technologies such as ELISA and flow cytometry. The EGFR/HER1 and HER3 assays displayed ~5-fold greater sensitivity than conventional IHC measurements. When compared with ELISA and flow cytometry, the rank order and accuracy of the HER1 and HER3 measurements were preserved over a wide dynamic range in well characterized cell line panels. Intra-assay and inter-assay reproducibility measurements demonstrated 7−12%CV and 13−20%CV, respectively. Current studies are underway to explore the predictive value of quantitative HER1 and HER3 biomarker measurements in a retrospective selection of patients for erlotinib, cetuximab, and lapatinib therapy, as well as to explore the potential additive value of HER1 and HER3 measurements in selecting patients for trastuzumab therapy. We believe these assays may provide the next generation of predictive assays for alternative or combination therapies for the treatment of solid malignancies.

104 POSTER

Role of P21 in sensitivity to DACH-platinum compounds, oxaliplatin and ProLindac, in human cancer cells

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Background: ProLindac (AP5346) is a novel DACH platinum prodrug with activity in a wide variety of solid tumors in preclinical models and in clinical trials. ProLindac was designed to be relatively non-toxic, with increased platinum release within the tumor environment in acidic pH. We previously showed that ProLindac yields antiproliferative effects, platinum-DNA incorporation, DNA strand-breaks and apoptosis, which were similar to that of oxaliplatin in most human cancer cell lines. This study evaluated the effects of ProLindac on the expression of several cell cycle and DNA repair-related genes aiming identifying biomarkers of sensitivity.
Methods: Antiproliferative effects of ProLindac, oxaliplatin and cisplatin were evaluated in human cancer cell lines by MTT assay after 72 hours of exposure. Gene expression was determined using q-RT-PCR.
Results: ProLindac displayed cytotoxic effects against human cancer cell lines (IC_{50} values: 0.3–2.2 μM), HT29 being the most sensitive. At equimolar concentrations, ProLindac and oxaliplatin displayed similar level of activity, that differs from that of cisplatin in our panel of human cancer cells. Treatment of HCT116 cells with ProLindac induced a cell cycle delay in both G1 and G2 phases. Similar results were observed using equitoxic concentrations of oxaliplatin while, conversely, cisplatin only induced a G2-arrest in cell cycle. In HCT116 colon cancer cells, the antiproliferative effects of ProLindac were associated with >5-fold increase of p21 expression and 10-fold decrease of Ki67 and NEK2 mRNA levels. The effects of ProLindac on gene expression were p53-dependent. In cells with deletion of p53, the expression of p21 was 2.5-fold decreased and no significant effect of ProLindac was detectable. Oxaliplatin (but not cisplatin) displayed similar effects on gene expression, although the changes were observed earlier than for ProLindac. The role of p21 in cellular response to ProLindac was confirmed using isogenic $p21^{+/-}$ cell lines. ProLindac was more active in $p21^{+}$ than in $p21^{-}$ cells (IC_{50}s 0.5 and 1.1 μM, respectively). Oxaliplatin but not cisplatin also displayed increased cytotoxicity against $p21^{+}$ cells.
Conclusions: The antiproliferative effects of ProLindac are similar to that of oxaliplatin but with a lag due to the DACH platinum polymer release. These effects are associated with increased p21 expression, which appears to be necessary for G1 arrest and cellular response to DACH-platinum drugs.

105 POSTER

Evaluation of PET tracer uptake in mouse xenograft models of hormone-dependent prostate cancer

