and shown to be 100% concordant. Dilution experiments demonstrated an analytical sensitivity of 1% mutant DNA in a background of normal gene sequence and the pyrosequencing method showed an analytical sensitivity of 2−5%.
Conclusion: The experiments performed in these studies showed that both the ARMS and pyrosequencing methods could detect K-RAS mutations in formalin-fixed paraffin-embedded tissues in a reproducible and robust fashion. The assays showed comparable analytical sensitivity for the common codons examined.

PP109

Comparability and reliability of different approaches to obtain gene expression measurements from formalin-fixed, paraffin-embedded breast cancer samples

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Background: The use of formalin-fixed, paraffin-embedded (FFPE) tumour samples, a valuable resource for gene expression studies on archival specimens or on limited amount of tissue as that obtained from a core biopsy, is no longer restricted by the poor quality of the extracted RNA. New technologies allow the performance of reliable gene expression quantification from FFPE samples. The choice of the technique to use for such studies on FFPE samples depends mainly on practical issues such as the available amount of sample and the biological end-point of the study. However it is very important to understand if gene expression information obtained by different methods is comparable.
Materials and Methods: We selected 16 breast cancer samples for which FFPE and snap-frozen tissues were available. Gene expression data for the FFPE tissues were obtained using the microarray-based cDNA-mediated annealing, selection, extension and ligation (DASL®) assay from Illumina, the QuantiGene® Plex 2.0 Reagent System from Panomics and qPCR. Gene expression profiles were also obtained from high quality RNA drawn from snap-frozen tissues (HumanHT-12® Expression BeadChip, Illumina). Frozen tissues were also analysed using the QuantiGene® Plex 2.0 Reagent System and qPCR.
Results: After adequate pre-processing of data, we used a Pearson correlation analysis to assess DASL® and QuantiGene® reproducibility and intra-assay discrepancy when using frozen or FFPE derived RNA. We also correlated data for the same genes derived from different methods in order to elucidate inter-assays differences. Finally using available patho-biologic data of the samples and hierarchical clustering methods, we verified the biological reliability of results. Both DASL® and QuantiGene® assays provided highly reproducible and biologically reliable data when compared with either whole genome and qPCR expression measurements, nevertheless we found differences related on sensibility and dynamic range.
Conclusion: These data, together with technical issues, such as amount of material required and number of measured genes, can provide guidance for the choice of the strategy to apply in studies involving gene expression analysis of FFPE samples.

PP108

Genes associated to development of distant metastases in node negative breast cancer patients

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Background: In the group of node-negative breast cancer patients patients, about 30% will develop distant metastasis. Adjuvant treatments used for high-risk patients are poorly target-oriented and are characterized by high toxicities. Linking the risk prediction to specific biological mechanisms would therefore improve the ability to eventually plan target-oriented treatments
Materials and Methods: 63 tumors from women with resectable primary node-negative breast cancer, who developed distant metastases within 5 years from surgery and 64 tumors from women free of distant metastases for at least 5 years, were subjected to whole genome profiling. Differentially expressed (DE) genes among patient groups were investigated by significance analysis of microarrays
Results: In the 104 women with ER+ tumors 113 DE probes corresponding to 101 genes were found between primary tumors from women who developed metastases compared to those from women disease-free (FDR = 0.34), while in the 23 ER− primary tumors 594 probes were DE in two subsets, with a FDR = 0.49. Interestingly, in ER+ samples, at the same FDR level (0.34) a higher number of DE probes was found by separate analysis according to metastatic site (629 DE probes for bone, 707 for lung and 656 for liver metastases).
Clusters of correlated genes ($r > 0.40$) were identified and interrogated for biological function Compared to primaries from metastasis-free women, ER+ primary tumors from patients developing distant metastases over-expressed a cluster of interferon-related genes (IFN). In the 19 tumors from women with bone metastases beside IFN gene also a set of immunoresponse-related genes was over-expressed while extracellular matrix/cell adhesion genes and morphogenesis-related genes were down-regulated. In women developing lung metastases (7 cases) IFN-related and immunoresponse genes behaved oppositely and were down-regulated compared to disease-free controls, suggesting a different molecular mechanism promoting distant spread according to the site of metastatization.
In the 23 ER− primaries, genes related to glucoronization, morphogenesis and immunoresponse were up-regulated in patients developing distant metastases.
Conclusion: Metastatic dissemination can be fostered by the ability of tumors to recruit immune cells promoting an inflammatory environment which favours distant spread. Definition of involved tumor features would allow 1) prediction of distant spread risk and 2) identification of treatment targets.

