Late Breaking Abstracts

P93

Quantitative RT-PCR assays for the determination of urokinase-type plasminogen activator and plasminogen activator inhibitor type 1 mRNA in primary tumor tissue of breast cancer patients: comparison to antigen quantification by ELISA

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Introduction: uPA and its inhibitor PAI-1 play a key role in tumor-associated processes such as the degradation of extracellular matrix proteins, tissue remodeling, cell adhesion, migration, and invasion. High antigen levels of uPA and PAI-1 in tumor tissue of various solid malignant tumors, including breast cancer, are associated with poor patient prognosis. In the present study, we examined whether analysis of uPA and PAI-1 mRNA expression represents an alternative to the measurement of the respective antigen levels in breast cancer.

Main Message: Highly sensitive quantitative real-time PCR (QPCR) assays, based on the LightCycler technology, were established to quantify uPA and PAI-1 mRNA expression in breast cancer cell lines as well as in tumor tissue of breast cancer patients. mRNA concentrations were normalized to the expression level of the housekeeping gene h-G6PDH. The respective uPA and PAI-1 antigen concentrations were determined by established ELISA formats. QPCR mean interassay variation coefficients were 11 % (uPA) and 8 % (PAI-1). In breast cancer cell lines mRNA and antigen values were highly correlated for both uPA and PAI-1 (each: rs = 0.95; p < 0.001). In contrast, correlations between uPA/PAI-1 mRNA and protein in the breast cancer samples were found to be distinctly weaker or not significant. Thus, quantitative determination of mRNA expression for both factors does not mirror antigen levels in breast cancer tissue, possibly due to posttranscriptional regulation. Except for nodal status being inversely correlated with uPA mRNA levels, no significant interrelations were observed between uPA or PAI-1 mRNA expression and clinico¬pathological parameters. On the protein level, elevated uPA and PAI-1 values were associated with a negative steroid hormone receptor status.

Conclusion: The implementation of mRNA quantification of uPA and PAI-1 in breast tumors can not serve as one-to-one substitution for antigen determination by ELISA.

P96

RT-PCR based 3-gene expression signature related to prognosis for early-stage squamous cell lung cancer (SqCLC)

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Introduction: Adjuvant chemotherapy has shown a significant but modest benefit in stage II-IIIA NSCLC. Selection of patients with high risk of distant relapse is a cost-effective approach and will spare low-risk patients from treatment toxicity. No clinical factors are available to this end. RT-PCR is accurate for routine assessment of prognostic genes expression in tumor biopsies.

Main Message: The study included surgically treated SqCLC patients (42 stage I, 20 stage II and 2 stage IIIA). Thirty-three patients developed distant metastases after surgery, and the remaining 33 were recurrence free after median follow-up of 37 months (range: 24-64 months). The fresh frozen tumor biopsies used for gene expression analysis were confirmed to contain at least 60% of tumor cells. Total RNA was extracted with AllPrep kits (Qiagen). RNA quantity was assessed in Nano-drop and quality tested on agarose gel. cDNA was synthesized from 1 µg of RNA using High-Capacity cDNA Kit (Applied Biosystems). Quantitative RT-PCR reactions for 29 genes were done using (TLDAs) in an ABI PRISM 7900 HT (Applied Biosystems). Gene expression values were normalized according to expression of 18S RNA and obtained by ÄÄCt method. Ten genes: CSF1, FN1, CA IX, PH4, KIAA0974, ANLN, VEGFC, ISNR, NTRK1, EGFR were found to be significantly correlated with survival in the univariate Cox regression analysis. Biologically meaningful correlations were observed among these genes. High expression of PH4 were related to low or no expression of CA IX (r = -0.33; p=0.007). CSF-1, EGFR, CA IX expression and tumor size were independent prognostic factors in multivariate analysis. To assess the prognostic impact of the expression of those 3 genes simultaneously a risk score was generated as follows:

Risk score (RS) = [0.93xCSF + 1.4xCA IX + 1.1xEGFR]. Median survival for high-risk patients was 24 months (95% CI, 17.1-30.9), while it was not reached in the low-risk group (p <0.00001). The accuracy of prediction was 70%. This model also performed well in predicting development of distant metastases, with 64% sensitivity and 73% specificity. The prognostic value of the model was confirmed in an independent cohort of SqCLC patients (n=26) stage I and II (USA). The difference in survival between high and low risk score patients was statistically significant (p=0,05).