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Previous mouse PET studies with the human hormone-independent cell lines PC-3 and DU145 showed, also compared to clinical findings in humans, very different pharmacodynamics and uptake characteristics for [18F]FLT, [18F]FDG, [11C]Choline and [18F]FECh in both xenograft models. Subsequently, the aim of this study was to investigate PET tracer uptake dynamics in mice for the CWR22 and PAC120 hormone-dependent human prostate-tumour models before and after castration. A $2\times2\times1\,mm^3$ viable tumour outgrowth was grafted subcutaneously into BALB/c-nude male mice. After tumour formation followed baseline PET imaging on four consecutive days with the four tracers. After this baseline scan the animals were surgically castrated to mimic an androgen ablation therapy. On three different time points after castration mice were again imaged with the four different tracers on four consecutive days. After the last scan tumours were harvested for histology and immunohistochemistry. Dynamics of Tracer uptake was assessed by analyzing the time activity curves (TAC). Results from static scans were recorded as percent injected dose per cc (%ID/cc) and standard uptake value (SUV). Table 1 displays the [18F]FLT and [18F]FDG tracer uptake values for the baseline scans and imaging performed 3 weeks after castration. While we found faint uptake in tumours imaged with [18F]FECh, no tumour tracer uptake was achieved with [11C]Choline.
We observed only a moderate [18F]FLT and [18F]FDG uptake. Castration induced a decrease of [18F]FDG and [18F]FLT tumour-to-muscle ratios in the CWR22 model. For the PAC120 we found a decrease in tumour uptake with [18F]FDG and for [18F]FLT an increase. Currently we focus on cross-validation of the PET data using Ki67 immunohistochemistry.

Table 1

	FLT		FDG	
	Baseline	3 w post castration	Baseline	3 w post castration
CWR22	n = 7	n = 5	n = 5	n = 5
SUV-tumour	0.30±0.15	0.27±0.13	0.60±0.13	0.30±0.10
%ID/cc − tumour	1.20±0.68	1.09±0.58	2.36±0.43	1.24±0.40
%ID/cc − muscle	0.68±0.59	1.11±0.57	1.23±0.67	1.58±0.38
PAC120	n = 7	n = 4	n = 6	n = 3
SUV-tumour	0.22±0.10	0.45±0.10	0.42±0.05	0.33±0.05
%ID/cc − tumour	0.83±0.40	1.87±0.50	1.56±0.15	1.34±0.16
%ID/cc − muscle	0.63±0.51	1.54±0.58	1.02±0.25	0.75±0.07

106 POSTER

−765G>C COX2 polymorphism and bladder cancer onset: implications for chemoprevention in a Portuguese population

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Background: Urothelial cell carcinoma (UCC) is relevant in matter of health care treatment and life quality loss. BCG immunotherapy comes to improve recurrence free survival of this disease. Although the efficacy of this therapy, 30% of treated patients recur and present numerous side effects. Cyclooxygenase-2 (COX-2) overexpression in UCC has been associated with unfavourable overall survival of patients with superficial high risk tumors. Furthermore, COX-2 selective inhibition has been suggested as having antitumor activity against bladder cancer and being a potential mechanism for improving the efficacy of BCG immunotherapy. Even though, the *−765G>C* polymorphism in the *COX2* promoter region has been associated with the development of several epithelial tumors, no report regarding the involvement of this genetic variation in bladder cancer has yet been published. Therefore, the aim of this study was to assess the influence of the *−765G>C* COX2 polymorphism in the development of bladder cancer and tumor recurrence after BCG immunotherapy.
Material and Methods: DNA extracted from peripheral blood of 387 individuals (136 bladder cancer patients treated with BCG and 251 healthy controls) was genotyped by Polymerase Chain Reaction – Restriction Fragment Length Polymorphism (PCR-RFLP) for *−765G>C COX2* polymorphism.
Results: We found that *−765GC* genotype was overrepresented in the cancer patients group (42.6% vs 28.3% in control group, $P = 0.003$). A nearly two-fold increased risk for bladder cancer onset was observed for *−765C* allele carriers (GC and CC genotypes pooled together) (Odds Ratio (OR) = 1.98; 95% confidence interval (CI): 1.27–3.00). This increased risk was even more pronounced in individuals younger or with 56 years carriers of *−765C* allele (OR = 3.09; 95%CI: 1.50–6.38). When correlating the genotypes with the clinical data, we observed that an increased risk for development of multifocal tumors was found for C allele carriers (OR = 2.70; 95%CI: 1.20–6.07). We found no statically significant differences between *765G>COX2* genotypes and disease recurrence after BCG immunotherapy.

