quantitatively studied at the proteomic and transcriptomic levels using Reverse Phase Protein Arrays (RPPAs) and qRT-PCR, respectively. Direct targeting of miRNAs is validated by luciferase assays as well as site-directed mutagenesis, and identified miRNAs/targets will be studied in different cancer-relevant cell-based assays (e.g. invasion and BrdU/7-AAD assays). Finally, we will test the effects of miRNAs/targets in combination with several ERBB receptor-targeting drugs e.g. gefitinib or trastuzumab in different cell line models (drug sensitive vs. resistant). Hence, combining bioinformatics, functional genomics, proteomics as well as molecular and cellular biology approaches, we integrate miRNAs into the well-studied ERBB network for combinatorial targeted therapy.

831 Applying JISTIC to different stages for breast cancer

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Motivation: We wanted to compare array CGH DNA profiles of various stages breast cancer as a surrogate of the course of tumourigenesis and identify significant aberrations that may be driving the process forward at each stage.

JISTIC (http://www.c2b2.columbia.edu/danapeerlab/html/jistic.html) is a bioinformatic tool useful to analyze datasets of genome-wide copy number alteration that detects driver aberrations in cancer. JISTIC implements the standard, focal and arm-peel-off for the widely used GISTIC (Genomic Identification of Significant Targets in Cancer) algorithm developed by Beroukhim et al. (PNAS ref, 2007). We applied JISTIC to identify the regions that are likely to be driver aberrations of the carcinogenesis regions.

Material and Methods: DNA copy levels were obtained in the form of log R values from 244 K array CGH (Agilent). Total 588 profiles of pre-malignant and malignant breast tissue were gathered from five different clinical cohorts involving healthy tissues from mammographically dense breasts, breast tumours without invasive compontent (DCIS), and stage I−IV tumours.

We segmented the CGH logR by applying piecewise constant function and performed centering of the values to avoid differences by cohorts. The centered data were divided into different stages, normal, DCIS, stage I (T1), II (T2) and III (T3). JISTIC was applied to each stage. JISTIC identified significant aberration regions and peaks.

The sets of genes identified in the significant aberration regions, were subjected to GO analysis by DAVID http://david.abcc.ncifcrf.gov.

Results: The significant peaks were 20 for normal, 19 for DCIS, 103 for T1, 92 for T2 and 25 for T3, which were in focal region, in the preliminary analysis. The number of peaks increased with increasing stages from DCIS to T2 but leveled off at T3. For the genes identified in the loci of significant aberrations, we applied GO analysis. In the case of normal, phospholipid biosynthetic process for deletion was identified and no significant term for amplification. DCIS indicated C21-steroid hormone biosynthetic process for amplification, and a term for antigen processing and again lipid metabolic process for deletion. For the remaining cases, significant GO terms were 16 (T1), 11(T2), 2(T3) for amplification, and 15(T1), 6(T2), 7(T3) for deletion.

Conclusions: Applying JISTIC to different stages of breast cancer indicated some trends; specific aberrations were observed at each stage of breast cancer pointing to important biological processes. However, since the sample size for normal, DCIS and T3 was smaller than the sample size for T1 and T2, further studies will be needed to support these preliminary findings.

I would like to thank Vilde D. Haakensen, Simen Myhre and Lars O. Baumbusch for providing the CGH data.

832 Human liver regeneration and its clinical impact

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Background: Study concerning human liver regeneration (HLR) evaluates the hepatic extraction fraction (HEF) using radioisotopic methods as an indicator of HLR of patients underwent hepatectomy (HR).

Material and Methods: 95 patients with colorectal metastases ($n=69$), hepatocellular carcinoma ($n=16$) and other tumours ($n=10$) were included. 37% underwent major hepatectomy (*MA*HR) and 63% minor hepatectomy (*mi*HR). HLR was assessed after intravenous bolus injection of ^{99m}Tc-Mebrofenin. The HEF is calculated using deconvolution analysis of first pass curve coming from scintigraphic data. We evaluated the pre-operative HEF (T0) and in the 5th day (T5) and one month after PH (T30). We considered the HEF values of 98.8±0.4% as normal.

