[2] Aarøe J et al. (2006) The 97th AACR Annual Meeting, 1−5 April, USA.
[3] Aarøe J et al. (2006) The 19th EACR Conference, 1−4 July, Hungary.

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.

Results: One day after the last catumaxomab infusion 46 or 47 patients analysed in the OC or NC treatment group showed a statistically significant decrease in VEGF to total protein ratio when compared to the measurement before catumaxomab therapy (ANOVA $p = 0.034$ for OC and $p < 0.001$ for NC). These results are consistent with the tumor cell elimination previously assessed in these patients. In contrast, the OC control group showed a statistically significant increase of VEGF to total protein ratio ($p = 0.009$) which is accompanied by an increase in tumor cell numbers. In the NC control group VEGF to total protein ratio remained unaffected ($p = 0.096$).
Conclusions: Catumaxomab therapy significantly reduced VEGF protein levels correlating with tumor cell elimination in MA which in turn led to the prevention of fluid accumulation in the peritoneal cavity and finally to prolonged PuFS of patients suffering from MA.

1314 POSTER

Review of cancer diagnoses in BRCA 1/2 carriers: hypersensitivity of BRCA 1/2 patients to cisplatin may also aply to uterine carcinosarcoma (UCS)

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Background: When identified, BRCA1/2 carriers must be included in long-term follow-up studies. Yearly review of clinical data concerning BRCA1/2 carriers identified through our program is performed.
Matherials and Methods: Review of clinical files of all BRCA1/2 carriers identified up to 31st March 2009 for new cancer diagnoses. The following variables: sex, ages, age at all cancer (CA) diagnoses, duration of follow-up and outcomes of treatments will be updated in September 2009.
Results: Up to March 2009, 170 BRCA1/2 carriers were identified: 130 women (71 affected with CA and 59 healthy at risk) and 40 men (14 affected with CA and 26 healthy at risk). The following CA diagnoses were established during follow-up as BRCA1/2 carriers: *8 cases of Breast CA* (4 cases in previously healthy women and 4 in Breast CA survivors – 3 women and 1 man); *5 cases of Prostate* CA (4 in Breast CA survivors and 1 case in a Gastric CA survivor); *2 cases of Gastric CA* in male Breast CA survivors; *1 Peritoneal CA* in a previously healthy female; *1 Basocellular CA* in a male healthy carrier and *1 UCS* in a Breast CA survivor. The UCS case refers to a BRCA2 female Breast CA survivor (initial diagnosis at 42 yrs) whose uterine diagnosis was established when investigating uterine bleeding while on Tamoxifen treatment. After surgery (pathology revealed an uterine sarcoma with heterelogous elements) and radiotherapy, she was kept under surveillance and a retroperitoneal, irressectable UCS relapse was observed after 9 months (confirmed by surgery and pathology). After chemotherapy with Cisplatin and Ifosfamide a complete response confirmed by CT, PET scan and surgery was observed and pt is free of disease after 32 months of follow-up.
Conclusions: BRCA1/2 cancer survivors (female and male) should be included in surveillance programs due to their risk of second and third cancers. The remarkable Cisplatin hypersensitivity observed in BRCA1/2 patients may not be limited to ovarian cancer; known cases of breast cancer and the unexpected long-term survival without relapse of our pt with metastatic UCS suggests that platinum hipersensitivity may be a general feature of cancers in BRCA1/2 patients, even in neoplasms not belonging to the BRCA phenotype.

