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.

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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.

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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

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−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.