Results: Overall patient population: the mean HEF values in our series were 97.3±9.6% for T0, 97.5±8.3% for T5 and 98.7±4.1% for T30(ns). The mean Tmax values were 15.6±8.1 min for T0, 13.9±5.7 min for T5 and 14.1±5.4 min for T30 (ns). The mean T1/2 values were 35.7±22 min for T0, 86.4±105.1 min for T5 and 39.5±19.2 min for T30 ($p<0.0001$). Subgroup of patients treated by Major *MA*HR: For the 35 patients who underwent *MA*HR the HEF values were 97.2±5.3% (T0), 95.6±12.6% (T5) and 98.9±1.8% (T30) (ns); the mean Tmax values were 16.5±7.3 min for T0, 14.3±5.6 min for T5 and 15±4.7 min for T30 (ns); and the mean T1/2 values were 27±26.2 min for T0, 89.2±74.3 min for T5 and 42.2±8.7 min for T30. There are significant differences only for the values of T1/2 in T5 when compared with T0($p<0.035$). For the 60 patients who underwent *mi*HR the HEF values were 97.4±11% (T0), 98.8±2.4% (T5) and 98.6±4.7% (T30) (ns); the mean Tmax values were 15.2±8.5 min for T0, 13.6±5.9 min for T5 and 13.8±5.7 min for T30 (ns); and the mean T1/2 values were 39.1±19.4 min for T0, 84.6±122.4 min for T5 and 38.6±21.6 min for T30. There are significant differences only for the values of T1/2 in T5 when compared with T0($p<0.04$).

Conclusion: Our results strongly support the view that the HLR is early enough to normalize the HEF at day 5 after HR. We have demonstrated that HLR is early, fast, non-anatomical and functionally complete 5 days after liver resection. This fast functional liver recovery has high clinical importance because concerning adjuvant chemotherapy, possibly resulting in treatments administered much earlier after surgical resection than the three weeks dogma. Similarly, the same applies to liver resections following portal embolisation.

833 Proteomic technologies in brain tumours early diagnosis

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Background: One of the important goals of oncology is to develop biomarkers that can be identified through less invasive methods with the potential to identify cancer risk, early detection, utility in monitoring.

Several different proteomic methods have been discovered. One or a combination of methods needs to be chosen for an accurate cancer diagnosis.

Brain tumours are a heterogeneous group of neoplasms that appear to underlie individual patterns of growth, invasion, response to therapy and prognosis.

The aim of this study was to evaluate the protein profiling in brain tumours (gliomas and pituitary tumours) in order to establish a biomarker panel useful in early tumour detection.

Material and Methods: Our data were obtained using two cutting-edge proteomic profiling technologies: xMAP array – Luminex200 (cytokines panel) and SELDI-TOF-MS (protein profile). Serum samples from 3 groups: gliomas, pituitary tumours and controls were analyzed.

Results: From multiplex assay (Luminex200) strong overexpression was detected for IL-6, IL-1β, TNF-α (over 2 fold stimulation in patients vs. control). Significant up-regulation (1−2 fold) was found for VEGF and bFGF. Cytokines expression was significantly higher and strongly correlated with tumour stage, proliferation markers and clinical aggressiveness in glioblastomas and invasive pituitary adenomas. SELDI TOF proteomic profiling led to the selection of 110 protein peaks; a few differed significantly between brain tumours and controls (ROC curve; p value).

Conclusions: These techniques can be used for a rapid and efficient method in discovery of serum biomarkers in brain tumours diagnosis.

The advantages could be: less invasive techniques, screening for molecular markers, validation of putative therapeutic targets.

Tuesday 29 June 2010 09:45−17:30

Poster Session

Radiobiology/Radiation Oncology

834 A mouse model for the study of the radiobiology of medulloblastoma

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Medulloblastoma is the most common paediatric malignant brain tumour. The treatment of medulloblastoma involves a combination of surgery, radiation, and chemotherapy. While the overall survival rate for medulloblastoma is relatively good, those patients who experience a relapse of their tumour after initial treatment have a grim prognosis. Because medulloblastoma that relapses post-radiotherapy is nearly universally fatal, our goal is to develop an animal model to better understand tumour relapse in hopes of designing effective therapies. Little is known about the biological response of medulloblastoma to radiation, because clinically relevant doses of radiation are lethal to genetically engineered mouse models. We have developed a mouse model of relapsed medulloblastoma in order to study changes in the tumour after exposure to therapy, and to test novel treatments for their effectiveness after relapse.

