EGFR and PIK3CA alterations do not seem predict the efficacy of chemoradiation in SCAC patients, suggesting that other molecular markers should be investigated.
The presence of both EGFR gene deregulation and high frequency of PI3KCA mutation suggests that patients with SCAC could more benefit from tailored therapies against these two targets.

1090 POSTER

Plasma biomarkers for early prediction of chemotherapy response and toxicity in colorectal cancer

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Background: Accurate predictors of chemotherapy response and toxicity are required to improve the safety, efficacy and costs of cancer treatment in colorectal cancer (CRC). Our aims were to investigate the utility of plasma proteomic profiling using multiple reaction monitoring mass spectrometry (MRM-MS) for predicting early haematological toxicity and response for patients receiving chemotherapy.
Materials and Methods: Patients with locally advanced and metastatic CRC receiving chemotherapy were enrolled. Plasma collection was performed at day 1, day 3 and day 15 of treatment. Toxicity assessments (NCI Criteria version 3.0) were prospectively collected for all patients and treatment response (RECIST Criteria) for patients with metastatic disease. MRM-MS assays were designed for 39 peptides representing 31 liver derived plasma proteins with roles in inflammation and/or cancer. Two-sample t-test was used to assess statistically significant fold change differences ($p < 0.05$) between sample days for: (1) absence or presence of $\geqslant$Grade 2 neutropenia after two cycles and (2) responders (CR and PR) versus non-responders (SD or PD).
Results: Fifty one patients have been enrolled in the trial. Sixty one percent of patients were male with 45% having metastatic disease. Plasma proteomic profiling for 39 peptides was performed for 16 patients at Day 1, Day 3 and Day 15 selected due to their toxicity and response to treatment. The greatest change in protein levels was observed between Day 3 and 15 with approximately 9% of proteins showing a 1.5 fold or greater change. Some proteins such as serum amyloid A showed more than 200-fold change in level. Preliminary results indicate there are statistically significant differences in protein expression for patients with (1) neutropenia versus those without neutropenia and (2) clinical responders versus non-responders in those with metastatic disease.
Discussion: Our results are encouraging for the use of plasma biomarkers using this technique for early prediction of moderate to severe neutropenia and chemotherapeutic response in CRC. These data require validation in large prospective cohorts of colorectal cancer patients.

1091 POSTER

Associations between genetic KDR polymorphisms and survival in patients with metastatic cancer treated with antiangiogenic therapy

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Background: Vascular endothelial growth factor (VEGF) and its receptors KDR (VEGFr-2) have important roles in angiogenesis, predicting risk and prognosis in several solids tumor. VEGFr-2 located on chromosome 4 (4q11-q12) is organized into 30 exons separated by 29 introns. Recently the VEGF-2578 AA and VEGF-1154 AA genotypes were associated with a superior median overall survival when using bevacizumab in metastatic breast cancer. We investigated the association of VEGFr-2 polymorphisms to efficacy and toxicity in patients with antiangiogenic therapy.
Methods: We performed genotype for selected VEGFr-2 polymorphisms in promoter regions 5'UTR, 3'UTR; in exons 7, 8, 9, 11, 16, 17, 18, 21, 27, 30 and introns 9, 17, 20. DNA was extracted from venous blood of 44 patients with non-curable solid tumors who have received treatment with bevacizumab (B) N = 20 (45%) or raf kinase inhibitors 55%; vatalanib (PTK-787) N = 3, sunitinib (SU011248) N = 6, sorafenib (BAY 43–9006) N = 13, ZD6474 N = 1 and AMG706 N = 1. Kaplan-Meier survival analysis was used to assess the association between VEGFr-2 staining and either progression-free survival (PFS) or overall survival (OS).
Results: 44 patients have received a median of 6 (1−19) cycles of treatment, 72% was used simultaneously with QT. According to the criteria of NCI-CTC the severe toxicity G3−4 occurred in 47%, 9% with a definite suspension of the drug. The toxicity was not associated with VEGFr-2 genotypes. Efficacy; 5/44 patients (11%) had complete response and 11/44 (22%) partial responses by RECIST criteria. With a median follow up of 12 months, the ILP was 8.5 months dt (5.8). The analysis of VEGFr-2 polymorphisms identifies the variant AA of the intron-20 rs2219471 with a significant difference in PFS and OS regarding their ancestral variant AG.
Conclusions: Our data suggest that VEGF-R polymorphism can be a predictor of clinical outcomes in antiangiogénic therapy. However, these findings require further prospective investigation.

