transcribed blood miRNA samples by RQ-PCR. Advanced *QBase Plus* software and SPSS were used for biostatistical analysis of the data.
Results: Whole blood samples were preferable to serum and plasma for greater detection and relative quantification of circulating miRNAs. Of 7 breast cancer specific miRNAs investigated, across 130 whole blood samples (106 preoperative samples from breast cancer patients and 24 from healthy age matched female controls), the levels of two miRNAs were found to be significantly higher in blood from breast cancer patients compared to controls ($p < 0.001$). Increased systemic miRNA levels in breast cancer patients are also reflected in breast tumours. We also report significant associations between circulating miRNAs and the clinicopathologic variables ER and HER2/*neu* receptor status, and nodal status.
Conclusion: This study is the first to demonstrate that miRNAs are detectable and in the circulation of breast cancer patients, compared to controls. The circulating miRNA expression profile correlates with the tumour miRNA expression profile and with various biopathologic parameters of breast cancer. In this, lies the expectation that circulating miRNAs will be clinically useful as a novel minimally invasive tool to aid in the early diagnosis and monitoring of breast cancer.

1307 POSTER

CXCL12/SDF1 expression by breast cancers is an independent prognostic marker of progression

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Purpose: The cytokine CXCL12 is synthesized by metastasis target tissues and has been shown to attract tumor cells that express the receptor, CXCR4. However, epigenetic silencing of CXCL12 has recently been reported to increase the metastatic potential of breast cancer cells and the reintroduction of the cytokine gene into MDA-MB-231 breast carcinoma cells decreases the number of metastases formed *in vivo*. We therefore wished to know whether CXCL12 expression correlates with relapse free and overall survival in human breast cancer patients.
Experimental Design: CXCL12 and CXCR4 expression was analyzed in one hundred archival breast cancer samples by immunohistochemistry and in two breast cancer microarray datasets of 408 cases. Data were analyzed by uni- and multivariate COX regression analysis.
Results: CXCL12 and CXCR4 are expressed by epithelial tumor cells and by stromal and endothelial cells. Microarray gene expression analysis and immunohistochemistry revealed that CXCL12 but not CXCR4 expression significantly correlates with disease free and overall survival in estrogen receptor positive and negative cancers. Expression of the estrogen receptor α and CXCL12 does not correlate.
Conclusions: CXCL12 is a strong, independent prognostic marker. We propose that saturation of the receptor through autocrine CXCL12 production reduces chemotaxis towards CXCL12 releasing metastasis target tissues.

1308 POSTER

Evaluating the prognostic role of serum extracellular domain (ECD) of HER-2/neu (s-HER2) in patients (PTS) with metastatic (M) breast cancer (BC): results of an observational study

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Background: BC is the leading cause of cancer-related death affecting women worldwide. Since the last decades, many efforts have been made to identify different subsets of BCs on the basis of molecular markers that might help in defining the prognosis. HER2/neu belongs to a family of 4 transmembrane tyrosine kinase (TK) cell membrane receptors mediating cell growth, differentiation and survival, its overexpression occurring in about 20%-30% of BCs and being associated with a more aggressive tumour behaviour and a poor prognosis. The ECD can be cleaved from the cellular surface and measured in the peripheral blood. A potential prognostic and predictive role of elevated s-HER-2 levels has been previously reported.
Material and Methods: we prospectively measured the s-HER2 levels of consecutive MBC pts [ELISA immunometric test (cut-off 13 ng/ml)], in order to investigate the possible correlation with the clinical situation. The assessment was done at the first visit (bs-HER2) and then every 3 months. All pts gave their written informed consent.
Results: 46 consecutive MBC pts have been enrolled (median age 63 ys (35−85) so far; ER+ 30 pts (65%); PgR+ 21 pts (46%); IHC evaluation of HER2 was available in 37 pts [16 pts (35%) positive (IHC3+ or IHC2+ FISH+), 21 pts (46%) negative, 9 pts (19%) unknown]. All pts were treated with standard hormone (8 pts) and/or chemotherapy (28 pts), including antracyclines and taxanes. Trastuzumab was given as needed (13 pts).
A total of 325 samples have been tested (mean = 7/pt). Elevated bs-HER2 were found in 27 (59%) pts, of which 12 HER2 positive and 12 HER2 negative; median ECD values were significantly higher in pts with HER2 positive ($P < 0.0001$).
A significant correlation was found between elevated bs-HER2 and number of M sites ($p < 0.05$), as well as between high ECD levels and visceral/nodal disease ($p < 0.05$). Noteworthy, a significant correlation has been found between the increase or the decrease of ECD values and PD or non-PD respectively ($p = 0.0001$). Moreover, with regard to OS, a statistically significant difference, regardless of HER2 status, has been observed between pts with elevated and lower ECD levels (log-rank test $p = 0.003$).
Conclusions: Our data seem to suggest that serum HER2 levels might be used as a "real-time" non invasive marker, allowing repeated evaluations of HER2 status during the whole disease history, with possible future therapeutic implications.