In this model, primary tumour cells derived from genetically engineered mouse medulloblastomas (*Ptc*$^{+/-}$ or ND2:SmoA1) are orthotopically transplanted into wild type host mice. Hosts are monitored for early tumour development by MRI or bioluminescence imaging. To model medulloblastoma relapse, implanted tumours are established and then treated with a dose of radiation that leads to near-complete regression by MRI. Mice bearing implanted tumours survive radiotherapy, in contrast with the lethality observed with genetically engineered mouse models. While this radiation treatment provides a significant survival advantage as compared to untreated mice, the tumours eventually relapse, allowing for further analysis of medulloblastoma recurrence.

The initial tumours that form after transplant are histologically indistinguishable from spontaneously occurring medulloblastoma. In contrast to the uniform masses formed by xenografts, tumours that result from the implantation of primary tumour cells display perivascular niche architecture resembling that of spontaneous tumours. Radiation-resistant nestin-positive stem-like cells reside near blood vessels, and the tumour bulk is comprised of nestin-negative cells that are sensitive to radiation, undergoing apoptosis within 6 hours after exposure to a single dose of 2 Gy.

We are currently using tumours generated in this model to study treatment-acquired phenotypic alterations in medulloblastomas, and to identify pathways that can be targeted in relapsed medulloblastomas in order to better provide treatment for this devastating disease.

835 Pancreatic stellate cells modify tumour growth and radioresponse of pancreatic cancer

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Background: Pancreatic ductal adenocarcinoma (PDAC) is characterised by an abundant stromal response also known as desmoplastic reaction. One of the main actors in promoting the desmoplastic reaction is a stromal cell type known as pancreatic stellate cells (PSCs). There is accumulating evidence that PSCs influence the malignant phenotype of PDAC. The aim of our study is to analyse the tumour response to radiation treatment in the presence of PSCs and to determine the stromal and tumour factors involved in this response.

Material and Methods: PSCs were used in a coculture system with Panc-1 and PSN-1 PDAC cell lines *in vitro*. Their effect on radiation survival was tested using clonogenic survival assays. Conditioned media from the monocultures and from the coculture were analysed for the expression of different factors using a proteomic approach. *In vivo*, subcutaneous and orthotopic injection of pancreatic cancer cells with or without PSCs were used for the evaluation of the tumour growth. Tumour regrowth was measured on subcutaneous tumours after irradiation. All animal experiments were carried out in accordance with U.K. Home Office regulations.

Results: PDAC cell lines showed decreased radiosensitivity when cocultured with PSCs. Coinjection of PDAC with PSCs in nude mice enhanced tumour proliferation. Furthermore, tumour regrowth experiments after irradiation showed that tumours from coinjection of PDAC with PSCs respond less to radiotherapy than tumours from PDAC only. At last, we identified three factors differentially expressed *in vitro* in the coculture supernatant compared to the monocultures.

Conclusions: These data demonstrate that PSCs promote tumour growth and decrease radiation response in PDAC. Further investigations of the mechanisms of communication between tumour and stromal cells may lead to a better understanding of pancreatic cancer biology and to new targets for multimodal therapy.

836 Effects of irradiation on viability, growth, metastatic properties and expression of Eph receptors and their ephrin ligands in human melanoma cells

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Background: It is accepted that X-ray irradiation influences growth, viability and metastatic potential of tumour cells. Furthermore, it is supposed that tumour cell invasion and metastasis is regulated by Eph receptors and their ephrin ligands. The aim of our study was to investigate the influence of irradiation on cell viability, growth, and metastasis in human melanoma cells and whether this is mediated by dysregulated Eph receptor or ephrin ligand expression.

Material and Methods: Primary (Mel-Juso) and metastatic (A375, A2058) human melanoma cell lines were irradiated with 5 or 10 Gy. Up to 7 days after irradiation we examined cell viability (MTT test). At 1 day and 7 days post irradiation we further analyzed cellular growth, motility (scratch assay), adhesion to fibronectin, and migration through a porous membrane. Furthermore, the mRNA expression of 8 different Eph receptors and 6 ephrin ligands was analyzed using RT-PCR.

