mice. Treatment of mice with sphingosine (25 mg/kg) resulted in a time-dependent rise in 5-HIAA.
Conclusions: The results, together with those from work in progress, are consistent with the hypothesis that ASA404 activates the ceramide pathway. Ceramides and/or sphingosine may activate downstream targets including p38 MAP kinase, leading to vascular damage and consequent release of 5-HT from platelets. In addition to its role in ASA404 action, sphingosine may be a potentially useful biomarker for the assessment of patients treated with ASA404 in combination therapy.

97 POSTER

Analysis of EGFR and KRAS mutations in circulating tumor DNA (cTDNA) from plasma of NSCLC patients in phase 2 trials of XL647

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Background: EGFR and KRAS mutations play a critical role in the response to EGFR tyrosine kinase inhibitors (TKI) in NSCLC. Obtaining adequate tumor tissue in patients (pts) with non-operable disease, especially prior to initiation of a later line of therapy when the molecular profile of a cancer may have evolved, is a key element for the development of targeted anticancer agents and remains challenging. Sensitive assay methodology, eg allele specific PCR, to analyze cTDNA may facilitate development of targeted agents such as XL647, a TKI which potently inhibits EGFR (including the T790M resistance mutation), HER2 and VEGFR2 in vitro.
Methods: EGFR and KRAS mutations were determined in plasma cTDNA by the sensitive Scorpions ARMS technology (DxS Ltd, Manchester, UK). Samples were collected from pts enrolled in NSCLC clinical trials of XL647 either in a first line or late line of therapy after previous EGFR TKI benefit. EGFR and KRAS mutations in plasma were compared to those in available tumor samples.
Results: Plasma samples (n = 67) from 55 pts were analyzed in the DxS EGFR29 panel. A subset of 46 samples was analyzed with the DxS KRAS kit. EGFR ($p < 0.0001$) and KRAS ($p = 0.0003$) DNA levels were lower in plasma from late line pts compared to those with newly diagnosed disease. The spectrum of EGFR mutations detected included Exon19 deletions, L858R, T790M, L861Q, and G719X. Assay failure was more likely when testing KRAS (19/46) than EGFR (3/67), $p < 0.0001$ and more common when testing KRAS in the late line setting (16/23 vs 3/23), $p < 0.0001$, thought secondary to lower PCR performance specific to the KRAS sequence. When testing an individual patient over time, variability was noted. The significance of this observation is being explored. Data from 11 paired plasma and tumor samples in first line pts showed concordance for EGFR status at 82%, specificity at 100%, while the sensitivity to detect EGFR mutations was limited at 50%. In the late line, EGFR mutations were detected in plasma from 11/39 pts. Correlation of these results relative to direct analysis of archival tumor is ongoing.
Conclusions: cTDNA can be informative for EGFR and KRAS mutation testing; however, the current technology performs better on samples from newly diagnosed patients than on those with disease recurrence/persistence. Optimal timing for plasma sampling and larger studies are required to better understand the limitations of mutational analysis conducted exclusively in plasma.

98 POSTER

DUSP4 expression level in colorectal primaries predicts overall survival benefit in Kras wild-type and Kras mutant colorectal cancer after treatment with cetuximab for metastatic disease

