1091 POSTER

Association Between Serum Adipokines Levels and Quality of Life in Advanced Non-small Cell Lung Cancer

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Background: Serum adipokines are produced by adipose tissue and have an important role in cancer cachexia. They have been studied in several cancer patients with cachexia, but have not been studied in patients with non-small cell lung cancer (NSCLC) for prognosis and quality of life. Therefore, the aims of the present study were to evaluate the association between serum adipokines (adiponectin, resistin, leptin and ghrelin) levels and prognostic factors and also quality of life in patients with NSCLC.
Methods: Sixty seven patients (42 pts with weight loss and 25 without weight loss) and 20 healthy subjects were included in this study. The clinicopathological features were recorded. Antropometrical, laboratory data and serum adipokines levels were measured. The evaluations of quality of life in both patients and healthy subjects were assesed by EORTC QLQ-C30.
Results: Serum albumin ($p = 0.01$), adiponectin ($p = 0.78$) and leptin ($p = 0.03$) levels were lower in the patients, whereas CRP ($p = 0.00$), LDH ($p = 0.01$), resistin ($p = 0.00$) and ghrelin ($p = 0.34$) levels were higher than the healthy subjects. In patients with weight loss, serum albumin ($p = 0.02$) and leptin ($p = 0.04$) levels were significantly lower and serum ghrelin levels were higher than patients without weight loss. We also found a significantly increased serum ghrelin levels in non-squamous histology ($p = 0.02$). On multivariate survival analysis, high LDH ($p = 0.01$), high ferritin ($p = 0.02$) and ghrelin levels ($p = 0.00$) had significant independent effects on disease progression. In EORTC QLQ-C30 assessments, there were significant correllations between the scores of low physical functioning, low role functioning, low cognitive functioning, high fatigue, nausea/vomiting, dyspnoea and pain and decreased albumin, increased LDH and ferritin levels in the patients. In addition, while low adiponectin level was correlated with high fatique and pain scores, increased resistin level was correlated with low global quality of life score, and also increased ghrelin levels were significantly associated with low role functioning and low cognitive functioning scores.
Conclusions: Our study showed that low leptin and high resistin and ghrelin levels were found in NSCLC patients. Adipokines, particularly ghrelin, may have an effect on progression. Also, the importance of serum albumin, LDH, CRP remained in NSCLC. There were significant differences in quality of life in the patient groups. Confirmation of the roles of adipokines in NSCLC with further studies is needed.

Poster Presentations (Sat, 24 Sep, 09:30−12:00)
Translational Research

1092 POSTER

Trastuzumab Binds to HER2 Non-amplified Breast Cancer Cells and Induces ADCC

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Background: HER2 is over-expressed in approximately 25% of breast cancers. Trastuzumab is a monoclonal antibody used to treat HER2 positive (HER2 gene amplification/IHC 3+, >30% cells), but not HER2 "negative" (non-amplified/low expression) breast cancer. Abrogation of HER2 signaling through the PI3K/Akt and MAPK pathways and antibody-dependent cell-mediated cytotoxicity (ADCC) are two major mechanisms of actions of trastuzumab. A retrospective study on the NSABP B-31 adjuvant clinical trial suggested a possible clinical benefit for the addition of trastuzumab to chemotherapy in patients with HER2 "negative" disease. In this study we investigated the possibility that trastuzumab might induce ADCC against HER2 negative breast tumour cells.
Materials and Methods: HER2 protein levels were determined by ELISA in HER2 amplified (SKBR3, HCC1954) and non-amplified breast cancer cell lines (CAL-51, CAMA-1, MCF-7, T47D, EFM19, MDA-MB-468), and in tumour and autologous normal tissue samples from patients. Fluorescence microscopy was used to examine Q-dot-labelled trastuzumab bound to cell surface HER2. CD56+ natural killer cell-mediated ADCC was assessed in trastuzumab-treated, HER2 amplified and non-amplified breast cancer cell lines.
Results: HER2 protein levels were significantly lower in the HER2 non-amplified cell lines (6.2 ± 1.9 − 55.1 ± 23.4 pg/μg) than in the HER2 amplified cell lines (SKBR3 – 748 ± 296 pg/μg; HCC1954 − 511.3 ± 80.9 pg/μg), consistent with the levels of HER2 observed in the HER2 "negative" patient samples (3.8 ± 6.1 pg/μg – 61.5 ± 34.2 pg/μg). HER2 protein was undetectable in 12/15 normal autologous tissues. Using fluorescence microscopy we showed that trastuzumab can bind to HER2 on each of the breast cancer cell lines examined including the MDA-MB-468 cell line, which has the lowest levels of HER2. Trastuzumab induced a significant ADCC response in the HER2 positive HCC1954 and SKBR3 cell lines, and in five of the non-amplified, low HER2 expressing cell lines examined, but not in the MDA-MB-468 cell line.
Conclusions: Our results suggest that HER2 non-amplified breast cancer cells, with low but detectable levels of HER2 protein can bind trastuzumab and initiate ADCC and that there is a significant difference between HER2 expression in normal versus HER-2 "negative" tumour tissue. These results warrant further investigation of the ADCC response to trastuzumab, and other HER2 targeted mAb therapies, particularly in early stage HER2 negative breast cancer.