1092 POSTER

Detection of cytokeratin-19 mRNA-positive cells in peripheral blood and bone marrow of patients with operable breast cancer

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Purpose: To compare the detection rates and evaluate the clinical relevance of cytokeratin-19 (CK-19) mRNA-positive cells in bone marrow (disseminated tumor cells;DTCs) and peripheral blood (circulating tumor cells;CTCs) of patients with early breast cancer.
Patients and Methods: Paired samples of peripheral blood and bone marrow were concomitantly obtained from 165 patients with stage I/II breast cancer before the initiation of adjuvant chemotherapy. In 84 patients, paired blood and bone marrow samples were available post-chemotherapy. The detection of CK-19 mRNA-positive CTCs and DTCs was assessed by real-time PCR.
Results: CK-19 mRNA-positive CTCs and DTCs could be detected in 55.2% and 57.6% of patients pre- chemotherapy, respectively. Post-chemotherapy, CTCs and DTCs were identified in 44 (52.4%) and 43 (51.2%) of the 84 patients, respectively. There was a 93.9% ($p = 0.344$) and 72.6% ($p = 0.999$) concordance between blood and bone marrow samples pre- and post-chemotherapy, respectively, when classifying the results as either positive or negative. The detection of CK-19 mRNA-positive CTCs and DTCs before chemotherapy was associated with decreased overall survival ($p = 0.024$ and $p = 0.015$, respectively), whereas, their simultaneous detection was associated with an increased incidence of disease-related death and decreased overall survival ($p = 0.016$). The detection of either CTCs and/or DTCs was an independent factor ($p = 0.040$) associated with decreased survival.
Conclusions: The above results indicate a strong correlation between the presence of CTCs and DTCs evaluated by RT-PCR for CK-19 mRNA in patients with early breast cancer. The detection of CTCs using this assay is able to deliver clinically relevant information that is not inferior to the detection of DTCs in the bone marrow.

1093 POSTER

Low or undetectable levels of MPL (thrombopoietin receptor gene) mRNA expression on tumour cell lines and primary tumours compared with EPOR, ERBB2, and IGF1R

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Background: A better understanding of the effects of recently discovered thrombopoietin receptor (TpoR) agonists on tumors is needed. By binding to different parts of TpoR, TpoR agonists signal differently. Eltrombopag, a non-peptide TpoR agonist, has been shown to decrease proliferation of leukemia and lymphoma cells in vitro. Although TpoR expression on megakaryocytic cells is well documented, little quantitative data exist for expression on tumors.
Materials and Methods: Quantitative RT-PCR (qRT-PCR) was performed on 378 tumor cell lines available from ATCC and the German Collection of Microorganisms and Cell Cultures (DSMZ). Microarray data were examined from 118 breast cancer, 29 non-small cell lung cancer (NSCLC), and 151 renal cell carcinoma (RCC) samples. Robust multiarray average (RMA) analysis was used to determine relative mRNA expression levels. In addition, qRT-PCR analysis was performed for *MPL* (TpoR gene) expression on ~160 tumor samples each, from subjects with prostate, ovarian, lung, and breast cancers. Protein levels were determined by western blot analyses on several tumor cell lines.
Results: *MPL* was consistently expressed at low or undetectable levels in the tumor cell lines with the exception of 3 cell lines with >9500 normalized abundance: HEL 92.1.7, KG-1 (2 erythroleukemia cell lines), and NCI-H510 (lung cancer cell line). Western blot analyses showed that the high levels