1309 POSTER

Invasive lobular carcinoma: Preliminary study of the efficacy of a blood based gene expression test for early detection of breast cancer

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Background: Mammography has limited efficacy for the detection of breast cancer of lobular origin. Invasive lobular carcinoma is particularly difficult to detect since this cancer type shows diffuse infiltration and often presents on mammography as a subtle distortion rather a solid mass. In addition, this cancer type is rarely associated with microcalcifications which are characteristic signs of malignancies. Due to the later detection of lobular cancers the likelihood of surviving lobular cancer is less than that seen for ductal cancer.
We have developed a blood based gene expression test for the detection of breast cancer using samples from women with ductal carcinoma due to the higher prevalence of this cancer type. We have previously reported detection of ductal carcinoma with accuracies ranging from 75−82% [1−3]. Here we report the findings from a preliminary study using lobular cancers and healthy controls (without mammographic findings) with a blood based gene expression signature developed from ductal breast cancer.
Materials and Methods: A 96-assay format gene expression signature developed from samples from 294 women was used to establish a PLS classifier. The resultant signature was used with blood samples from women with lobular cancer and healthy controls from a European/US cohort. Whole blood was collected in PAXgene™ tubes and shipped on dry ice to a central laboratory. RNA extraction was performed using PAXgene™ Blood RNA kit according to the manufacturer's instructions.
Results: Of the 14 lobular cases, 11 were correctly classified as cancer. Of the 14 healthy controls, 11 of 14 were correctly classified. These results are consistent with the accuracy of the test for ductal tumours.

	Mean age (y)	Lesion diameter (mm)	Correctly predicted
Stage 1 N = 10	65	8−20	8/10
Stage 2 N = 4	69	15−50	3/4
Healthy controls	51	NA	11/14

Conclusions: The present study provides an indication that lobular cancers are detected by the blood based gene expression test with implication that lobular and ductal cancers elicit a common response in whole blood which is recognised by the test.
The study suggests the test has a potential for detecting a form of cancer which is difficult to detect with normal mammographic procedures. Additional cases of lobular cancer are required to support these preliminary findings.

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1310 POSTER

Association of Her2Neu Ile655Val polymorphism with clinical characteristics, response to neoadjuvant chemotherapy and cardiac toxicity in locally-advanced breast cancer

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Background: Locally-advanced breast cancer (LABC) is a heterogeneous group requiring different treatments for disease control and enhancement of survival. The aims of our study were to evaluate the frequency of Her2Neu Ile655Val polymorphism in patients with LABC, and to study it's association with clinical characteristics, response to neoadjuvant chemotherapy (CT) and cardiac toxicity.
Methods: Women with LABC with or without her2Neu overexpresion (h2N-OE), who received neoadjuvant CT with FAC 4 cycles followed by paclitaxel (80 mg/m^2) for 12 weeks were included. Patients with h2N-OE received trastuzumab load dose of 4/mg/kg and 2 mg/m^2 weekly during neoadjuvant CT. HER2 polymorphism was determined by RFLP, using BsmA1 enzyme. Cardiac toxicity was measured with MUGA at baseline and at the end of treatment. The study was approved by local ethics committee.
Results:114 patients with LABC and 107 healthy controls were included, median age was 46.4±9, ER and PR positive were 46.5% and 24.6% respectively, h2N-OE 3+ or FISH+ was 43.9%. The frequency of Ile655Val in controls was of 20.6% and in LABC of 25.4% (NS). The patients who had h2N-OE presented the polymorphism in 36% vs 17.2% who did not (OR 1.6, CI 95% 1.1−2.7, p = 0.021). We did not find association between age, nodal status, clinical and pathologic response, nuclear grade and the polymorphism. In the HER2 subgroup we found a higher prevalence of cardiac toxicity (45% vs. 21% p = 0.05), in patients with genotype Ile/Ile, Ile/Val, Val/Val cardiac toxicity was 11/53 (21%), 4/10 (40%) and 1/1 respectively, being a trend (p = 0.09).
Conclusion: Patients with HER2/neu overexpresion and Ile655Val polymorphism might have more cardiac toxicity. The presence of Val allele may be can cause more aggressive tumors. The following study showed a little perspective to individualized treatment on a genomic alteration besides able to let known pharmagenomic of some drugs like trastuzumab in susceptible persons.