Conclusions: This preliminary study seems to indicate that the presence of *−765C COX2* allele could be an important marker to yield the identification of higher risk individuals for bladder cancer development that might beneficiate from chemopreventive strategies with COX inhibitors.

107 POSTER

Novel epitopes presented by the HLA-A*0201 are recognized by the cytotoxic T lymphocytes of breast cancer survivors

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Cellular immune mechanisms detect and destroy cancerous and infected cells via the human leukocyte antigen (HLA) class I molecules that present peptides of intracellular origin on the surface of nucleated cells. The identification of novel, tumor-specific epitopes is a critical step in the development of immunotherapeutics for breast cancer. In order to directly identify peptide epitopes unique to cancerous cells, secreted human class I HLA molecules (sHLA) were constructed by deletion of the transmembrane and cytoplasmic domain of HLA A*0201. The resulting sHLA-A*0201 was transferred and expressed in breast cancer cell lines MCF-7, MDA-MB-231, and BT-20 as well as the immortal, non-tumorigenic cell line MCF10A. Stable transfectants were seeded into bioreactors for production of >25 mg of sHLA-A*0201. Peptides eluted from affinity purified sHLA were analyzed by mass spectroscopy. Comparative analysis of HLA-A*0201 peptides revealed 5 previously uncharacterized epitopes specifically presented by the tumorigenic cell lines. These peptides are derived from intracellular proteins with either well defined or putative roles in breast cancer development and progression: Cyclin Dependent Kinase 2 (Cdk2), Ornithine Decarboxylase (ODC), Kinetochore Associated 2 (KNTC2 or HEC1), Macrophage Migration Inhibitory Factor (MIF), and Exosome Component 6 (EXOSC6). From a small HLA-A*0201 positive cohort including 8 breast cancer survivors and 9 controls, cellular recognition of the ODC, MIF, KNTC2, EXOSC6, and Cdk2 peptides by circulating CD8+ cells from 3 cancer survivors was demonstrated by tetramer staining, IFN-γ ELISPOT, intracellular cytokine staining, and CD107a cytotoxicity assays. The identification and characterization of peptides unique to the class I of breast cancer cells provide putative targets for the development of immune diagnostic tools and therapeutics.

108 POSTER

Screening Plasma Thioredoxin-1 (Trx-1) to potentially guide clinical development of the Trx-1 Inhibitor PX-12

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Background: PX-12, an irreversible thioredoxin (Trx-1) inhibitor, is currently being evaluated in a number of clinical trials. Its molecular target Trx-1 is over-expressed in many human cancers and is associated with aggressive tumor growth, poor prognosis and contributes to resistance to chemotherapy. Tumors which over-express Trx-1 also secrete the protein, and plasma Trx-1 levels of cancer patients have been found to be elevated over that of healthy individuals. After surgical removal of tumor burden, these plasma levels drop to the normal range. In previous and ongoing clinical trials, in some patients treated with PX-12, circulating levels of Trx-1 were lowered and a Trx-1 decrease of 25% or greater was associated with increased survival in the Phase 1 study. This current study evaluated the plasma of 135 patients from 7 different indications to determine if there may be potential to select patients based upon thioredoxin levels.
Methods: Frozen plasma samples were obtained from consented patients through the NCI's Cooperative Human Tissue Network and included 17−20 from each of prostate, renal, melanoma, endometrial, sarcoma, pancreas and colon cancers. Plasma Trx-1 protein was measured by a sandwich ELISA developed by Oncothyreon. Other angiogenic proteins regulated by Trx-1 were also evaluated by standard commercial ELISA. Trx-1 in ng/mL was determined for each samples and the data is reported by indication with comparisons to other proteins such as VEGF and FGF-2.
Results: A total of 135 patient samples from 7 different indications were evaluated for Trx-1, VEGF, FGF-2 and other angiogenic proteins. It was found that 37 of the 135 had elevated circulating levels of Trx-1 (>3-fold of the previously determined normal donor median, 5.4 ng/mL). Of the indications where samples were obtained, sarcoma, melanoma, pancreatic and endometrial had the highest percentage of patient plasmas with elevated Trx-1 values (50%, 45%, 35%, and 32% respectively). The plasma Trx-1 levels will be presented along with trends with other Trx-1 controlled plasma protein and patient status information where available.
Conclusions: It has been found that patients with elevated plasma Trx-1 treated with PX-12 were more likely to have lowering of Trx-1 levels and possibly a better clinical outcome than patients with plasma Trx-1 in the normal range. The results from the current study show that approximately 30% of patients across a number of indications have elevated plasma Trx-1. The data suggests clinical studies of a Trx-1 inhibitor such as PX-12 may benefit from patient selection.

