to GSK1120212 was also observed. For breast cancer cell lines, unbiased transcript analysis revealed high expression of both progesterone and estrogen receptors correlates with resistance suggesting that ER/PR/HER2 negative tumors might respond positively to this inhibitor.

**100** POSTER

**The Expression of c-erb B2 and p53 in human gastric cancer: Correlation to clinicopathologic features and cancer recurrence**

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**Background:** Although UICC TNM stage has been adopted as a predicting factor for recurrence of gastric cancer, there's no satisfactory and affirmative prognostic factors proved to be associated with recurrence rates of gastric cancer. With the advancement of biologic markers, several researchers suggested that expression of these biomarkers could predict the prognosis of gastric cancer, which is still controversial. Therefore, we investigated the expression of c-erb B2/p53 protein in 126 Korean patients who were subjected to curative resection of gastric carcinoma. The aim of this study was to determine if the expression of c-erb B2/p53 is related to clinicopathologic factors in gastric cancer after curative resection, which can be a candidate for predicting factors of tumor recurrence.
**Material and Methods:** The surgical specimens of 126 patients underwent curative resection at St. Mary Hospital, the Catholic University of Korea between January 2000 and June 2003 were investigated. The frequency of c-erb B2 and p53 expression were examined by immunohistochemistry using formalin fixed, paraffin embedded gastric carcinoma specimens.
**Results:** 73 specimens (57.9%) showed the immunoreactivity to p53, while 30 specimens (23.8%) were positive to c-erb B2 expression. When investigating relevance between the expression of these molecules and clinicopathologic features, the expression of p53 was associated with tumor invasion ($p = 0.029$) and c-erb B2 with tumor invasion ($p = 0.019$), lymph node metastasis ($p = 0.003$) and lymphatic invasion ($p = 0.022$). The patients were followed for mean 47 months. When patients with gastric cancer showed the expression of c-erb B2, they had higher recurrent rates in univariate analysis ($p = 0.051$). Especially, the disease-free survival rate of patients with the simultaneous expression of c-erb B2 and p53 was significantly lower than that of other patients ($p = 0.006$). Multivariate analysis revealed expression of both c-erb B2 and p53 in gastric cancer was associated with high rates of tumor recurrence (OR = 3.186, 95%C.I.; 1.289–7.876).
**Conclusions:** Expressions of c-erb B2 and p53 were related to pathologic advancement of gastric cancer. Immunoreactivity to both c-erb B2 and p53 were independent predicting factors for tumor recurrence. These results suggest that an adjuvant antibody therapy targeted to c-erb B2 might be needed in patients who underwent the curative gastric cancer surgery and had tumors expressing c-erb B2 as well as p53.

**101** POSTER

**Identification of alpha-enolase autoantibody as a novel biomarker in non-small cell lung cancer**

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**Background:** Lung cancer is the leading cause of cancer death and non-small-cell lung cancer (NSCLC) accounts for approximately 80% of cases. There is a continuing need for the identification of novel biomarkers to facilitate the detection of NSCLC. Serum autoantibodies against tumor antigens represent potential biomarkers for both cancer detection and monitoring of disease progression. In this study, we used a proteomics-based approach to identify tumor antigens specific to NSCLC.
**Material and Methods:** Proteins from NSCLC tumor tissues were separated by 2-D gel electrophoresis, transferred onto membrane and incubated with subject sera. Proteins that reacted specifically with serum antibodies were identified by mass spectrometry and further evaluated by Western blotting and ELISA using recombinant proteins.
**Results:** Proteomics-based screening demonstrated the presence of serum autoantibodies to alpha-enolase in a subset of NSCLC patients. The prevalence of this autoantibody was 27.7% in NSCLC patients (26 of 94), 1.7% in healthy controls (1 of 60) and was not detected in sera of 15 patients with small cell lung cancer, 18 patients with gastrointestinal cancer and nine patients with mycobacterium avium complex infection of lung. Immunohistochemical staining showed increased expression of alpha-enolase in lung tissues of NSCLC patients. FACS analysis confirmed the expression of alpha-enolase on the surface of cancer cells. Sensitivity to detection of NSCLC using current biomarkers (carcinoembryonic antigen and cytokeratin 19 fragment) was increased with the combined use of alpha-enolase (from 58.9% to 69.3% detection).
**Conclusions:** By proteomic approach, we observed a specific association between the presence of serum autoantibodies to alpha-enolase and NSCLC. Autoantibodies to alpha-enolase may represent a novel and specific biomarker for the enhanced detection of NSCLC.

**102** POSTER

**Quantitative clinical biomarker measurement using multiplexed quantum dot immunhistochemistry**

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The recent widespread use of global gene expression profiling has identified a plethora of prognostic biomarkers for cancer. These biomarkers can direct targeted therapy and, in order to inform drug discovery, a method is required for their measurement and localisation in clinical tumour biopsy samples. Quantum dots (QD) are fluorescent semiconductor nanocrystals possessing wide excitation and narrow, symmetrical emission spectra; their emission is bright and resistant to photobleaching. As such, their application for quantitative multiplexed immunohistochemistry in clinical samples, along with the capability to measure co-localisation, is of significant interest due to the limited quantity of clinical tissue available. Traditional methods of multiplexed staining using QDs involved sequential staining approaches, which are lengthy and operator-intensive, so consequently are not practical in a high-throughput clinical trial setting. This study has investigated systematically the problems associated with sequential multiplex staining and developed a method using conjugation of QDs to biotinylated primary antibodies, enabling simultaneous multiplex staining of up to three antigens. An endothelial cell marker (CD34), an epithelial cell marker (cytokeratin 18) and an apoptosis biomarker (cleaved Caspase 3) were triplexed in tonsillar tissue using a 24 h protocol, with localisation of each to separate cellular compartments. Using spectral imaging technology, the average signal intensity/pixel and percentage co-localisation of each were measured. This study demonstrates utility of the method for clinical biomarker measurement, particularly in scarce or small tissue samples, enabling quantitative measurement of multiple co-localised biomarkers on single paraffin tissue sections within one day, of importance for future (high-throughput) clinical trial studies.

**103** POSTER

**Development of highly quantitative, sensitive, and reproducible immunoassays for the detection of EGFR/HER1 and ErbB3/HER3 in formalin-fixed, paraffin-embedded (FFPE) tumor tissue**

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The overall response rate of most ErbB family targeted therapeutics is generally quite low. While measurement of drug target levels is often performed prior to therapy as a means to identify potential responders, the available immunohistochemistry (IHC) methods for detecting HER family members are relatively insensitive and not quantitative. Consequently, the selection of patients likely to respond to therapy may not be optimal. To address this issue, we have developed highly quantitative, accurate, precise, sensitive, and reproducible EGFR/HER1 and HER3 total protein assays. Our method is based on the VeraTag technology platform which utilizes a proximity-based release of a fluorescent tag bound to a specific antibody and the subsequent quantification of this tag by capillary electrophoresis. In the most common format of the VeraTag platform, the dual-antibody approach provides significantly increased selectivity and sensitivity above single antibody-based IHC methods. Antibodies targeting HER1 and HER3 proteins were selected in screens of over 20 commercially available antibodies as well as from an in-house effort to generate novel monoclonal HER3 antibodies. Measurements of EGFR/HER1 and HER3 total protein levels, in an FFPE format, were established in well characterized cancer cell lines as well as a variety of solid malignancies including breast, ovary, lung, and head and neck. Accuracy of EGFR and HER3 protein measurements were determined by using cross-validating

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.