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,

including malignant and non-malignant lung tissue, obtained surgically or at bronchoscopy, and blood.
Materials and Methods: A tissue collection team was established comprising thoracic surgeons, pathologists and the laboratory research team. Project implementation required (i) approval from local Ethics and Hospital R&D Committees; (ii) preparation and implementation of standard operating procedures (SOPs) for tissue collection, processing and storage; and (iii) the establishment of dedicated databases for recording clinical and pathological patient data. Donors were selected on the basis of having a radiological lung abnormality; planned rigid bronchoscopy or lung surgery; age $\geqslant$18 years; and written informed consent. Patients were not excluded if clinical suspicion of malignant histology was low. Surgical tissue samples were snap frozen, bronchial biopsies frozen in OCT medium, and blood samples fractionated, buffy coat and plasma separated and then frozen. Frozen material was cryopreserved at −80ºC pending molecular analysis.
Results: Over a 12 month period we have collected samples from 81 donors whose characteristics include: M/F, 54/46%, median age 64 years, current or former smokers 88%, previous asbestos exposure 12%. In 60% of donors malignancy was confirmed, including NSCLC 45%, metastatic colorectal cancer 5% and carcinoid 2%; benign conditions included pleural fibrosis 5%, adenochondroma 2%, and sarcoidosis 2%. Lung tissue, bronchial biopsies and peripheral blood have been collected from 48%, 68% and 88% of donors, respectively. Twenty-one lung cancer tissue specimens and paired non-cancerous lung tissue specimens or peripheral blood have been collected so far. No adverse events were associated with the study procedures.
Conclusions: We have established a successful lung tissue bank. Challenges that had to be overcome included obtaining consent in busy clinical environments, co-ordination of sample collection with changes in theater lists and outside normal working hours, and the need for a designated pathologist to process tissue samples. A designated person to act as tissue collector, and good links with clinicians, pathologists and operating theater staff were identified as vital to the success of establishing a tissue repository.

1118 POSTER

Diet-induced obesity modulates signaling through the Akt/mTOR pathway in colon and colon cancer xenografts

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Obesity has recently been linked to mortality from the majority of cancers. For colon cancer, in particular, several epidemiological studies have supported the concept that high energy intake, obesity, and/or hyperinsulinemia are the major risk factors for its incidence and severity. However, the exact molecular mechanism linking obesity and colon cancer is not fully understood. Insulin, via activation of insulin receptors expressed ubiquitously in normal and neoplastic cells as well as IRS-1, Akt, mTOR and p70 activation may enhance the anabolic state necessary for cell growth. By the other hand, markers of chronic inflammation like IKKβ and COX-2 are in the centre of colon carcinogenesis by promoting cell proliferation, inhibiting apoptosis and stimulating angiogenesis.
Western blotting assay was performed to evaluate IR, IRS-1, Akt, mTOR, p70S6K, COX2 and IKKβ in colon from diet-induced obesity (DIO) wistar rats and *ob*/*ob* mice as well as tumor extract from DIO SCID mice xenografted with HT-29 colon cancer cells.
Insulin-stimulated phosphorylation of IR, IRS-1, Akt, mTOR and p70S6K in colon tissues were enhanced in diet induced obesity (DIO) rats and *ob*/*ob* mice compared with lean counterparties. In contrast, the insulin signaling pathway demonstrated reduced phosphorylation rates in the muscle of insulin stimulated DIO and ob/ob animals compared to lean controls. DIO increased the activation of mTOR pathway in vivo and induced tumor growth in colon cancer cell xenografts. We also observed an increased in protein expression of COX-2 and phosphorylation of IKKβ in the colon or colon cancer xenografts extracts from obese animals compared to that observed in lean controls.
This study provides direct measurements of insulin signaling in colon and colon cancer xenografts, and documents an increased sensitivity to insulin, despite the activation of inflammatory signaling pathways, in colon and colon cancer xenografts.

Drug development − Preclinical and phase I

Oral presentations (Wed, 23 Sep, 09:00−11:00)
Drug development − Preclinical and phase I

1200 ORAL

Drug responses and predictive markers for sensitivity in colorectal cancer cell lines

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Background: Colorectal cancer (CRC) is one of the main causes of death from malignant disease and new strategies have to be found to target therapy better to patients. This study uses a large panel of 85 CRC cell lines and correlation analysis to detect markers for drug response and to elucidate the mechanism of action of drugs.
Material and Methods: Drug response is tested using serial dilutions of various drugs in a 96-well format and assayed using the SRB method. An algorithm is used to group cell lines objectively into clearly distinct categories of response. Subsequently, correlations with genetic, epigenetic and protein expression data are detected by statistical analysis to find significant associations. Combination treatment with different drugs as well as 3D assays, InCell Western and Western blotting are used for further investigation.
Results: We were able to find both well known and new associations. For example, response to 5-fluorouracil (5-FU) correlates with replication error (RER) status and mutations in kras and TGFβRII: insensitive cell lines tend to be RER positive (replication error defective) ($p = 0.0027$, Fisher's Exact test) and to have mutations in those two genes ($p = 0.0173$ and 0.0198, respectively). Sensitivity against a novel, specific MEK1/2 inhibitor (MEKi; GSK1120212) was found to be associated with mRNA levels and the degree of methylation of LY75 (CD205): resistant cell lines show high levels of methylation of LY75 and in concordance with this, very low message levels. But response to MEKi does not correlate with the degree of inhibition of Erk phosphorylation, its direct downstream target in the MAP kinase pathway. This suggests that resistance is caused further downstream in the pathway or by an altogether different mechanism.
Conclusions: CRC cell lines show differential responses to a variety of drugs. These correlate with other cell line characteristics and could therefore be used as predictive markers for a certain cell line/drug combination. Large cell line panels as used for this study prove to be powerful tools for finding associations, as demonstrated by the confirmation of clinical data published for 5-FU.

1201 ORAL

New fluorine-18 radiolabeled Cdk4/6 inhibitors: potential radiotracers for tumour imaging by positron emission tomography

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Background: Cyclin-dependent kinases 4 and 6 (Cdk4/6) are important components of cell cycle activation in G_1 phase and play critical roles in dysfunction of growth control during cancerogenesis. The aim of our study was the evaluation of new fluorine containing pyrido[2,3-*d*]pyrimidin-7-one derivatives (CKIC, CKID and CKIE) concerning their efficacy and suitability as Cdk4/6 inhibitors and, after fluorine-18 radiolabeling, as radiotracers for imaging of tumors by positron emission tomography (PET).
Materials and Methods: Small molecule inhibitors CKIC, CKID and CKIE were analyzed concerning their biological and radiopharmacological properties in human tumor cell lines HT-29, FaDu and THP-1. Cell cycle distribution of cells was determined by flow cytometry DNA analysis and effects on cell growth were measured. Phosphorylation of retinoblastoma protein (pRb) at Ser^{780} was analyzed by Western blotting. mRNA expression of the pRb affected genes E2F-1 and PCNA was measured with quantitative RT-PCR. Stability and radiotracer uptake studies with [^{18}F]CKIE were performed.
Results: Cell cycle analyses showed a concentration-dependent (50 nM to 10 μM) increment of percentage of tumor cells in G1 phase after 24 h of incubation with CKIC, CKID and CKIE, with CKIE to be more potent than CKIC and CKID. Cell growth studies indicated reduced tumor cell numbers after 48 h of treatment with 1 μM (<75%) and 10 μM (<30%) CKIE and 10 μM (<70%) CKIC or respectively CKID. Cdk4 specific phosphorylation at pRb-Ser^{780} is decreased in a concentration dependent manner after