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Gene Signature of Bevacizumab Effects in Primary Breast Cancer

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Background: At today, no proven predictive biomarkers of anti-vascular endothelial growth factor (VEGF) therapies are available but the need of them is becoming critical. To determine the mechanisms of early anti-angiogenic tumour response might be the first step toward the identification of potential predictive biomarkers. Bevacizumab, a recombinant humanized monoclonal antibody targeting VEGF, was administered to previously untreated patients to evaluate its effects on gene expression.
Material and Methods: 73 patients (pts) with histological proven breast cancer were prospectively enrolled in IMAGING phase II clinical trial (ML22197) designed to determine molecular biomarkers for bevacizumab therapy. Bevacizumab (15 mg/kg) (C1) was administrated 3 weeks prior to the beginning of chemotherapy consisted of 4 cycles of docetaxel (60 mg/mq), adriamycin (50 mg/mq) and bevacizumab (15 mg/kg) every 21 days (C2-C5). Eco-guided tumour core biopsies were performed at the baseline (BL) and two weeks after bevacizumab treatment (C1). RNA was extracted from paired samples (BL and C1) and examined using an Affimetrix Exon 1.0 ST array. Analysis included differential gene selection methods as fold change and signature p-value filters of t-paired test correcting for multiple comparisons.
Results: At the time of the analysis, the accrual of this trial is completed but data of response are not yet available. Pts were age 29−70 (mean: 48) years, 58 (79%) and 15 (21%) clinical stage II and III, respectively. Differential gene expression analysis of 55 paired samples showed that 434 genes significantly changes (FDR < 0.01) after bevacizumab treatment. Using Gene Ontology (GO) pathway analysis, we identified 176 gene categories, the most notably involved in angiogenesis (including VEGF activity and Notch signaling), immune response (including lymphocyte mediated immunity and leukocyte activation) and cell death.
Conclusions: GO pathway analysis identified a number of gene categories influenced by bevacizumab administration. Our data confirmed the relevant role of bevacizumab in angiogenesis and showed new GO pathways involved in bevacizumab mechanism of action including the activation of immune response. This analysis might be an informative method for identifying new genes involved in response to bevacizumab and so to identify new potential predictive biomarkers for this treatment.

1094 POSTER

EpCAM+ Tumour Cells Are Frequently Detected in Malignant Ascites Samples- Results From a Randomized Phase IIIb CASIMas Study

