NT analog was shown to exert minor effects on proliferation of the cancer cell lines in MTT assays. The NT analog stimulated Na^+- and amiloride-sensitive proton flux of the Na^+/H^+-exchanger 1 (NHE1). Activity of NHE1 is regulated by phosphorylation and, ERK1/2, p38α MAPK and mitogen- and stress-activated kinase1/2 (MSK1/2) were identified as responsible kinases in phosphoprotein arrays. Functional involvement of these kinases was proved with inhibitors PD 98059, SC68376 and dimethyl fumarate (DMF), respectively. Downstream targets of are MSK1/2 are CREB and NFκB and DMF was reported to inhibit metastasis of melanoma cells in experimental animals. In BxPC-3 and PANC-1 cells, lys-ψ-lys-NT(8−13) enhanced the production of IL-8, an important inducer of tumor cell dissemination, and these cells were acquired the ability to evade from an extracellular matrix gels. The NT analog upregulated expression of genes encoding cytoskeletal and adhesion proteins, glycolytic enzymes and metalloproteinases.

Conclusion. In conclusion, NT stimulated the aggressiveness of pancreatic cancer cells by induction of intracellular alkalinisation/extracellular acidosis and increased production of IL-8, in addition to its minor growth promoting effects.

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1111 POSTER

Pharmacogenetics of peripheral neuropathy in elderly patients (>65years) with advanced gastric cancer receiving oxaliplatin based chemotherapy within a randomized phase II study

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Background: Peripheral neuropathy (PNP) is a dose-limiting side effect of oxaliplatin based chemotherapy. High grade PNP may compromise quality of life especially in elderly patients (pts). A randomized multicenter phase II study was conducted to compare fluorouracil, leucovorin, oxaliplatin with or without docetaxel (FLO vs. FLOT, respectively) in elderly pts with advanced gastric cancer (AGC). Our purpose was to identify pharmacogenetic markers as predictors of high grade PNP within this study.
Methods: 143 pts were enrolled in this study. Pts. were numerically >65 years or numerically >59 years but classified biologically >65 years as defined by an *Instrumental Activities of Daily Living* score of <8. PNP was classified according to an oxaliplatin specific scale. Genotyping was performed using PCR-based RFLP or TaqMan®-based allelic discrimination. 20 polymorphisms in 13 genes being part of the metabolism of the applied drugs or DNA repair were analyzed. Statistical analyses were based on stepwise multivariate cox regression models and included genotypes and clinical parameters.
Results: Median age was 71 years (range 60−83). Pts received in median 6 cycles of treatment (range 1−12). 130 pts were evaluable for PN at time of analyses. Of these, 68 received FLO and 62 received FLOT. Cumulative grade 3 PNP occurred in 49% of pts without a significant difference between FLO and FLOT receiving pts (44% and 53%, respectively, $p = 0.4$). Genotypes of TS and MTHFR could be identified as independent risk factors for grade 3 PNP by multivariate analyses. Pts carrying a TS promoter genotype known to be associated with low TS expression (2R/2R, 2R/3RC, 3RC/3RC) were at higher risk for developing grade 3 PNP compared to pts without one of these genotypes (OR 3.0 [95%CI 1.27; 7.06], $p = 0.01$). Pts carrying MTHFR1298AC or CC genotypes were also at higher risk for experiencing grade 3 PNP compared to pts with the wildtype MTHFR-1298AA genotype (OR 3.1 [95%CI 1.26; 7.60], $p = 0.01$). In fact, 89% of pts that experienced grade 3 PNP were carriers of at least one of these risk genotypes.
Conclusion: Polymorphisms of TS and MTHFR might be associated with grade 3 PNP in AGC pts receiving oxaliplatin based chemotherapy.

1112 POSTER

The effect of adjuvant chemotherapy with a taxane and a bisphosphonate on bone mass and bone strength in an animal model

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Background: Taxane-containing chemotherapy is becoming a standard in the treatment for many different tumors such as breast cancer. The purpose of the present animal study was to investigate the direct effects of docetaxel as a modern chemotherapy agent on bone strength and bone imaging parameters and wether a bisphosphonate in an osteoporosis equivalent dose mitigates the putative effects of the chemotherapy on bone.
Materials and Methods: 45 female Sprague-Dawley rats were randomized to three experimental groups. All groups underwent a sham ovariectomy. The first group received a placebo treatment with saline injections subcutaneous while the second and third group (each n = 15) were treated with 6 cycles of docetaxel in a 3 week term. One chemotherapy group received in addition daily subcutaneous application of 1 μg/kg ibandronate while the second group received a placebo treatment.
Following methods were used in order to characterize the effects of the different treatments on bone mass and strength: Peripheral Quantitative Computer Tomography (pQCT) bone density scans and structural analysis (μCT) scans were performed at the center of the femoral neck and shaft. After bone density and structural analysis the right femora were tested in 3 point-bending, while the left femora were tested in compression mode of the femoral neck. For both tests the load displacement curve was analyzed for stiffness and ultimate load.
Analysis of the vertebral bodies included μCT and a compression test of LVB 5.
Results: 6 cycles of taxane-containing chemotherapy caused a significant decrease in almost all parameters determining bone mass and bone strength. The effects followed the same pattern in all used methods. The treatment with ibandronate was able to preserve those parameters significantly compared to the negative effects in the group treated with chemotherapy only.
Conclusion: Since hypogonadism is not the result of the treatment with docetaxel it is likely that a direct negative effect on bone is the reason for decreased bone mass and bone strength. Estrogen might even protect the bone from more devastating destruction due to chemotherapy. Further experimental investigations are underway to clarify the protective role of estrogen in cancer treatment. Furthermore, we were able to show that an osteoporosis equivalent dose of the bisphosphonate ibandronate is able to mitigate the negative effects of chemotherapy in the animal model used.