of *MPL* mRNA in NCI-H510 cells did not correlate with detectable TpoR protein expression. Erythropoietin receptor (*EPOR*) mRNA was expressed at low-to-moderate levels, while *ERBB2* and *IGF1R* were expressed at higher levels. Microarray analysis showed undetectable *MPL* mRNA levels in all breast cancer and RCC samples and low levels in 48% NSCLC samples. In contrast, *EPOR* was expressed in 75–100% of the breast cancer, NSCLC, and RCC samples. *ERBB2* was expressed in 81–100% of the samples and *IGF1R* was expressed in 54–100% of the samples. For breast tumors, the levels of mRNA expression were as follows: *MPL* < *EPOR* < *IGF1R* < *ERBB2* < *epor* < *igf1r* < *erbb2* </>. By qRT-PCR, there were also low or undetectable levels of *MPL* expression in these samples from subjects with prostate, ovarian, lung, and breast tumors. *EPOR*, *ERBB2*, and *IGF1R* expression vary according to tumor type, but were generally higher than *MPL*.
Conclusions: In summary, low or undetectable levels of *MPL* mRNA expression were observed in most tumor cell lines and in most samples of patient tumors, compared with *EPOR*, *ERBB2*, and *IGF1R*.

1094 POSTER

Influence of TGFB1+869T>c polymorphism in non-small cell lung cancer (NSCLC) risk

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Background: Lung cancer (LC) is the third most common type of cancer in Europe and was the first cause of death by cancer in 2006. Non-small cell lung cancer (NSCLC) accounts for 75%–85% of all histological types of lung cancer. Lung carcinogenesis is complex and a multi-step process, resulting from exposure to environmental and genetic factors. The transforming growth factor beta 1 (TGFβ1) is a multifunctional regulatory polypeptide that controls many aspects of cellular function (cellular proliferation, differentiation, migration, apoptosis, immune surveillance). Nevertheless, TGFβ1 has been suggested to play a dual role, acting as a suppressor in early stages and as a tumor promoter in later stages. *TGFB1+869T>C*, is a functional polymorphism described in *TGFB1* gene responsible for a T-to-C substitution at nucleotide 29 of codon 10. This transition has been associated with higher circulating levels of TGFβ1, that may influence LC development and prognosis. Our purpose was to investigate the role of *TGFB1+869T>C* functional polymorphism in NSCLC risk.
Material and Methods: DNA was extracted from peripheral blood cells of 1099 individuals: 305 patients histopathologically diagnosed with NSCLC and 794 healthy individuals without evidence of neoplastic disease. Genotyping of *TGFB1+869T>C* polymorphism was performed by Real-Time PCR allelic discrimination method. The odds ratio (OR) and its 95% confidence interval (CI) were calculated as a measure of the association between *TGFB1+869T>C* genotypes and NSCLC risk.
Results: The frequency of the TT genotype was lower in LC patients than in controls (29% and 37%, respectively). Conversely, we found an overrepresentation of C carriers in NSCLC group in comparison with normal controls (71% and 63%, respectively). Carriers of the C allele had an increased risk for NSCLC development (OR = 1.44, 95%CI = 1.07−1.94, $P = 0.012$). Stratification according to histological type, showed a statistical significant increased risk for epidermoid NSCLC development in the individuals with C carrier genotypes when compared to individuals with TT genotype (OR = 1.68, 95%CI = 1.05−2.72, $P = 0.023$).
Conclusions: Individual differences in cellular microenvironment may influence the susceptibility to cancer development and behaviour. Our results suggest that *TGFB1+869T>C* functional genetic polymorphism influence NSCLC susceptibility. This genetic profiling may help define higher risk groups for an individualized therapy.

