manual method, 5.2% and 8.1%; 2D automated, 7.6 % and 8.1%; and 3D automated, 8.2% and 8.8%, respectively.

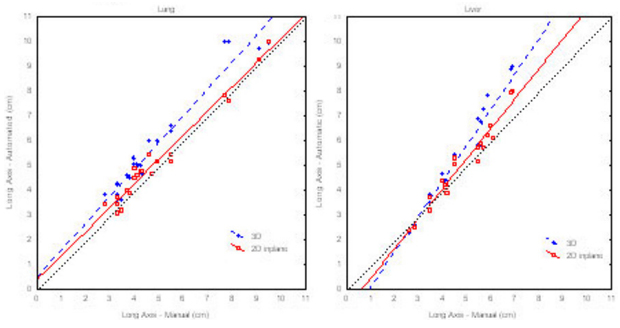

Fig. 1. Correlation of automated 2D *vs*. manual inplane long-axis measurements for lung (left) and liver (right) tumors (black dotted line represents perfect correlation).

Conclusions: Scan-rescan measurements of tumor size can be made with reproducibility in the range 5–9%, with no significant differences between manual and automated methods. There were no significant differences in assessments of size between inplane manual and 2D automated methods, but 3D derived measurements were significantly larger, which, for liver lesions, showed divergence from manual and automated inplane 2D.

141 POSTER

Effect of population and gender on chemotherapeutic agent-induced cytotoxicity

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Large inter-individual variance is observed in both response and toxicity associated with chemotherapy. Our goal is to identify factors that contribute to chemotherapy-induced toxicity. To this end, we used EBV-transformed B-lymphoblastoid HapMap cell lines derived from thirty Yoruban trios (African descent) and thirty CEPH trios (European descent) to evaluate population and gender specific differences regarding cytotoxicity of carboplatin, cisplatin, daunorubicin and etoposide using a high throughput, short-term alamarBlue™ assay. The IC_{50} was compared for population and gender specific differences for the four drugs. We observed large inter-individual variance in IC_{50} values for carboplatin, cisplatin, daunorubicin and etoposide for both Yoruban and CEPH populations (range from 8- to 433-fold). Statistically significant differences in carboplatin and daunorubicin IC_{50} were demonstrated when comparing Yoruban cell lines ($n = 89$) to CEPH cell lines ($n = 87$) ($p = 0.002$ and $p = 0.029$, respectively). This population difference in treatment induced cytotoxicity was not seen for either cisplatin or etoposide. In the Yoruban population, cell lines derived from females were less sensitive to platinating agents than males [median carboplatin IC_{50} 29.1 vs 24.6 M ($p = 0.012$); median cisplatin IC_{50} 7.0 vs 6.0 M ($p = 0.020$) in female and male, respectively]. This difference was not observed in the CEPH population. These results demonstrate that population and gender may affect risk for toxicities associated with certain chemotherapeutic agents.

142 POSTER

The role of the novel apoptosis related gene BCL2L12 in prognosis and individualized treatment of breast cancer: a molecular and clinical approach

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Background: Breast cancer is a major health problem. The currently most successful approach for combating breast cancer is by early diagnosis, good prognosis and administration of effective treatment. A multitude of markers have been discovered within the last three decades, including factors related to different cell functions, such as apoptosis, with many members of the *BCL2* family of apoptosis-related genes being found to be differentially expressed in various malignancies, and some regulating cellular fate after exposure to anticancer drugs. A new member of the *BCL2* gene family, *BCL2L12*, was discovered and cloned (Scorilas et al. 2001) and it was found to be expressed in mammary gland. It maps to chromosome 19q13.3 and is localized between the *IRF3* and *RRAS* oncogene. Our objective is to investigate the novel gene *BCL2L12*, as a novel molecular biomarker for prognosis and individualized treatment of breast cancer.
Materials and Methods: In the present study, we explored the research on the prognostic value of *BCL2L12*, as a novel breast cancer biomarker. Sixty specimens from patients with, histologically confirmed, epithelial breast carcinoma were analyzed for *BCL2L12* gene expression by RT-PCR using gene specific primers. Actin was used as a control gene. Their gene expression profile was associated with clinicopathological parameters and survival analysis regarding to relapse and death were evaluated by constructing Kaplain-Meier curves and developing a Cox proportional hazard regression model.
We also studied the possible alterations in the mRNA expression of the apoptosis-related gene *BCL2L12* after cell treatment with cisplatin or carboplatin, in the breast cancer cell lines MCF7 and BT-20. The cytotoxic effect of each drug was evaluated by the MTT method and trypan blue staining, whereas the expression levels of distinct apoptosis-related genes were analysed by RT-PCR, using gene specific primers.
Results: Increased expression of *BCL2L12* gene was found in estrogen receptors positive as well as in chemotherapy responded patients. In addition, *BCL2L12*-positive patients were found to be almost 4 times less likely to relapse or die in comparison to *BCL2L12*-negative patients. Furthermore, treatment of the breast cancer cell lines, MCF-7 and BT20, with well-known chemotherapeutic drugs induces distinct alterations in the mRNA expression levels of *BCL2L12* gene, giving some preliminary information about its value in chemotherapy response prediction.
Conclusions: *BCL2L12* is involved in both breast cancer progression and in chemotherapy response, implying a possible role in individualized medicine and its application into more successful therapeutic interventions.
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143 POSTER

