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

M.B. Flores[1], G.Z. Rocha[1], M.M. Dias[1], J.A. Saad[1], J.B. Carvalheira[1]. [1]*Faculty of Medical Science – Unicamp, Internal Medicine, Campinas, Brazil*

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

K. Bracht[1], A.M. Nicholls[1], O. Dikomitou[1], J. Wilding[1], W.F. Bodmer[1]. [1]*Weatherall Institute of Molecular Medicine, Cancer Immunogenetics, Oxford, United Kingdom*

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

F. Graf[1], L. Koehler[2], B. Mosch[1], J. Pietzsch[1]. [1]*Forschungszentrum Dresden-Rossendorf e.V. Institute of Radiopharmacy, Radiopharmaceutical Biology, Dresden, Germany;* [2]*Forschungszentrum Dresden-Rossendorf e.V. Institute of Radiopharmacy, PET Tracer, Dresden, Germany*

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

24 h of incubation with CKIE. Downregulation of E2F-1 and PCNA mRNA expression could be demonstrated after treatment with CKIE. [^{18}F]CKIE indicated high stability in physiological buffer and cell culture medium. Cellular radiotracer uptake using [^{18}F]CKIE increased with time amounting to 46.3±11.2%ID/mg protein in HT-29 and 46.2±13.8%ID/mg protein in FaDu cells, respectively, after 60 min at 37ºC. Uptake of [^{18}F]CKIE could be blocked with nonradioactive CKIE dependent on concentration (e.g., 23.5±3.7%ID/mg protein with 5 μM CKIE after 60 min at 37ºC).
Conclusion: CKIE was identified as the most potent fluorine containing pyrido[2,3-*d*]pyrimidin-7-one derivative analyzed in our study causing arrest of tumour cells in G1 phase due to inhibition of the Cdk4/6/pRb/E2F pathway. *In vitro* radiotracer uptake studies using [^{18}F]CKIE demonstrated tumor cell uptake, which is an important prerequisite for further PET studies in tumor-bearing mice.

1202 ORAL

In silico modelling of Doxorubicin penetration through multicell layers

R. Phillips[1], P.M. Loadman[1], C.J. Evans[2], P.F. Jones[3], S.W. Smye[4], B.D. Sleeman[5], C.J. Twelves[3]. *[1]Institute of Cancer Therapeutics, Tom Connors Cancer Research Centre, Bradford, United Kingdom; [2]University of Leeds, Leeds Institute of Genetics, Leeds, United Kingdom; [3]University of Leeds, Leeds Institute of Molecular Medicine, Leeds, United Kingdom; [4]St James University Hospital, Department of Medical Physics, Leeds, United Kingdom; [5]University of Leeds, Department of Applied Mathematics, Leeds, United Kingdom*

Background: Inadequate delivery of anti-cancer drugs to solid tumours is a significant factor limiting efficacy. Factors determining drug delivery to tumours are complex but the pharmacokinetic (PK) properties of the drug and its ability to leave the blood vessel then penetrate avascular tissue are critically important. The aim of this study was to develop an *in silico* model based on *in vitro* measurements that can predict how far a drug will penetrate from a blood vessel within its PK lifespan using the transport of doxorubicin through multicellular layers as a model and assessing the potential impact of efflux via P-Glycoprotein (PgP) on drug penetration.
Materials and Methods: Three cell lines were employed; DLD-1 (human colon carcinoma), MCF7 (human breast carcinoma) and NCI/ADR-Res (doxorubicin resistant and PgP over expressing OVCAR8 cells). Cells were cultured on Transwell culture inserts to thicknesses between 20 and145 μm as determined by microscope analysis of histological sections. Doxorubicin at concentrations of 100, 50 or 25 μM was added to the top chamber of the Transwell apparatus, and the concentration of drug appearing in the bottom chamber determined as function of time by HPLC-MS/MS.
Results: In all cell lines, the rate of drug penetration was inversely proportional to the thickness of the multicell layer; the presence of PgP (NCI/ADR-Res) did not alter the rate of doxorubicin penetration compared to the wild type MCF7 cells. We established a mathematical model based upon the fact that the transport of doxorubicin across cell membrane bilayers occurs by a passive "flip-flop" mechanism of the drug between two membrane leaflets with the Transwell setup treated as a series of compartments and the multicell layer as a series of cell layers, separated by small intercellular spaces. This initial model demonstrates good agreement between predicted and actual drug penetration rates *in vitro*.
Conclusions: We have developed an effective preliminary model. Further studies incorporating both real and simulated PK parameters are underway. Our ultimate objective is to make predictions of which dose and schedule of drug administration is likely to be the most efficacious; the model could also be used to identify and prioritise the development of those compounds in pre-clinical development most likely to achieve adequate tumour drug concentrations.

1203 ORAL

Pharmacokinetic and pharmacodynamic Phase I trial of ARQ 197 incorporating dynamic contrast-enhanced (DCE) and diffusion weighted (DW) magnetic resonance imaging (MRI) studies investigating the antiangiogenic and antitumor activity of selective c-Met inhibition

T.A. Yap[1], M.P. Lolkema[1], N. Tunariu[2], D.S.W. Tan[1], A.T. Brunetto[1], M. Germuska[2], E. Garmey[3], J. Li[3], N. deSouza[2], J.S. de Bono[1]. *[1]Royal Marsden Hospital, Drug Development Unit, Sutton, United Kingdom; [2]The Institute of Cancer Research, Cancer Research UK Clinical Magnetic Resonance Research Group, Sutton, United Kingdom; [3]ArQule Inc, Clinical Development, Woburn, USA*