1095 POSTER

Pharmacogenomic analysis of the triplet combination of gemcitabine, oxaliplatin and cetuximab as salvage therapy for metastatic colorectal cancer (mCRC) patients

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Background: We have previouslyreported that the combination of biweekly gemcitabine-based therapy wasactive in pretreated mCRC pts (De la Cruz et al, ASCO GI 2008, abstr377). We aimed to investigate whether germ line polymorphisms may bepredictors of clinical outcome in mCRC pts treated with thiscombination.
Material and Methods: We evaluated SNPs ofgenes involved in gemcitabine metabolism (CDA, dCDK, RRM1, DCTD, SLC28A1), DNA repair (XRCC1, XRCC 3, ERCC1, XPD) and two IgG Fragment CReceptor polymorphisms (FcgRIIa- H131R and FcgRIIIa-V158F) reported tobe predictive of cetuximab-based therapy, even in K-ras mutatedpts. Whole blood was collected and DNA extracted from peripherallymphocytes using a DNA isolation Kit (Qiagen, CA). Polymorphisms weredetected using the TaqMan genotyping assays (Applied Biosystems, CA). Clinical response was evaluated according to RECIST criteria. Univariate analysis (Fisher's exact test for response; log-rank testfor TTP and OS) was performed to examine associations betweenpolymorphisms and clinical outcome.
Results: Blood samplesof 35 out of 39 enrolled pts were tested for genomic analysis. Patient's characteristics are as follows; M/F: 26/13, median age: 59 years, median number of prior chemotherapy lines: 2 (1−4), Köhne riskgroups; low: 8 pts, intermediate: 18 pts, high: 13 pts. After a medianfollow-up of 20 months, median progression-free survival (PFS) is 6.7months (95% CI; 5.2−8.3) and median overall survival 15.4 m (95% CI;14.7−16.1). Overall response rate (ORR) was 53.8%. RRM1 R284R ($p = 0.06$),T741T ($p = 0.02$) and RRM1–524CT ($p = 0.04$) were linked to clinicalresponsiveness. All pts possessing 2 or 3 favourable RRM1 SNPsresponded. ORR was 53.3% for pts with no favourable SNPs versus 85% forthose pts with any favourable SNP ($p = 0.04$). ORR was also significantlyhigher in pts with any histidine allele in the FcgRIIa polymorphism (93% vs. 60%, $p = 0.034$).
Median PFS was adversely affected in ptsharbouring no favourable RRM1 SNPs (4.2m versus 6.7 months, $p = 0.019$) and in those pts with homozygous FcgRIIa-131R allele (4.4 vs. 7.5months, $p = 0.007$).
Conclusions: Polymorphic variants of RRM1 and FcgRIIa may play a key role in the efficacy of gemcitabine-based therapy for mCRC pts.

1096 POSTER

Molecular signatures of disseminated tumour cells in metastatic breast cancer patients

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Background: The critical step in breast cancer progression is the spreading of tumor cells to distant organs. Numerous studies have demonstrated that the presence of Disseminated Tumor Cells (DTC) in the bone marrow from patients with breast cancer is an independent prognostic factor for systemic relapse and breast cancer related death. Immunocytochemical methods for detection of DTC make it possible to identify single DTC in a population of normal cells. However, we still have limited knowledge about the biological and molecular characteristics of the DTC themselves. Complete genomic profiles and expression patterns have to be considered in order to understand tumor aggressiveness, clinical outcome and/or the disseminated tumor cell status.
Material and Methods: Mononuclear cells from the bone marrow of metastatic breast cancer patients are transferred to slides, DTC are identified by immunocytochemical staining and isolated by micromanipulation. Samples are amplified by single cell whole genome amplification and the resulting amplified DNA is applied to high density whole genome Agilent CGH arrays.
Results: We tested and established the Single Cell array Comparative Genomic Hybridization (SCaCGH) technique in our laboratories in order to investigate the molecular signatures of DTC in metastatic breast cancer patients. DTC from the bone marrow of metastatic breast cancer with variable numbers of DTC per mononuclear cells were selected and analyzed. In a first pilot of about 10 patients we compare the genomic profiles of 1−3 DTC per patient in relation to each other and among different patients. Our preliminary results show concordance of the genetic profiles from different DTC within a patient and different copy number variations among the DTC from different patients.
Conclusions: Due to the implementation of a technique called the Single Cell array Comparative Genomic Hybridization (SCaCGH) we are finally able to characterize single cells using high density microarrays. This will provide us with information about the properties of DTC important for the understanding of the metastatic cascade, apart from the potential to better understand tumor heterogeneity in general.