Conclusion: The proposed 3 gene expression signature is highly prognostic for SqCLC patients and may serve for selection of patients for adjuvant chemotherapy.

P102

Cantharidin induced apoptosis via p38 and JNK pathways associated with p53 and caspase-3 in U937 cells

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Introduction: Cantharidin is an active compound of blister beetles used for the treatment of cancer. It was known to exert antitumor activity via induction of apoptosis in cancer cells. However, its signaling pathway still remains unclear.

Main Message: XTT assay, wetern blotting(Caspase 3,8,9, PARP, cytochrome c, p53, p38, ERK,and JNK), inhibitor assay and flow cytometric analysis were used for the

elucidation of the mechanism of cantharidin induced apoptosis Cantharidin effectively activated ERK-1/2, p38 and JNK in U937 cells in a time and dose-dependent manner. Cantharidin also exhibited a strong cytotoxicity and induced apoptosis in U937 cells. For the evaluation of roles of MAPKs, PD98059, SB202190 and SP600125 were used as MAPK inhibitors for ERK-1/2, p38 and JNK in cytotoxicity and apoptosis assays. PD98059 didn¡¯t affect cantharidin-induced cytotoxicity and apoptosis, whereas SP600125 and SB202190 significantly interfered with cytotoxic and apoptotic activites induced by cantharidin. Cantharidin alone induced the apoptosis accompanied by p53 accumulation and caspase-3 activation, whereas SP600125 and SB 202190 caused the downregulation of p53 and caspase-3 after co-treatment with cantharidin by western blotting. Likewise, SP600125 and SB202190 significantly disturbed the caspase-3 activity induced by cantharidin by colormetric assay.

Conclusion: Cantharidin can induce apoptosis through the activation of p38 and JNK MAP kinase pathways associated with p53 and caspase-3.

P106

Comprehensive analysis of the main immunohistochemical markers used in ovarian cancers

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Introduction: Cancer of the ovary represents about 30% of all cancers of the female genital organs (WHO, 2003). In developed countries it is about as common as cancer of the corpus uteri and invasive cancer of the cervix. About 70-75% of patients with ovarian cancer have tumor spread beyond the pelvis at the time of diagnosis. The low survival in ovarian cancer is due, at least by the fact that the disease is occult in the first stages. The diagnosis is made too late, when the metastasis exists already and the prognosis is bad. Immunohistochemical study of the main epithelial tumors of the ovary-serous and mucinos adenocarcinoma-is a valuable adjunct in through histologic examination. Using a wide panel of antibodies, as ER (estrogen receptor), PGR (progesterone receptor), EGFR (epidermal growth factor receptor), cerbB2 (HER2NEU protooncogene), PCNA (proliferative cellular nucleolar antigen), CA125, Ki67, P53 (P53 oncoprotein) and CK7 (cytokeratin 7) we have tried to find a “happy combination” for an early and precisely diagnosis of ovarian epithelial cancers.

Main Message: We investigated immunohistochemical a group of 60 patients with serous (n=45, 45.75%), mucinous (n=12, 20%) adenocarcinomas and borderline tumors (n=3; 5%). We used the tristadium methods of Hsu (1981) Avidin-Biotin-Peroxidase. A number of 19 patients have had peritoneal implants (31.6%) and tumors were bilateral in 7 cases (11,66%). The method used revealed that the ER was positive in 70% cases, PGR in 60%, PCNA in 61,66% and p53 in 63,33%, CA125 in 81,66% and the perimembrane pattern of cerbB2 in 63,33%. The majority of cases were serous carcinomas.

Conclusion: The relevance of CA125 -a useful antibody in ovarian cancer, also for metastatic disease- is limited because it is positive in many conditions (mesothelial proliferations, metastatic carcinomas, etc). The ovarian serous carcinomas are a homogenous group from the standpoint of pathogenesis. The panel of antibodies was positive in more than 50% of serous carcinomas. None of the antibodies or combination has a clearly established role for staging and prognosis of the tumor. Only the three-tiered grading system has important prognostic and therapeutic implications.