1311 POSTER

Quantitative analysis of Pten conditional knockout mouse proteome reveals significant prognostic biomarkers for survival in metastatic castration resistant prostate cancer (CRPC)

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Background: Men with metastastic CRCP have a poor prognosis with a median survival of 16 to 20 months. The course of the disease is heterogeneous. Nomograms based on clinical parameters are often weak in prognostic accuracy. Rebiopsy is rarely indicated in CRCP. Therefore serum biomarkers are on high demand. The purpose of this study was to identify novel biomarkers for survival in the serum of patients with CRCP based on a screen of the murine Pten-dependent glycoproteome.
Methods: We established a novel platform for human biomarker discovery and validation based on a large-scale quantitative analysis of *N*-linked glycoproteins of the Pten conditional knockout mouse model for prostate cancer progression. Quantitatively comparing the glycoproteome of mice with homozygous Pten deletion with the corresponding control animals led to the identification of 153 candidate biomarkers. In serum samples from patients with CRCP we tested candidate biomarkers with ELISA for prognostic significance in survival.
Results: Serum samples of 68 patients with CRCP were retrospectively analyzed. Survival in the specific context was defined as the time between onset of the castration resistant state and death. We identified several candidates as biomarkers for survival in Kaplan Meier Plots. Three biomarkers with best significance in the log rank test showed p-values between 0.004 and 0.02. PSA and PSA doubling time were not significantly correlated with survival in our collective.
Conclusion: Our newly established biomarker-platform derived from a Pten conditional knockout mouse model showed high feasiblity for the identification of biomarkers for survival in CRCP. As the course of CRCP is heterogeneous prognostic serum biomarkers are likely to be helpful in the planning and scheduling of the therapeutic follow up.

1312 POSTER

Catumaxomab therapy eliminates putative CD133+ EpCAM+ cancer stem celles from malignant ascites: data from a pivotal phase II/III study

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Background: Putative cancer stems cells (CSCs) are defined as "tumor-initiating cells" that have the capacity to self-renew and to give rise to the variety of differentiated cells found in the malignancy. The CD133 membrane glycoprotein represents a CSC marker that has been previously demonstrated to be capable of identifying a cancer initiating subpopulation in brain tumors, melanoma and EpCAM+ solid tumors. The trifunctional anti-EpCAM x anti-CD3 antibody catumaxomab efficiently eliminates tumor cells from the peritoneal fluid of malignant ascites (MA) patients as demonstrated in a pivotal phase II/III trial (Parsons et al., ASCO 2008). Here we report on the presence of CD133+/EpCAM+ putative CSCs in MA and, more importantly, on the elimination of this cell population from the peritoneal fluid of MA patients by means of catumaxomab therapy.
Methods: 18 CTX-refractory patients with MA caused by a variety of primary carcinoma diseases (i.e. ovarian, pancreas and gastric cancer) were analyzed for the presence CD133+/EpCAM+ cells in peritoneal fluids by means of CD133+/EpCAM+ double staining on cytospin preparations. Analyses were performed before, 2 days after the first and 1 day after the last catumaxomab infusion, respectively. Double stained cytospin preparations were evaluated with a computerized image analysis system.
Results: Before therapeutic intervention, CD133+/EpCAM+ cells were detected in the peritoneal fluids of 14 from 18 patients suffering from MA. After the 1st infusion of catumaxomab (10 μg), 9 of these 14 patients showed complete elimination of the CD133+/EpCAM+ cells. After 4 i.p. catumaxomab infusions (10 μg day 0, 20 μg day 3, 50 μg day 7 and 150 μg day 10) the CD133+/EpCAM+ cells were completely eliminated from the peritoneal fluids of all 14 MA patients.
Conclusions: In a preliminary monitoring study, putative CSCs (CD133+/EpCAM+) were present in peritoneal fluids of 78% of analyzed MA patients with different underlying primary tumor entities. Catumaxomab efficiently destroyed CD133+/EpCAM+ cells within peritoneal fluids of MA patients. Consequently, catumaxomab-based therapeutic measures may offer an additional treatment opportunity to eliminate CSCs in EpCAM+ malignancies.

1313 POSTER

Catumaxomab treatment reduces VEGF protein levels within malignant ascites: data from a pivotal phase II/III study

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Background: Treatment with the trifunctional anti-EpCAM x anti-CD3 antibody catumaxomab efficiently eliminates tumor cells from the peritoneal cavity (ASCO 2007, Jäger et al.) and led to clinically relevant prolongation of puncture-free survival (PuFS) in patients with malignant ascites (MA) in a pivotal phase II/III trial (ASCO 2008, Parsons et al.). As vascular endothelial growth factor (VEGF) levels are markedly elevated in MA in comparison to cirrhotic ascites the question was addressed whether catumaxomab treatment impacts the expression or accumulation of VEGF within MA. Here we report that in addition to tumor cell depletion, VEGF protein levels in MA significantly decreased upon catumaxomab therapy. We propose that the strongly correlated tumor cell elimination and reduced VEGF protein levels are causative for the prolonged PuFS of patients suffering from MA.
Methods: VEGF and total protein levels were measured by ELISA and BCA from MA supernatants before catumaxomab therapy, after the 1st infusion (10 μg;day 3) and after the 4th infusion (150 μg;day 11). Data were statistically analysed for the ratio of the VEGF protein concentration versus the total protein concentration for the MA treatment groups with ovarian (OC) or non-ovarian cancer (NC) as underlying disease and the corresponding control groups that received paracentesis only.