RANKL/OPG ratio was upregulated in patients with breast and lung cancer and tended to decline after treatment with zoledronic acid whereas patients with prostate cancer presented with elevated OPG levels that persisted after treatment. CTX levels were significantly reduced in the whole study population at the second compared to the initial measurement ($p = 0.003$). Decrease in TRACP-5b levels tended to correlate with reduced incidence of SRE (HR = 0.39, 95%CI: 0.14−1.10, $p = 0.076$) and the model fit was improved when Performance Status (PS) at diagnosis was added in logistic regression analysis ($p = 0.051$). Tumor type (lung or breast vs prostate) and PS (PS >2 vs 0 or 1) were the only significant predictors for recurrence and death and none of the bone markers was able to improve predictive value when added to the model.
**Conclusions:** The RANKL/OPG axis is upregulated in patients with breast and lung cancer metastatic to the skeleton and tends to normalize after treatment with zoledronic acid, as reflected by decrease in serum bone resorption markers. Marker level responses are not predictive for disease progression or survival.

**1087** POSTER

**Expression of angiogenic genes: prognostic marker in patients with early-stage non-small cell lung cancer (NSCLC)**

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**Background:** NSCLC is a major cause of cancer-related death worldwide. The prognosis for lung cancer patients is poor with 5-years survival rates being less than 15%. It is known that angiogenesis is an essential event for solid tumour growth. Vascular endothelial growth factor (VEGF) family of ligand and receptors (VEGFR) are described as powerful angiogenic factors. VEGF ligands bound to their receptors at the membrane levels, gathering a cascade of intracellular events. Our objective was to evaluate the expression and prognostic significance of VEGFA and VEGFR1 determined by real-time PCR (RTqPCR) in resectable NSCLC patients.
**Methods:** We performed RT-qPCR analysis to assess the expression of VEGFA and VEGFR1 (FTL1) in 151 frozen lung cancer specimens from untreated NSCLC patients who had undergone surgical resection. For this purpose, RNA was extracted using Trizol® and RTqPCR was performed using TaqMan® probes. Relative quantification was calculated by Pfaffl formulae, using GUS-B (endogenous control gene) for normalization. We correlate the expression of both angiogenic genes between them and with survival variables. All statistical analysis were done using the SPSS 13.0 software.
**Results:** Our results show a strong positive correlation between the expression of VEGFA and VEGFR1 in tumour samples ($p < 0.000$, Spearman's test). When patients were grouped according to tumor size, there was a trend in the way that bigger tissues express relative higher amounts of VEGFA and VEGFR1 ($p = 0.000$). We used the median as a cut-off value for both variables, therefore, cases were scored as high (H) or low (L) according to this criteria. There were 71.5% (108/151) of concordant results (both variables H or L). Kaplan Meier plots show that the group of patients expressing high levels of VEGFA and VEGFR1 (HH) has a worse prognosis than the other groups (HL or LL). The median OS for the HH group was 24.27 months, compared with the 38.03 months for the HL + LL group.
**Conclusion:** Our results reveal that, in NSCLC tumour samples, there is a correlation between the expression of VEGFA and VEGFR1 mRNA. Bigger tissues express relative higher amounts of VEGFA and VEGFR1. In addition, determination of these two genes by RTqPCR would be a useful clinical test to assess prognosis in NSCLC, due to the fact that higher levels of expression of both genes correlates with shorter OS.

**1088** POSTER

**Serum levels of vascular endothelial growth factor receptor 2 (VEGFR2): prognostic biomarker in advanced non-small cell lung cancer (NSCLC)?**

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**Background:** An increase in VEGF expression in tumour or some blood compartments (i.e. serum or plasma) has been found in solid tumours of various origins. Several studies have suggested that ligands and receptors of the VEGFs/VEGFR system play an important role in tumour growth and is associated with metastasis and poor prognosis. The aim of our study was to investigate the usefulness of serum VEGFR2 quantification as a new biomarker in advanced NSCLC.
**Material and Methods:** We studied 106 healthy controls (c) and 462 advanced NSCLC patients (p) (stage IIIB and IV) treated with cisplatin and docetaxel. Blood samples were collected before chemotherapy and the serum levels of the VEGFR2 were determined by ELISA.
**Results:** In the NSCLC group, the median age was 59.9, range (31−80); 82% were males. The histological subtypes were: 31.4% squamous, 49.8% adenocarcinoma, 15.3% large cell and undifferentiated and 3.5% other. There was a significant difference in the serum levels of VEGFR2 between c and p (mean±SEM): 6318±152 ng/ml and 8373±120 ng/ml, respectively ($p < 0.0001$). On the other hand, we found no statistical differences according to sex, histology, or stage. The area under the ROC curve was 0.744 indicating that VEGFR2 is an adequate biomarker for the discrimination between c and p. Dividing the cohort in two subgroups according to VEGFR2 levels: high (>9473.9 ng/ml) and low ($\leqslant$9473.9 ng/ml), we found significant difference in terms of Time to Progression (TTP). Patients with higher levels of VEGFR2 had a median TTP of 204 days whereas in the group with lower expression the median was 164 days, ($p = 0.039$).
**Conclusions:** In advanced NSCLC, we found higher levels of soluble VEGFR2 in p than in c. There was a correlation between higher expressions of soluble VEGFR2 with better prognosis, in terms of TTP, therefore a more thorough understanding in the role of the serum quantification of this angiogenic receptor in advanced NSCLC p seems to be an important task.

**1089** POSTER

**Does EGFR gene deregulation and PI3KCA mutations predict response to chemoradiation in squamous cell anal cancer (SCAC)?**

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**Background:** Chemoradiation is the standard treatment for locally advanced SCAC, and complete response can been achieved in 75–85% of cases. A meaningful question is whether molecular markers might predict the response to chemoradiation. Preclinical and clinical studies in several cancers have demonstrated that EGFR and PI3K alterations may impair the efficacy of radiotherapy or, limited to PI3K, of fluoropyrimidines. We analyzed the frequency of EGFR gene deregulation and PIK3CA mutations in patients with locally advanced SCAC who underwent concurrent chemoradiation, and we matched the results to clinical outcome.
**Methods:** Patients who underwent split course of mitomycin and 5-fluorouracil continuous infusion with concurrent radiation (total dose 59.4 Gy in two steps with a gap of two weeks), were considered for analysis. The EGFR gene status was assessed by Fluorescent In Situ Hybridization, PI3KCA mutations by direct sequencing. Objective tumor response was evaluated by radiological and endoscopic methods; if indicated, a confirmatory biopsy was performed.
**Results:** Data of 20 patients were recorded. Seventeen patients (85%) achieved a complete remission after chemoradiation. The EGFR gene copy number gain was detected in 2/19 (10%) evaluable cases, but did not correlate with response. A PIK3CA point mutation was detected in 7/20 (35%) patients: 6 patients were responders, while 1 patient did not achieve a response.
**Conclusions:** PI3K pathway could play a key role in the development of SCAC.

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