Comparison of cell death ELISAs applied as potential surrogate biomarkers in the clinical evaluation of AEG35156 (XIAP antisense)

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The protein XIAP is the most potent endogenous inhibitor of caspase function currently known, and its over-expression is associated with poor patient outcome. AEG35156 is a second generation 19-mer oligonucleotide targeting XIAP and is currently undergoing early clinical trials. One of the anticipated outcomes of AEG35156 treatment is the induction of tumour cell death. We have studied 3 different plasma ELISAs as pharmacodynamic biomarkers during a CRUK Phase I trial of AEG35156 administered as a 7 day infusion. M30 Apoptosense detects a caspase cleaved fragment of the epithelial cell protein cytokeratin 18 (CK18) as a selective marker of apoptosis. M65 detects both intact and caspase cleaved CK18 as markers of apoptotic and non-apoptotic cell death. Quantitation of circulating nucleosomal DNA (nDNA) offers a further approach to apoptosis measurement. The 3 assays were utilised to analyse plasma samples collected at multiple time points spanning the first three week treatment cycle from 20 patients who had received AEG35156 at multiple dose levels from 48 to160 mg/m^2/d, where dose limiting transaminitis was encountered. Baseline concentrations of M30 and M65 antigens exhibited a 15 fold range. Similar values to those of healthy subjects were seen in patients with non-epithelial tumours (60–300 U/L). Very high values of 1600–3200 U/L were recorded in 2 patients with breast cancer. Analysis of two independent pre-treatment samples showed only minor variations in M30 and M65 antigens (<15%), whereas greater variability was detected with the nDNA assay. Increases in M30, M65 and nDNA antigens occurred with greater frequency at the higher doses of AEG35156, normally reaching a peak during the 7-day drug infusion. Increases in M30 and M65 antigens were also detected in patients with non-epithelial tumours, suggesting that these assays may also detect toxicity in non-tumour tissues. In 50% of patients, the concentration-time profiles for all 3 assays showed close temporal agreement. In a further subset of patients good temporal agreement was observed between nDNA and M65. In conclusion, the 3 ELISA assays appear to detect drug induced changes in circulating levels of their

respective antigens and may have potential as biomarkers of cell death. Further studies are required to define if these pharmacodynamic effects correlate with tumour responses and clinical outcome and whether these assays are also potential markers of drug induced toxicity.

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Novel Virtual Patient technology for predicting response of breast cancer and mesenchymal chondrosarcoma patients to mono- and combination therapy by cytotoxic and targeted drugs

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Introduction: Virtual Patient (VP) is a predictive biosimulation technology, comprising computer-implemented mathematical algorithms of key physiological, pathological and pharmacological processes in the body of the patient. Calibrated with available patient-specific data, the VP can accurately retrieve preclinical and clinical trials and predict short- and long-term effects of drugs.
Materials and Methods: The VP's solid tumor model was calibrated to retrieve the dynamics of breast cancer (BC) and mesenchymal chondrosarcoma (MCS) xenografts. Growth curves of untreated human tumor xenografts, derived from a lung metastasis of an MCS patient and histopathological results of this metastasis were used to create the MCS model. Published data served for modeling BC, PK/PD of three targeted therapies (Bevacizumab, Sunitinib, Sorafenib) and PK of four chemotherapeuteutics (Docetaxel, Gemcitabine, Doxorubicin and Irinotecan) in mice. *In vitro* proliferation assays of the MCS patient's tumor cells were used for establishing patient-specific concentration-effect curves for the chemotherapeutics. 'Administration' of the virtual drugs as single-agents and in combination was simulated and compared to corresponding experimental growth curves of treated and untreated MCS tumors for evaluating prediction accuracy. Optimal treatment was calculated.
Results: Significant superiority of Bevacizumab +Docetaxel combination, and Sunitinib, on other therapies, notably Gemcitabine, was shown for the MCS patient's xenografts. Over the simulated treatment period of up to 41 days, combinations with Bevacizumab are predicted to greatly enhance the treatment efficacy in comparison to the corresponding monotherapies in both cancer types. The average accuracy of the VP's predictions is 82%.
Conclusions: The VP showed high precision in predicting the growth pattern and response of xenografted MCS patient's tumor cells to various mono- or combination therapies. Our results suggest that, in general, treatments involving antiangiogenic drugs greatly improve MCS as well as BC tumor growth inhibition. In particular, Bevacizumab+Docetaxel regimens of reduced doses and inter-dosing intervals proved superior to other tested regimens for both indications. These results support the use of the Virtual Cancer Patient as a powerful tool for personalizing patients' treatment, especially when the application of new drugs is anticipated or when treatment of patients with rare diseases is considered.