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Background: The epithelial cell adhesion molecule (EpCAM) is a tumour-associated marker frequently expressed on various carcinoma tissues. The

trifunctional antibody (AB) catumaxomab (anti-EpCAM x anti-CD3) has been approved for the i.p. treatment of patients with malignant ascites due to EpCAM+ carcinomas. By eradication of EpCAM+ tumour cells in the peritoneal cavity, catumaxomab effectively controls malignant ascites (MA). The aim of the reported investigation was to assess the frequency of EpCAM expression on tumour cells in malignant ascites.
Methods: The clinical phase IIIb study CASIMAS (NCT00822809, Fresenius Biotech) investigated a 3 hour i.p. infusion of catumaxomab with and without prednisolone premedication in MA patients.
Before treatment, ascites samples of 193 patients were collected for detection of EpCAM+ tumour cells. Cells were harvested, spun onto slides and labeled with the EpCAM-specific AB Ber-EP4. Cell-bound Ber-EP4 was detected with a biotinylated horse anti-mouse IgG and visualized with an avidin/biotinylated horseradish peroxidase complex and 3-amino-9-ethylcarbazole as substrate. Mayer's hemalaun was used as counterstaining. EpCAM+ cells were evaluated by light microscopy. The cytological data were related to the histology of the primary tumour.
Results: The main primary tumours of 193 patients evaluated were gastric, colon, pancreatic, breast, ovarian, lung and endometrial carcinomas.
In the majority of evaluable patient samples (n = 183, 95%) EpCAM+ tumour cells were detected (n = 170, 93%). In 2 patient samples, tumour cells were EpCAM-negative (1.1%) and in 11 patient samples tumour cells were not detectable (6.0%). 5.2% of the test samples were not evaluable. With regards to the primary tumour, in 100% of evaluable ascites samples from gastric, colon and endometrial, 98% from ovarian, 86% from breast, 80% from pancreatic and 85% of other carcinomas EpCAM+ tumour cells were detected.
Conclusions: The presented method for detection of EpCAM+ tumour cells in MA can be performed with standard laboratory equipment. In the ascites samples of the vast majority of patients, over all primary tumours investigated EpCAM+ tumour cells were detected at a high frequency. Based on these data it is concluded that the vast majority of patients with MA are indicated for an EpCAM targeted treatment with catumaxomab.

1095 POSTER

Growth Factor Receptor-targeted Therapy for Cancer

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Deregulated growth factor signaling aggressively stimulates the expression of oncogenes and is central to tumour growth and development. By selectively targeting the inhibition of FGF receptor (FGFR) signaling, our study is aimed at developing a potent treatment for cancer. To assess the sensitivity of cancer cells to FGFR-targeted therapy, we first screened the protein expression of FGFR by Western Blot analysis in seven lines of human osteosarcoma cells (including one primary osteosarcoma) and four lines of breast carcinoma cells, and compared their expression profiles with that of normal human osteoblasts and mammary gland epithelial cells. A neutralizing antibody against FGFR was developed and its effect on cancer cell growth and proliferation determined. Antibody specificity was evaluated by ELISA assays and the ability to neutralize FGFR activity examined using a Receptor Tyrosine Kinase (RTK) array. Lastly, the mechanism by which FGFR controls cancer cell growth and survival was determined by Western Blot analysis and Taqman Realtime PCR. Our data shows that FGFRs are upregulated in cancer cells, which prompted us to develop a novel antibody that significantly impairs FGF signaling and renders tumour cells unable to proliferate. This inhibition of cell growth is due to enhanced activation of several tumour suppressors, e.g. p53, Rb and FOXO3 as well as their target genes p21 and Bim. Our study demonstrates that FGF plays a key role in supporting cancer cell growth and that inactivating FGF signalling is a promising therapy to treat cancer. Future studies seek to determine whether the antibody is suitable for the treatment of cancer by examining its effect on animal tumour models.

1096 POSTER

Expression of Treg Associated Markers Have Prognostic Implications in Early-stage NSCLC