Results: In all cell lines a dose dependent decrease in viability and cell growth for up to 1 week after irradiation was demonstrated. Analysis of metastatic properties 1 day after X-ray showed decelerated scratch closure, slight increase in migration, and increased adhesion to fibronectin in all investigated cell lines. In contrast, 1 week after irradiation we detected faster scratch closure in irradiated primary Mel-Juso cells but unaltered motility in metastatic cell lines and, moreover, decreased migration in primary Mel-Juso cells and, by trend also in metastatic A375 cells. In addition, in Mel-Juso and A375 cells capability to adhere to fibronectin remained elevated. RT-PCR analysis revealed that Eph receptors and ephrins investigated have similar mRNA expression levels in primary and metastatic cell lines, with exception of both EphA2 and ephrinA5 showing enhanced expression in metastatic A375 cells. After irradiation changes in mRNA expression were not detected with exception of an increase in EphA2 and EphA3 in A375 cells and ephrins A1 and A5 in A375 and Mel-Juso cells 7 days after treatment.

Conclusion: Irradiation considerably influences viability and metastatic properties of melanoma cells. The different effects depending on time after irradiation observed suggest an involvement of cell-cell interaction via A-type Eph receptors and ephrins in irradiation-induced metastatic potency of melanoma cells.

837 Concomitant targeting of cyclooxygenase-2 and oxidant stress pathways for radioprotection of normal vascular tissue

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Background: Radiotherapy of various cancers is closely associated with increased cardiovascular morbidity and mortality. Arachidonic acid metabolites are supposed to play a key role in radiation-induced vascular dysfunction, inflammation, and injury. This study was designed to evaluate the effects of novel selective cyclooxygenase-2 (COX-2) inhibitors on radiation-induced formation of arachidonic acid metabolites via cyclooxygenase-2 and oxidant stress pathways in endothelial cells.

Materials and Methods: Acute effects (1 d, 3 d) of X-ray radiation at moderate doses (2 to 10 Gy) without or with presence of selective COX-2 inhibitors (cyclopentene/indole/indomethacin derivatives (2 each); 1 μM, 10 μM) in human arterial (HAEC) and microvascular (HDMEC) endothelial cells compared to sham-irradiated controls were assessed. Therefore, the following parameters were measured: COX-2 induction; secretion of cytokines tumour necrosis factor-α, interleukin-6, and monocyte chemoattractant protein-1; release of prostaglandins PGE_2 and PGI_2; release of isoprostanes 8-iso-PGE_2 and 8-iso-$PGF_{2\alpha}$; and oxidative stress (lipid peroxides).

Results: Irradiation of endothelial cells without presence of COX-2 inhibitors resulted in a dose-dependent augmentation of all parameters studied. When endothelial cells were exposed to COX-2 inhibitors during and for 24 h post irradiation, indole derivatives showed highest potency to inhibit release of both prostaglandins and isoprostanes. Furthermore, when irradiated cells were treated with indole derivatives a significant decrease of lipid peroxide formation and cytokine secretion could be observed, which indicates a direct interaction with oxidant stress-pathways. By contrast, both cyclopentene and indomethacin derivatives majorily inhibited prostaglandin release, but showed only slight effects on formation of isoprostanes, lipid peroxides and cytokines. Model experiments using human low density lipoproteins oxidized by radiolytically generated oxygen radicals showed that indole derivatives differently interact with peroxidation of polyunsaturated fatty acids, than the cyclopentene/indomethacin derivatives, suggesting a physico-chemical rationale for observed anti-oxidant activity.

Conclusion: Indole-based selective COX-2 inhibitors substantially decreased radiation-induced formation of vasoactive isoprostanes 8-iso-PGE_2 and 8-iso-$PGF_{2\alpha}$ by endothelial cells. These findings may have particular importance in radiation-induced processes in which COX-2 is induced and oxidant stress occurs. The reduction of radiation-induced vascular dysfunction by antioxidative COX-2 inhibitors may widen the therapeutic window of cyclooxygenase-2 targeted treatment.

838 Withdrawn

839 Withdrawn

840 Influence of irradiation on para- and autocrine regulation of extracellular S100A4 (metastasin) and its receptor RAGE in B16 mouse melanoma cells

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Background: Malignant melanoma is one of the most invasive and metastatic tumours. A common therapeutic approach towards metastases will combine