PP111

Examination of the expression profiles of PGIS and TXS in NSCLC: Regulation of tumor cell growth and invasive potential

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Background: Prostacyclin Synthase (PGIS) and Thromboxane synthase (TXS) metabolize the cyclooxygenase product, prostaglandin H (2), into prostacyclin (PGI2) and thromboxane (TXA2) respectively. PGIS over-expression inhibits cancer growth in a murine model, while TXS over-expression has the opposite effects. TXS over-expression has been reported in a number of cancers and is associated with a poor prognosis. The aim of this study was to determine the individual roles of these enzymes in NSCLC.
Materials and Methods: Stable cell lines over-expressing PGIS and TXS were generated and their effect on tumor cell survival was examined (BrdU, FACS, Invasion Assay). PGIS and TXS expression were examined in human lung tumors and matched normal controls by western analysis and IHC. In a separate study, a 200-patient NSCLC TMA was generated and stained for TXS and PGIS expression. Staining intensity was correlated with clinical parameters and Kaplan-Meiers survival curves were constructed. Cell growth was examined in NSCLC cell lines following selective TXS inhibition. Apoptosis was assessed by High Content Screening (HCS) and validated by DNA laddering and Cell Death ELISA.
Results: Tumor cells over-expressing PGIS grew significantly slower than controls, were less invasive, and more sensitive to apoptosis following serum-starvation. In contrast, over-expression of TXS resulted in opposing effects. Examination of PGIS/TXS expression profiles revealed PGIS to be down-regulated/absent in tumor protein samples relative to normal, while TXS was up-regulated in tumors. TMA analysis revealed TXS expression to be significantly higher in adenocarcinoma tumor tissue, relative to squamous and in females, relative to males. A direct contrast in PGIS expression profile was observed, with significantly reduced expression in adenocarcinoma, relative to squamous and in females, relative to males. No correlation between TXS expression and patient survival was observed. Selective TXS inhibition significantly reduced tumor cell growth and increased apoptosis.
Conclusion: Overexpression of TXS increased proliferation and invasiveness of NSCLC cells, while PGIS overexpression had contrasting effects. Expression patterns of these enzymes are altered in NSCLC. The balance in PGIS/TXS expression may underlie the pathogenesis of NSCLC. While TXS does not appear to be prognostic, it may be a potential therapeutic target in NSCLC. In contrast, PGIS over-expression may be a novel strategy for chemoprevention studies.

PP86

Sister chromatid exchanges as marker of genomic instability in familial breast cancer patients

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Background: Cancer predisposition is correlated with spontaneous chromosomal instability. The aim of the present study was to determine whether there are an increased in number of SCE in peripheral blood lymphocytes of familial breast cancer patients compared to sporadic ones.
Materials and Methods: SCE were evaluated in 29 familial breast cancer patients with one first degree relative with a history of breast and/or ovarian