Background: ARQ 197 (ARQ) is a selective non-ATP competitive inhibitor of c-Met, a receptor tyrosine kinase implicated in tumor cell proliferation, migration, apoptosis and angiogenesis. Promising preclinical data and declines in circulating endothelial cell (CEC) levels in patients (pts) receiving ARQ support antiangiogenic potential of c-Met inhibition.
Materials and Methods: ARQ was administered orally twice daily (bid) to pts with advanced solid tumors. Pre and post-therapy tumor biopsies were mandated in all patients (n = 16) for c-Met and FAK immunohistochemical pharmacodynamic (PD) studies during dose escalation. CEC enumeration was evaluated at multiple timepoints. 12 pts are being investigated in the maximum tolerated dose (MTD) expansion cohort with DCE and DW MRI studies.
Results: 29 pts (14F/15M; mean age 54.4 yrs; mean of 4.4 prior therapies) received ARQ at doses 100 (n = 3), 200 (n = 6), 300 (n = 16) and 400 (n = 4) mg bid. 3 pts experienced dose limiting toxicities: CTCAEv3 grade (G) 3 fatigue at 200 mg bid (n = 1); G3 hand-foot syndrome and G3 mucositis at 400 mg bid (n = 1); G3 febrile neutropenia at 400 mg bid (n = 2). This established the ARQ MTD/recommended phase 2 dose (RP2D) at 300 mg bid. Other toxicities were G1–2, such as fatigue (n = 5); diarrhea, nausea and vomiting (n = 3). Mean $AUC_{0-12\,h}$ and C_{max} increased linearly through the MTD. Statistically significant post-ARQ inhibition of high baseline phosphorylated c-Met and FAK expression in tumor tissue was seen in all dose cohorts confirming target inhibition. Disease stabilization (SD) was seen in 11 pts for up to 23 weeks with tumor regressions up to 12.4% (metastatic gastric cancer pt). 13 of 20 pts had post-ARQ CEC declines of up to 100%, supporting antiangiogenic effects of ARQ. In the DCE-MRI cohort to date, preliminary analyses of k_{trans} histograms from pelvic and liver lesions showed a reduction in k_{trans} values on day 7 of ARQ, consistent with antiangiogenic effects.
Conclusions: ARQ is well tolerated with MTD/RP2D of 300 mg bid, linear pharmacokinetics and c-Met and FAK PD inhibition. Promising antitumor activity was observed. CEC and preliminary DCE-MRI data support antiangiogenic effects of c-Met inhibition with ARQ. Correlation with DCE parameters and DW changes will be presented. Following preliminary antitumour activity, a cohort expansion of 10 castration resistant prostate cancer patients is ongoing.

1204 ORAL

A Phase I study evaluating the pharmacokinetics (PK) and pharmacodynamics (PD) of the oral pan-phosphoinositide-3 kinase (PI3K) inhibitor GDC-0941

R. Kristeleit[1], D. Sarker[1], M. Forster[1], M. Lolkema[1], D. Olmos[1], K. Mazina[2], M.V. Dolezal[2], J.A. Ware[2], Y. Yan[2], J.S. de Bono[1]. *[1]The Royal Marsden NHS trust – Institute of Cancer Research, Drug Development Unit, Sutton Surrey, United Kingdom; [2]Genentech Inc., 1 DNA Way, San Francisco, USA*

Background: The PI3K-PTEN-AKT signalling pathway is deregulated in a wide variety of cancers. GDC-0941 is a potent and selective oral pan-inhibitor of class I PI3K, with 3nM IC50 for the p110-alpha subunit in vitro and 28nM IC50 in a cell-based phosphorylated AKT (pAKT) assay, and demonstrates activity in several preclinical models (breast, ovarian, lung and prostate).
Materials and Methods: Patients (pts) with histologically confirmed advanced solid tumours and Eastern Cooperative Oncology Group performance status 0–1 were enrolled in a Phase I study of GDC-0941 (sponsored by Genentech), using a 3+3 escalation design at a single institution. Treatment was a single dose of GDC-0941 with a 1-week (wk) washout, followed by GDC-0941QD on a 3-wk on, 1-wk off schedule. Objectives were to determine the maximum tolerated dose and dose-limiting toxicity (DLT), evaluate PD endpoints in surrogate tissue (pAKT in platelet-rich plasma [PRP]) and tumour tissue (pAKT and pS6 in paired tumour biopsies and fluorodeoxyglucose (FDG) uptake via positron emission tomography imaging), and describe any observed antitumour activity.
Results: Eighteen patients have been enrolled in 5 successive cohorts (15–80 mg QD). GDC-0941 was generally well tolerated with no drug-related Grade 3–4 adverse events or DLT to date. Grade 1–2 diarrhoea, nausea, vomiting, fatigue, dysgeusia, peripheral sensory neuropathy, dry mouth, thrombocytopenia and increased alanine and aspartate aminotransferase levels have been observed. Preliminary PK data suggest GDC-0941 is rapidly absorbed (T_{max} range 1–2 hrs) and displays dose-appropriate increases in fasting mean C_{max} and AUC_{inf}. At current GDC-0941 doses we have exceeded exposures associated with efficacy in preclinical models. Preliminary surrogate PD data demonstrate decreased levels of pAKT in PRP associated with GDC-0941 plasma concentrations. Moreover, a good correlation between *ex vivo* and *in vivo* inhibition constants for pAKT exist with greatest inhibition (approximately 80%) occurring following the 80 mg dose. Evaluation of GDC-0941 effects on PI3K pathway modulation in paired tumour biopsies is currently underway.
Conclusions: GDC-0941 is generally well tolerated when administered QD at doses associated with inhibition of pAKT in surrogate tissues and displays linear PK from 15–80 mg. Evidence of surrogate tissue PD activity has been observed. Dose-escalation continues and updated PK/PD data will be presented.