Drug delivery

145 POSTER

Marked therapeutic efficacy of a novel poly(ethylene-glycol) conjugated SN38 conjugate in xenograft models of breast and colorectal cancers

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Background: SN38 (10-hydroxy-7-ethyl-camptothecin) is the active metabolite of CPT-11 (Camptosar®). The clinical utility of SN38 has been severely limited due to its poor solubility. We have generated a novel water soluble conjugate, PEG-SN38, by linking SN38 with a multi-arm high molecular weight polyethylene-glycol (PEG). PEG-SN38 conjugate is readily soluble and has *in vitro* potency equivalent to that of the free drug on a panel of tumor cell lines. Here we evaluate the pharmacokinetics and therapeutic efficacy of PEG-SN38 in xenograft models of human breast and colorectal cancer.
Material and Methods: Therapeutic efficacy of PEG-SN38 was evaluated in nude mice implanted with MX-1 breast tumor fragments or HT-29 colorectal cells. Pharmacokinetics of PEG-SN38 was determined in naïve (tumor free) ICR mice.
Results: In the MX-1 breast model, treatment with either a single dose of 20 mg/kg or multiple doses of 5 mg/kg (q2d × 6) PEG-SN38 led to 100% tumor growth inhibition and complete cures of all the animals. At equivalent dose levels, treatment with CPT-11 caused a 26 and 44% TGI when given as a single dose or multiple injections, respectively. In the HT-29 colorectal xenograft model, treatment with a single suboptimal dose of 12 mg/kg PEG-SN38 caused a TGI of 47%, while CPT-11 at the same dose-level caused only a 3% TGI. In the same model, PEG-SN38 when given as multiple 3 mg/kg doses (q2d × 5) caused a TGI of 60% and treatment with PEG-SN38 was significantly better than that with CPT-11 or pegamotecan (a PEGylated prodrug of camptothecin) ($P < 0.05$). The pharmacokinetic profile of PEG-SN38 in mice was biphasic showing a rapid plasma distribution phase during the initial 2 hrs followed by a 18−22 hrs terminal elimination half-life for the conjugate and a concomitant 18−26 hrs terminal elimination half-life for SN38.
Conclusions: PEG-SN38 demonstrated excellent antitumor activity in xenograft models of breast and colorectal cancer that, under our conditions, was significantly better than CPT-11. PEG-SN38 also provides a longer circulation half life compared to the native drug, SN38. These results merit further investigation of PEG-SN38 in the clinic.

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In vivo tumor targeting and radionuclide imaging with self-assembled nanoparticles: mechanisms, key factors, and their implications

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Background: The development of more selective drug delivery systems is one of the most important goals of current anticancer research. We herein describe a highly effective tumor-targeting strategy utilizing self-assembled nanoparticles.
Material and Methods: By combining different hydrophobic moieties and hydrophilic polymer backbones, various self-assembled nanoparticles were prepared, and their *in vivo* distributions in tumor-bearing mice were studied by radionuclide imaging.
Results: The most striking result was that only one type of nanoparticles (fluorescein isothiocyanate-conjugated glycol chitosan (FGC) nanoparticles) among many nanoparticles exhibited highly selective tumoral localization while all the others showed poor tumor selectivity. Scintigraphic images obtained 1 day after the intravenous injection of FGC nanoparticles clearly delineated the tumor against adjacent tissues. The mechanisms underlying the tumor targeting with self-assembled nanoparticles were investigated in terms of the physicochemical properties of nanoparticles and tumor microenvironments. FGC nanoparticles were preferentially localized in perivascular regions, implying their extravasation to tumors through the hyperpermeable tumor vasculature. The magnitude and pattern of tumoral distribution of self-assembled nanoparticles were influenced by several key factors: (i) *in vivo* colloidal stability: nanoparticles should maintain their intact nanostructures *in vivo* for a long period of time, (ii) particle size, (iii) intracellular uptake of nanoparticle: fast cellular uptake greatly facilitates the tumor targeting, (iv) tumor angiogenesis: pathological angiogenesis permits access of nanoparticles to tumors.
Conclusions: We believe that this work can provide insight for the engineering of nanoparticles and be extended to cancer therapy and diagnosis, so as to deliver multiple therapeutic agents and imaging probes at high local concentrations.

147 POSTER

A new Taxol delivery system for the treatment of brain primary or metastatic tumors

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Background: Brain tumors are among the most vascularized and resistant tumors. The blood–brain barrier (BBB) is frequently a rate limiting factor for the penetration of anticancer drugs into the central nervous system. In the present study, we investigated the utilization of a new peptide based drug delivery technology that provides a non-invasive and flexible platform for transporting drugs into the central nervous system. Taxol, which is normally impeded to reach its target in the brain by the presence of the P-glycoprotein (P-gp) efflux pump at the BBB, has been conjugated to these vector-peptides (Angiopeps). The efficacy of this Taxol-Angiopep conjugate has been assessed *in vitro* and *in vivo* using different experimental approaches.