cancer, in 32 sporadic ones before surgery and in 17 healthy donors. For the analysis of SCE, 500 μl of peripheral blood were cultured in 5 ml of Chromosome medium P (Euroclone); after a period of 48 h at 37ºC, 25 μl of a 1 mg/ml 5-bromo-deoxyuridine solution was added to the blood culture. During the incubation, cells were protected from direct light to prevent photolysis of BrdU-containing DNA. Cells were arrested in metaphases by colcemid. After harvesting, they were treated with hypotonic solution at 37ºC and fixed with methanol:acetic acid 3:1. Slides were air dried, placed in 90ºC hot plate for 1 h, stained with Hoechst for 15 min and exposed to UV light for 1 h. Subsequently, the slides were incubated with water at 56ºC for 10 min and stained with Giemsa. For each patient 10−30 metaphases were evaluated. Every metaphase was scored for SCE and individual mean value per cell was calculated. SCE baseline values of familial breast cancer patients were compared to those of sporadic ones and to those of a control group of healthy donors.
Results: Results were expressed as means ± standard deviation (sd). Intergroup comparisons were performed using Kruskall Wallis non parametric test. However, SCE was significantly increased ($P < 0.05$) in familial breast cancer patients (5.3 ±1.2 sd per metaphase) respect to sporadic (3.9 ±1.2 sd) ones and to controls (4 ±1.5 sd). No correlation was found between clinic pathological data and number of SCE.
Conclusion: We suggest that the increased number of SCE in familial patients respect to sporadic ones reflects an intrinsic genomic instability that is an essential process in carcinogenesis.

PP55

A MAGE-A3 specific quantitative RT-PCR assay used for patient accrual in MAGRIT, a Phase III ASCI (Antigen-Specific Cancer Immunotherapeutic) trial in adjuvant NSCLC

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Background: Over the last 20 years, the field of active cancer immunotherapy has been extensively investigated but no products have been registered to date. GSK Biologicals is developing an approach to cancer therapy, Antigen-Specific Cancer Immunotherapy (ASCI) that combines a tumor-specific antigen, delivered as a recombinant protein, with a potent immunological Adjuvant System. The first compound of this new class of anticancer agent contain MAGE-A3 tumor-specific protein that is expressed in a number of cancer types including non-small cell lung cancer (NSCLC). The MAGE-A3 antigen has been produced as a recombinant protein and combined with an immunological Adjuvant to treat NSCLC patients. Since only a fraction of NSCLC tumors (about 35%) express the MAGE-A3 protein, patients' tumor must be screened for MAGE-A3 expression to evaluate patient eligibility for enrolment in clinical trials.
Materials and Methods: A MAGE-A3 specific quantitative RT-PCR assay has been developed to measure mRNA expression of this protein in macro-dissected, formalin-fixed paraffin embedded (FFPE) tumor samples. Since paraffin embedded tissues are the most common source of human tumor samples, a method using this type of material rather than fresh or frozen tissues, has been developed by Roche Molecular Systems. The use of FFPE tumor biopsies reduces the potential for handling challenges related to testing fresh or frozen tissues and enables easy use in the frame of clinical trials. Design, cut-off and performance criteria have been established and verified for the use of this assay in a Phase III trial.
Results: The assay is being applied in MAGRIT, a large randomized, double-blind, placebo controlled Phase III trial evaluating MAGE-A3 ASCI in NSCLC. To date, over 3000 samples have been tested with the assay. The fraction of MAGE-A3-positive patients identified on FFPE material in MAGRIT is comparable to that identified on fresh frozen biopsies in a Phase II trial (Vansteenkiste et al., ASCO 2007). The large number of samples collected in MAGRIT allows a detailed analysis of MAGE-A3 expression in this patient population.
Conclusion: A critical aspect of the development of this immunotherapy approach is the co-development of the ASCI and the dedicated screening assay. This work reports on the feasibility of using quantitative RT-PCR on FFPE material in large Phase III immunotherapy trials.