1315 POSTER

Clinical role of human epididymis protein 4 (HE4) in epithelial ovarian cancer

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Background: HE4 is one of the most promising novel ovarian cancer biomarker. The aim of this study was to evaluate the potential role of serum HE4 (sHE4), in comparison with serum CA125 (sCA125), in diagnosis and monitoring of epithelial ovarian cancer (EOC) patients. Moreover, we investigated the relationship between sHE4 and clinicopathologic characteristics of EOC patients, in order to assess if sHE4 could influence EOC biology.
Material and Methods: For ovarian cancer detection study we included 45 healthy women, 45 patients with benign pelvic masses, and 87 EOC patients. All samples were collected before any treatment. For ovarian cancer monitoring study, we selected 13 EOC patients who underwent second look laparotomy procedures with no clinical evidence of disease following primary chemotherapy. Two out of 13 patients had macroscopic persistence of cancer, 5 had microscopic persistence of cancer while 6 had no evidence of disease after second look pathological evaluation. sCA125 and sHE4 were quantified by Architect CA125 II immunoassay kit (Abbott Diagnostics, IL, USA) and by HE4 EIA assay (Fujirebio Diagnostics, Malvern, PA), respectively. Patient charts were reviewed to obtain data regarding diagnosis, histology and response to treatment.
Results: ROC curves and statistical tests showed that sHE4 and sCA125 had comparable ability to discriminate EOC from benign masses (ROC curve AUC: HE4 = 0.95, CA125 = 0.92; AUC difference: not statistically significant $p = 0.29$) whereas sCA125 was better than sHE4 in distinguishing EOC from healthy controls (ROC curve AUC: HE4 = 0.93, CA125 = 0.98; AUC difference: statistically significant $p = 0.03$).
We found that sHE4 levels were significantly lower in clear cell histotype compared to serous and endometrioid histotypes ($p = 0.0003$) and that sHE4 levels were positively associated to FIGO stage ($p = 0.0003$).
When we compare the biomarker levels between preoperative and second look times, all patients showing microscopic or no residual disease displayed 95−99% decrease in both serum marker levels. More interestingly, in the only 2 patients experiencing macroscopic cancer persistence sCA125 diminished of 99% while sHE4 of 30% and 70%.
Conclusions: In conclusion, our results showed that sHE4 could identify patients with macroscopic persistence of cancer, following primary chemotherapy. These patients will be eligible for stronger therapeutic interventions.
Finally, the positive association between sHE4 and FIGO stage suggested a possible role of sHE4 in ovarian cancer progression.

1316 POSTER

Prognostic value of EGFR, COX-2, Ki-67, thymidylate synthase (TS) and cell cycle regulators expression in sporadic colon carcinoma

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Background: EGFR, COX-2, TS and Cell Cycle Regulators are involved in colorrectal carcinogenesis and may be useful predictors of clinical outcome. This retrospective study was carried out in order to assess biomarkers expression, relationship with clinical features and prognostic value.
Patients and Methods: 123 tumor samples from patients with histologically confirmed colon carcinoma in stages I-IV TNM system were studied. All patients were treated with surgery and adyuvant FPs-based chemotherapy (CT) as indicated by stage or risk factors. Overexpression of protein tumor markers were analyzed by immunohistochemistry assay in PEFF archived tumor samples with specific MoAbs: EGFr clon H11; Cox-2 CX294; TS TS106; p53 DO7; Ki-67 MIB1; ciclina D1 DCS6; p21 SX118 and p27 SX53G8, respectively and evaluated with semiquantitative method (score 0−3).
Results: EGFR protein expression was positive in 55 tumors (43.9%), COX-2 in 53 (43.1%); TS in 81 (57.7%); p53 in 78 (63.4%); cyclin D1 in 48 (39%); Ki67 in 75 (61%), p21 in 26 (21.1%) and p27 in 46 (37.4%) cases. No significant correlations between biomakers and conventional prognostic factors were confirmed except TS that was associated with positive nodes and stage III (chi-square test, $p < 0.05$). We found a positive correlation between EGFR with p53; COX-2 with cyclin D1, p27, Ki-67 or TS and cyclin D1 with p21 or p27 expression (Spearman test, $p < 0.05$). There were not differences in DFS or OS by biomarkers expression in all cohort of patients or in subgroups treated with CT (log rank test $p>0.20$). In multivariate analysis, age, grade, LVI and TNM stage were statistically significant prognostic factors (Cox model; $p < 0.05$).
Conclusions: In our experience, biomarkers were overexpressed in similar ranges previously reported in the literature except p21, they were found independent of conventional clinicopathological features and none of them had prognostic value.