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Background: DUSP4 dephosphorylates and inactivates ERK and is upregulated by Kras mutations (MUT). Higher expression of DUSP4 was found in epithelial cell cultures with activated ras (Bild et al. Nature 2005). Microarray analysis on metastases of colorectal cancers (CRC) treated with cetuximab (CTX) (Khambata-Ford S et al. JCO 2007) identified DUSP4 as a top resistance marker. We correlated Kras MUT state and DUSP4 mRNA expression level in 197 primary CRC of patients (pts) treated with CTX +/− irinotecan (IRI) with objective response (OR), progression-free survival (PFS) and overall survival (OS).
Material and Methods: Tumor areas were manually dissected on FFPE samples. Kras codon 12&13 MUT were analyzed by an allelic discrimination assay. We measured DUSP4 mRNA expression by real-time qRT-PCR. Relative expression levels were calculated comparing average values of duplicate reactions with a calibrator and normalizing them to 3 housekeeping genes: GAPDH, RPL13, UBC. Levels were expressed as calibrated normalized relative quantities. We used the median of these values as cut-off to distinguish high from low expression.
Results: 45% had a Kras MUT. Kras wild type (WT) was associated with OR ($p < 0.0001$), PFS ($p = 0.0005$) and OS ($p = 0.0001$). Low DUSP4 was associated with Kras WT ($p < 0.0001$). DUSP4 was associated with OR: 72% (33/46) of responders had a low DUSP4 compared to 44% (66/151) of non-responders ($p < 0.0012$). Overall, there was no association between DUSP4 and PFS ($p = 0.33$). In Kras WT, there was a trend towards longer median PFS in low DUSP4 ($p = 0.10$). Overall, OS was associated with low DUSP4 [41w (95%CI [36−49]) vs 33w high DUSP4 (95%CI [27−46]) ($p = 0.06$)]. In Kras WT, OS was associated with low DUSP4 [54w (95%CI [430−72]) vs 34.5w high DUSP4 (95%CI [25−54]) ($p = 0.01$)]. In Kras MUT, OS was associated with high DUSP4 [32w (95%CI [15−33]) vs 18w low DUSP4 (95%CI [21−39]) ($p = 0.02$)]. A Cox regression model for PFS and OS was built using Kras, DUSP4 and skin toxicity (table).

	PFS				OS			
	Sig.	HR	95% CI		Sig.	HR	95% CI	
			Lower	Upper			Lower	Upper
Kras WT								
DUSP4 (< or > median)	0.0308	0.614	0.395	0.956	0.0024	0.507	0.327	0.786
Skin tox (gr 2−3 vs 0−1)	<0.0001	0.345	0.223	0.533	<0.0001	0.407	0.265	0.623
Kras MUT								
DUSP4 (< or > median)	0.1030	1.514	0.920	2.492	0.0772	1.582	0.951	2.631
Skin tox (gr 2−3 vs 0−1)	0.1576	0.678	0.395	1.162	0.0060	0.443	0.248	0.792

Conclusions: DUSP4 expression levels influence OS in both Kras WT and Kras MUT CRC treated with CTX +/− IRI. In Kras WT low DUSP4 levels are favourable, while in Kras MUT high DUSP4 levels are. Kras WT with high DUSP4 may have other oncogenic MUT e.g. Kras codon 61. The extent of ERK signalling is shown not to be the same in all Kras MUT. The prognostic effect of DUSP4 levels needs to be looked into, but the use of DUSP4 expression level as a substrate for the extent of ERK signalling may make it possible to select CRC likely to benefit from ERK-inhibitors.

99 POSTER

Models for response to the MEK inhibitor GSK1120212 confirm RAS and BRAF mutations as predictive biomarkers and suggest other, unexpected tumor types for clinical evaluation

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The growth factor stimulated RAS/RAF/MEK/ERK signaling pathway is involved in many aspects of cancer progression, and is activated in a large percentage of tumors evidenced by frequently occurring RAF and RAS mutations in cancer. BRAF mutations occur in ~45% of melanomas and KRAS in ~90% of pancreatic adenocarcinomas. GSK1120212 is a potent and selective allosteric inhibitor of the MEK1/2 enzymes that exhibits antiproliferative effect in cell lines and xenograft models. To identify predictive biomarkers to facilitate the clinical development of this compound, sensitivity profiling was carried out for over 300 cancer cell lines. The cell lines were from diverse tumor types to confirm existing hypotheses and explore the sensitivity of other tumor types that were not initially considered to be sensitive to MEK inhibition. As expected, BRAF/RAS mutation status emerged as a strong predictor of response. Cell lines from tumor types that have high occurrence of BRAF/RAS mutations (e.g., melanoma, pancreas and colon cancer) showed a higher rate of sensitivity. We further refined the predictors by grouping cell lines into cytotoxic and cytostatic groups. GSK1120212 has a cytostatic effect in cell lines that are RAF/RAS mutant with co-occurring PI3K/PTEN mutations but cytotoxic in the absence of PI3K/PTEN mutations, suggesting mutant PI3K/PTEN are cytotoxicity resistance markers for GSK1120212. However, for certain tumor types that do not carry BRAF or RAS mutations, sensitivity

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.

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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.

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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