PP56

Prostate-specific antigen doubling time in metastatic castration-resistant prostate cancer: a clinically useful prognostic factor

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Background: Metastatic castration-resistant prostate cancer (mCRPC) is a heterogenous disease. In the present study PSA-velocity (PSAV) and prostate-specific antigen doubling time (PSA-DT) in mCRPC progressing after first-line chemotherapy was evaluated at time-1 (emergence of castration-resistance), time-2 (before first-line chemotherapy) and time-3 (after further PSA-progression).
Materials and Methods: Data from 25 mCRPC patients treated with chemotherapy between June 2003 and Jun 2009 were retrospectively analyzed. PSAV and PSA-DT were measured by considering at least three determinations of PSA. Survival rates according to PSAV and PSA-DT were reported and the behaviour of PSA-DT in PSA-responders was evaluated.
Results: The majority of patients received a docetaxel-based regimen (20/25 cases). PSA response rate was 31.8% (7/22). Median PSAV is increasing from time-1 (0.07 ng/ml/day) to time-2 (0.48 ng/ml/day) and time-3 (1.29 ng/ml/day), median PSA-DT is stable (71.6 days, 78.3 days and 71.42 days).
Median overall survival (OS) was 18.1 months and median survival from emergence of mCRPC was 28.8 months. Median OS was 28.8 mos in PSA responsive patients vs 19.4 mos in PSA-stable vs 13.6 mos in PSA non-responders. At time-1, median OS was 21.1 mos vs 11.8 mos ($p = 0.08$) in patients with PSA-DT >60 days and <60 days. At time-2, median OS was comparable (19.2 mos vs 18.1 mos) in patients with PSAV <1 ng/mL/day, and PSAV >1 ng/mL/day, respectively. Median OS was 23.4 mos vs 5.6 mos ($p = 0.01$) in patients with PSA-DT >50 days and with PSA-DT <50 days, respectively. At time-3, PSAV was comparable in responders and non responders (1.23 vs 1.29 ng/mL/day). However, PSA-DT was 65 days in responders compared to 88 days in non responders ($p = 0.20$). Median OS was 19.4 mos vs 28.8 mos in patients with PSA-DT >60 days or <60 ($p = 0.14$).
Conclusion: PSAV, despite the progressive increase in this parameter during the course of disease, is not a reliable predictor of individual prognosis at any time. It appears that a significant reduction in PSA-DT prior to first-line chemotherapy (time-2) is the best predictor of OS.

PP25

Correlation between clinical prognostic factors and MR spectroscopy-based biomarkers determined at 3.0 T without endorectal coils for patients with localised prostate cancer

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Background: The value of biomarkers such as Choline, determined with Magnetic Resonance Spectroscopy (MRS) remains questionable. We aimed to investigate if data from MRS could be related to prognostic parameters for patients (pts) with localised prostate cancer (PCa).
Materials and Methods: Seventy-two pts (mean age: 67.8±6.2 years) with biopsy-proven prostate cancer were referred for MRS. Mean PSA value was 12.9 ng/ml (range: 2.8–68 ng/ml). Pts and tumor characteristics were (number of pts between parentheses): TNM: T ⩽ 2a (46), T2b−c (15), T ⩾ 3 (11), Gleason score ⩽6 (50), 7 (20), ⩾8 (2), PSA ⩽10 ng/ml (44), 11–19 ng/ml (15), ⩾20 ng/ml (13).
The following prognostic classes were proposed according to the classification of D'Amico: Low risk (26): T ⩽ 2a, Gleason ⩽6, PSA ⩽10 ng/ml; intermediary risk (27): T2b−c, Gleason 7, PSA 11–19 ng/ml; high risk (19): T ⩾ 3, Gleason ⩾8, PSA ⩾20 ng/ml.
MRS was performed at 3 T using a phased-array coil. The following metabolites ratios were registered: Choline/Citrate (C/C), Choline+Creatine/Citrate (CC/C) and Choline+Creatine+Spermine/C (CCS/C). Mean ratio of the most pathological voxels and number of pathological voxels (i.e. voxels with a C/C ratio >0.5) were also determined. Wilcoxon and Kruskall-Wallis tests were used to compare mean values.
Results: Mean values of C/C, CC/C and CCS/C of the most pathological voxels were significantly higher for tumors ⩾T2b−c vs. ⩽T2a: 7.5 (±13.6) vs. 2.3 (±5.6), $p = 0.018$; 8.9 (±14.5) vs. 2.5 (±5.7), $p = 0.016$ and