1113 POSTER

Correlation of Sodium Iodide Symporter (NIS) and Retinoic Acid Receptor Alpha (RARA) expression in breast cancer

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Background: Sodium Iodide Symporter (NIS) expression in the thyroid gland supports imaging and treatment of thyroid disease using radioactive iodide. NIS expression also occurs in malignant breast tissue suggesting potential for radioiodide in breast cancer imaging and therapy. Both in vitro and in vivo animal studies have shown that NIS expression in breast cancer is regulated by retinoic acid.
Aim: The aim of this study was to quantify NIS and retinoic acid receptor-a (RARA) gene expression in normal, benign and malignant breast tissue using RQ-PCR, and to correlate levels with clinicopathological details.
Method: Breast tissue specimens (n = 92) harvested at surgery were homogenised and RNA extracted using the Qiagen RNeasy Mini Kit. Following Nanodrop RNA quantification and reverse transcription, the corresponding cDNA was interrogated for NIS and RARA expression using RQ-PCR. To determine relative quantification (RQ) values, levels of NIS and RARA expression were normalised using the average levels of endogenous control genes PPIA and MRPL19, and expressed relative to the lowest detectable level for correlation of data. To compare individual breast cancer subtypes, results were expressed relative to levels detected in normal breast tissue. Statistical significance was analysed using the Student t test and correlation between NIS and RARA was determined using the Pearson Correlation Coefficient.
Results: NIS expression was detected in 74/76 breast cancer tissues analysed (Mean ±SEM, 1.17 ± 0.06 $\log_{10}$ RQ). There was a significant positive correlation between NIS and RARA expression in all breast tissues samples (Pearson correlation coefficient = 0.215, $p < 0.05$). The highest

levels of both NIS and RARA expression were detected in benign breast tissue (NIS: 1.65±0.25 $\log_{10}$ RQ; RARA: 1.01±0.13 $\log_{10}$ RQ). Analysis based on hormone receptor status, menopausal status, tumour grade or stage revealed no significant differences in NIS or RARA expression. However, when analysed on the basis of epithelial subtype there was a trend towards higher levels of NIS expression in more invasive epithelial subtypes, with the Luminal B group having significantly lower expression than the Her2 group ($p < 0.05$).
Conclusion: This study is an important first step to further understand the presence, regulation and relevance of NIS expression in breast tumour tissue.

1114 POSTER

Pazopanib-induced hyperbilirubinemia is associated with Gilbert's syndrome UGT1A1 polymorphism

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Background: Pazopanib, an oral multikinase inhibitor, has demonstrated antitumor activity in several tumour types. Despite an overall acceptable and tolerable safety profile, treatment associated elevations in transaminases and bilirubin have been observed. As pazopanib inhibits UGT1A1 activity, we sought to determine the effect of a *UGT1A1* polymorphism on bilirubin elevation in pazopanib treated patients.
Material and Methods: Association between the *UGT1A1* TA repeat polymorphism and bilirubin levels was examined in 112 Caucasian patients from a Phase II pazopanib monotherapy study (VEG102616) for metastatic renal cell carcinoma (RCC). A replication analysis was carried out in an independent sample of 124 Caucasian patients from a Phase III RCC study (VEG105192). The data were analyzed both as continuous variables (quantitative trait analysis) and as discrete values according to predefined thresholds (case-control analysis).
Results: The *UGT1A1* TA repeat polymorphism was strongly associated with pazopanib-induced hyperbilirubinemia (defined as total bilirubin levels $\geqslant$1.5 upper limit of normal) in patients from the Phase II study ($p = 7.3 \times 10^{-6}$). This association was replicated in patients from the Phase III study ($p = 2.4 \times 10^{-3}$). Of the 38 Caucasian patients with hyperbilirubinemia, 32 (84%) were carriers of one or two TA7 alleles. Overall, when compared to other genotypes, the odds ratio (95% CI) of the TA7/TA7 genotype for developing hyperbilirubinemia was 13.1 (5.3−32.2), with positive and negative predictive values of 0.49 and 0.90. All results were confirmed in analyses treating TBL as a continuous measure.
Conclusions: These data suggest that most cases of pazopanib-induced isolated hyperbilirubinemia are benign manifestations of Gilbert's syndrome, therefore support continuation of pazopanib monotherapy for mild to moderate isolated indirect bilirubin elevation without the need for population-based prospective *UGT1A1* screening. For specific patients of concern, bilirubin fractionation or *UGT1A1* genotyping should be conducted to elucidate the nature of the bilirubin elevation which might enable differentiation of the risk of progression of drug induced liver injury.