109 POSTER

Promoter hypermethylation of the NORE1A occurs in endometrial cancer

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Background: Inactivation by promoter hypermethylation of the tumor suppressor gene RASSF1A and NORE1A have been reported in a variety of human cancers. Activating point mutations of the K-ras oncogene are a common feature of many tumor types.
Materials and Methods: We have analysed the K-ras mutational status and promoter hypermethylation of RASSF1A, NORE1A and hMLH1, and MSI of 78 endometrial cancers.
Results: Promoter hypermethylation of RASSF1A, NORE1A and hMLH1 was detected in 29.5% (23 of 78), 20.5% (16 of 78) and 26.9% (21 of 78) of endometrial cancers respectively. The absence of hMLH1 methylation and the absence of microsatellite instability (MSI) were almost exclusively confined to those without RASSF1A methylation in endometrial cancers respectively in this study. We demonstrate that RASSF1A promoter methylation was more frequently present in wild-type K-ras, and that NORE1A methylation was exclusively confined to those without hMLH1 methylation in the endometrial cancer.
Conclusions: Our results suggest that RASSF1A, NORE1A and hMLH1 are inactivated in a subset of endometrial cancers. The mutual exclusivity of the epigenetic and genetic alterations in these genes suggest that they play a critical and cooperative role in the carcinogenesis of endometrial cancer.

110 POSTER

TM9SF2, a novel cell surface potential target in breast cancer

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Cell surface proteins of tumor tissues are believed to have different expression from their counterparts of normal tissues. Thus, cell surface proteins can be attractive therapeutic targets important to optimize treatment strategies. To detect differentially expressed cell surface markers in breast carcinoma, we analyzed gene expression profiles of eight human breast cancer cell lines and normal breast tissue with oligonucleotide microarray. The array chips were fabricated in our laboratory so that the design of probes was implemented primarily to include the DNA sequence coding the transmembrane region or the GPI-anchor attachment site; excluding any alternative splicing possibility. Besides, probes of control RNAs were allowed to hybridize to confirm the reliability of the hybridization reactions. We followed a two-level analysis for the study of this complex gene expression data. This analysis summarizes the data by the SOM component plane, and then clusters the SOM to investigate the feature gene expression patterns. The SOM reduced the nine dimensions of the data to cluster the genes on a two-dimensional sphere and thereby allows to easy display the data and reveal the gene expression patterns. As a result, we detected genes whose expression was significantly consistent in all the cancer cell lines used and higher than that of normal breast tissue. The genes were transmembrane 9 superfamily protein member 2 (TM9SF2), vitelliform macular dystrophy 2-like protein 3 (VMD2L3), adenylate cyclase type III (ADCY3), membrane-spanning 4-domains subfamily A member 2 (MS4A2), and short transient receptor potential channel 5 (TRPC5). Also, CD24 and ErbB3 were significantly upregulated; evidence that supports the integrity of both arrays and samples. We selected TM9SF2 which is related to cell-to-cell signaling and functions as a channel or small molecule transporter; along with CD24 and ErbB3 as monitors, to validate their expression levels by real time quantitative PCR. The gene expression levels of TM9SF2 were significantly higher in all carcinoma than in normal breast tissue. TM9SF2 protein expression was evaluated by immunoflorescence and it showed concordance with the PCR results. Treatment by siRNA has enhanced TM9SF2 knockdown and less viability of MDA-MB-231 cells when compared to those treated with scrambled control siRNA. Here on, more siRNA treatments are to be done on the other cell lines.