world. It is a leading cause of cancer death in men and women in the United States and throughout the world. Lung cancer can be triggerd by many factors, such as lysophosphatidic acid (LPA). LPA is a type of G protein-coupled receptor ligand, and a bioactive mediator that promotes cancer cell proliferation and motility through activation of cell surface G protein-coupled receptors. LPA activates NF-kappaB, which is an important transcription factor and plays critical roles in tumourigenesis, such as tumour migration and invasion. We have previously reported that CARMA3 (CARD and MAGUK domain-containing protein 3) is indispensable in LPA-induced nuclear factor kappa B (NF-kappaB) activation in mouse embryonic fibroblast cells. However, it remains unknown whether the CARMA3 plays an important role in LPA-induced lung cancer cell migration and invasion. In the present study, using CARMA3 shRNA, we knockdowned its protein expression level in lung cancer cell lines. Consistent with previous reports, we found that down-regulation of CARMA3 strikingly impaired LPA-induced IKK activity and NF-kappaB activation in lung cancer cells. In addition, *in vitro* transwell migration and matrigel invasion assays demonstrated that CARMA3 shRNA significantly attenuated LPA-induced lung cancer cell motility and invasiveness. Together, our results provide the evidence that CARMA3 serve as a critical regulator in LPA-induced, NF-kappaB-mediated lung cancer cell migration and invasion. Therefore, we speculate that CARMA3 may represent an attractive therapeutic target for lung cancer cell and many other alignancies.

189 Sensitive detection of KRAS and BRAF mutations using mutant-enriched PCR and reverse-hybridization teststrips

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Background: KRAS and BRAF are key players in growth factor receptor induced signalling pathways. Somatic mutations in the two genes are known to play a role in oncogenesis and are found in various types of tumours, including colorectal, pancreatic, thyroid, lung and skin cancer. The most critical region of the KRAS gene for oncogenic activation are mutations in codons 12 and 13. Among BRAF mutations, V600E is by far the most frequently observed. KRAS and BRAF mutations are also known to be predictive for the response to cancer therapy with certain anti-EGFR monoclonal antibodies.
Materials and Methods: We have developed a reverse-hybridization StripAssay targeting ten KRAS codon 12/13 mutations as well as BRAF V600E. The test is based on PCR in the presence of KRAS/BRAF wild-type suppressors (mutant-enriched PCR), followed by hybridization of PCR products to teststrips presenting a parallel array of allele-specific oligonucleotide probes. The hybridization and detection steps can be carried out fully automated using commercially available instrumentation. StripAssay performance was evaluated on genomic DNA obtained from cultured cell lines, formalin-fixed paraffin-embedded (FFPE) tissue and stool.
Results: Using serial dilutions of DNA from various KRAS- and BRAF-mutant tumour cell lines into normal DNA, each mutation was shown to be detectable