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Background: Tumour infiltration of lymphocytes is considered to be a manifestation of host immune reactions against cancers. CD4+, CD25high, CD127low and FOXP3+ regulatory T cells (Tregs) are believed to regulate T-cell immunity and to be the main obstacle in immunotherapy. A body of evidence suggests that Tregs within the tumour microenvironments might play a significant role in the suppression of local antitumour immune responses. The aim of this study was to determine the expression of these genes by RT-PCR and to correlate them with clinico-pathological and prognostic variables in resectable NSCLC.
Materials and Methods: RNA was isolated from 150 frozen lung specimens (tumour and normal lung) from untreated NSCLC patients (stages I-IIIA). RT-PCR was performed to analyze the expression of: CD4, CD25, CD127 and FOXP3 genes. Relative expression was normalized by an endogenous gene (GUSB) using the Pfaffl formulae. Statistical analyses were considered significant at $p < 0.05$.
Results: Tumour samples had significantly higher expression of CD25 (x2.1) and lower expression of CD127 (x0.42) than normal lung tissues, reflecting a Treg phenotype infiltrating the tumour. On univariate analysis, age (>65), PS and tumour size were associated with OS rates. Higher intratumoral levels of FOXP3 were associated with adverse prognosis ($p = 0.017$ for TTP and $p = 0.036$ for OS). On the other hand, the group of patients with higher CD4 tumour expression has increased TTP and OS. The combination of both variables (ratio FOXP3/CD4) allows the discrimination of a group of patient with poor prognosis, characterized by higher FOXP3/CD4 ratio ($p = 0.015$ for TTP and $p = 0.004$ for OS).
Conclusions: Our results show that the expression of Tregs markers seems to be associated with an increased risk of relapse and shorter OS. We also observed that the ratio FOXP3/CD4 can be used as prognostic marker in early-stage NSCLC patients (Supported by ISCIII PS09–01149 grant).

1097 POSTER

Correlation of Serum Biomarkers and Clinical Outcomes to Identify Predictors of Response to the Multi-targeted Kinase Inhibitor E7080 in Patients With Advanced Melanoma

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Background: E7080 is an oral TKI targeting VEGFR1−3, FGFR1−4, PDGFRβ, RET and KIT, which affects tumour cell proliferation and tumour vascularization in laboratory models. Tumour response (RECIST) and prolonged disease stabilization (>6 months) were observed in melanoma patients (pts) treated in phase I. The present study sought to identify biomarkers predictive of response to E7080 in melanoma.
Materials and Methods: 26 pts with metastatic melanoma received E7080 10 mg twice daily continuously on a phase I study. The pts responses were: 2 partial responses (PR), 14 stable disease (SD) >3 months, 1 SD >2 months, 5 progressive disease (PD) and 4 pts non evaluable. Serum was collected in these pts at baseline, 2hr after first dosing, C1D8, C1D15 and C1D22 for testing of five angiogenesis-related markers (PDGF-BB, soluble Tie-2, angiopoietin1, soluble e-selectin, soluble c-kit) and two apoptosis-related markers (cytochrome C and M30) by ELISA. The predictive value of lactate dehydrogenase (LDH) level, diastolic and systolic blood pressure (DIABP and SYSBP), hematocrit and hemoglobin at baseline and in the first cycle of treatment were also analyzed.
Results: The data were analyzed using Student's T-test based on clinical benefit (PR or SD $\geqslant$6 months); regression analysis for the continuous measure of tumour shrinkage and by Cox proportional regression based on progression free survival (PFS). The analysis identified baseline DIABP and SYSBP, PDGF-BB and angiopoietin1 level change at 2 hr after first dosing, change from baseline of soluble c-kit, M30, hematocrit and systolic BP at cycle 1 day 8 are associated with PFS by univariate analysis. By multivariate Cox Regression analysis, the ratio of PDGF-BB at 2 hrs to baseline (>1.15) and the baseline SYSBP (>132 mm/Hg) were significant predictors of PFS. The Kaplan–Meier curves illustrated that two parameter biomarkers (PDGF-BB 2hr ratio and baseline SYSBP) were better at stratifying low- and high-risk pts in terms of PFS than baseline LDH level for melanoma pts treated with E7080.
Conclusion: PDGF-BB 2hr ratio and baseline SYSBP appear to identify pts with advanced melanoma who may benefit from E7080 treatment. These predictive parameters will be integrated with on-going proteomic analysis of melanoma cells, gene expression data from tumour and vasculature cells and genotyping from these same tumours to model the response to E7080. The markers will be validated for their predictive value in an ongoing Ph 2 trial.