1115 POSTER

Development of cancer genetic timeline analysis for identification of cancer founder mutations and driver mutations

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With an incidence of 12.3 million and a mortality of 7.6 million, cancer increasingly presents as a serious health issue worldwide. Although there is great progress in cancer research, the genetic basis of oncogenesis is still not well understood. Increasingly powerful genomic sequencing technologies have yet to identify the causal mutations for oncogenesis and the driver mutations responsible for cancer progression. We have developed a novel cell-ontology-based strategy, Cancer Genetic Timeline Analysis (CGTA), to determine the mechanistic relevance of genetic mutations in the formation and progression of an individual tumor. RNA from matched normal and tumor specimens from a uterine cancer patient was sequenced by mRNA-seq and bioinformatic filtering identified 246 somatic non-synonymous single-nucleotide variants in the tumor transcriptome. The Sanger method was used to re-sequence these variants in genomic DNA from coisogenic normal and tumor specimens, and 26 were validated to be somatic mutations in the tumor genome. Thirty single cancer cells were acquired through laser-captured micro-dissection from frozen sections of the tumor. The genomic DNA of each cell was extracted and amplified separately. The 26 mutated genes were re-sequenced to investigate their occurrence in single cells and a phylogenetic tree was thus constructed based upon maximum parsimony and statistical partitioning of the distribution of mutations. Five mutations were ubiquitous among all 30 cells and were imputed to be present in the cancer founder cell, and thus are considered to be the oncogenic pathway for tumorigenesis. Additionally, through an analysis by a phylogenetic probability model, two mutations were identified to be driver mutations, potentially responsible for the emergence of a dominant clone. Further analysis of the identified oncogenic pathway in an additional ten uterine tumors suggested that human uterine tumors may have multiple distinct oncogenic pathways, which is consistent with recent reports in breast, colorectal, pancreatic cancer and glioblastoma, and these findings collectively provide strong evidence against the notion of a single oncogenic pathway for any type of human cancer. Without relying on the prevalence of mutations in other tumors, the CGTA method can identify the oncogenic pathway and the driver mutations in individual tumors, which could serve as the etiological and mechanistic basis for novel molecular classification and drug development.

1116 POSTER

Differential expression in inflammatory-related genes after preoperative chemoradiation (CRT) in normal rectal tissue compared with rectal carcinoma

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Background: Radiation therapy (RT) initiates molecular and cellular events affecting both the tumor and the tissue microenvironment. Pre-clinical growing evidence suggests that the interaction and balance between those compartment effects rather than a single action of RT over the tumor is responsible of the tumor response and normal tissue tolerance. Novel preoperative CRT strategies in rectal cancer look for improving response without increasing toxicity. Identification of differential profile of radiation-effect in tumor and normal peritumoral tissue may be useful to achieve such goal. The purpose of this study is to compare the expression profile in inflammatory-related genes between tumor and peritumoral normal tissues in a series of rectal cancer patients treated with preoperative RT.
Material and Methods: 92 inflammation-related genes and 4 *housekeeping* genes were studied by Q-RT-PCR by using Taq-Man Low Density Array in tumoral and normal tissue obtained from 15 patients homogeneously treated with oxaliplatin followed by preoperative CRT (45 Gy and oral Tegafur). In order to obtain more reliable results, we assessed the normalization data using three different approaches: global median-normalization (similar to microarray analysis), 18 s rRNA (the most stable housekeeping gene in our CRC samples) and the geometric-mean of 4 *housekeeping* genes analyzed. To identify genes with significantly differential expression between tumoral and normal samples, we performed Class Comparison test, a multivariate permutation test provided in BRB-ArrayTools package.
Results: We identify 8 common genes whose expression were different ($p < 0.01$, FDR < 0.05) with the three different normalization approaches, suggesting that tumoral presence could affect the inflammation process. 4 of them were down-regulated in tumoral tissues: 3 members of secreted serine-protease-endopeptidases kallikreins (KLK) family (KLK3, KLK15 and KLKB1) and the mitogen-activated protein kinase MAPK8. The 4 up-regulated genes included 3 receptors (ADRB1, LTB4R and MC2R) and the adhesion molecule ICAM1.
Conclusions: This study describes a differential expression in inflammatory-related genes after preoperative CRT in normal rectal tissue and rectal tumor. Further studies to confirm whether this pattern of expression may play a role in the tumor response and side effects to RT are warranted.

1117 POSTER

Identifying the challenges in establishing a lung cancer tissue repository for translational research: a single institution experience

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Background: As part of a study of molecular abnormalities associated with the development of lung cancer, we had to establish a tissue repository,