P107

Extensive utility of immunohistochemistry in colorectal cancer; importance in targeted therapy

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Introduction: Colorectal cancer (CRC) is one of the major cancer diseases in the world. Each year there are 300.000 new cases of colorectal cancer and 200.000 deaths from the disease in Europe and in the United States. For surgical resected malignancies, the pathologic stage is widely recognized as the most accurate predictor of survival and it determines the appropriateness of adjuvant treatment as well. Studying tumors of patients across all stages of disease identifies molecular markers. In the same time, molecular tumor markers are often studied in CRC using immunohistochemistry to determine their prognostic or predictive value. Protein expression is typically assigned as a positive score based on a predetermined cutoff. The aim of our study was to find out the statistical correlations between different molecular markers and tumor grade and stage.

Main Message: We investigated histopathological and immunohistochemistry 40 patients who undergone surgery for colorectal cancer. The study included 19 female and 21 males. We analyzed using ABC method for immunohistochemistry the following markers for 10 selected cases: vascular endothelial growth factor (VEGF), CD44, carcinoembrionic antigen (CEA), transforming growth factor beta (TGFβ), cytokeratin 20 (CK20), epidermal growth factor receptor (EGFR) and oncoprotein p53. Then, we analyzed statistical the results using Student-t test. Six cases out of 40 patients with CRC presented perforation (15%) and lymph nodes invasion (85%). 23 cases (57.5%) were moderate differentiated, 6 cases had distant metastasis, and only 9 cases were mucinous cancers. We noticed positive correlation between VEGF1 and CK20 ($r=0.4$, $p=0.05$), and between VEGF1 and CEA ($r=0.88$, $p=0.001$). Also, our results have demonstrated a positive correlation between tumor grade and CEA ($r=0.43$, $p=0.009$), and no relation among the other markers.

Conclusion: Our present study shows that CK20 and CEA were positive no matter the stage (100%). The p53 oncoprotein has been negative in T1 and T2 stages, but in advanced stages has been positive only in a half of cases (50%). Regarding the tumor location, p53 and VEGF showed positivity, and VEGF was positive whatever the topography. We have noticed a direct proportional relation in VEGF expression and CEA, and CEA and tumor grade, also ($r = 0.88$, $p < 0.001$).

P111

Pioneering the use of new technology for an improved assessment of the HER2 status in breast cancer.

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Introduction: Inconsistencies in the results of HER2 testing when using well established protocols such as immunohistochemistry have been recognised. Alternative techniques such as frontline fluorescent or chromogenic in situ hybridisation (FISH or CISH) have been suggested. Recently a variation of CISH using a silver chromogen (SISH) has been released on to the market.

Main Message: 672 breast cancer cases (~50:50 surgical excisions:core biopsies) were assessed for HER2 status using a fully automated system which performs the assay in a little over 6 hours. The slides were assessed by experienced pathologists. 88 of 672 (13%) breast cancers showed an amplification of the HER2 gene. In 22% (19 cases) of the positive samples it was necessary to perform a chromosome 17 correction to ensure that it was low level amplification and not polysomy that had caused the increase in HER2 gene copies; two cases showed a high level of polysomy (>6 copies of chromosome 17). Approximately 7% cases required a repeat assessment, usually due to insufficient digestion of the tissue in the first assay.

Conclusion: SISH is a reliable, robust and time-efficient way to assess the HER2 status. The chromosome 17 correction is not required in the vast majority of cases.

P112

RT-PCR can predict melanoma recurrence in histological by negative sentinel node biopsy

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Introduction: SLNB is the best predictor of melanoma recurrence. Up to 10% of patients with histo-negative SLN still develop subsequent LN metastases indicating that melanoma cells may still have been present in the SLN but not detected even with extended histopathological examination. RT-PCR for molecular identification of melanoma cells within the SLN has shown conflicting results and many false positives. Specific tumour markers may address this problem.

Main Message: To demonstrate the accuracy of RT-PCR in the identification of histopathological SLN negative patients who will develop further disease. Melanoma specific markers were analysed by RT-PCR on a consecutive series of 77 paraffin embedded sentinel lymph nodes. Follow up and histological status were known for each cases. 63 cases were histologically negative, 14 were histologically positive. Molecular positivity was seen in 12 cases, 7 in the histo positive group and 5 in the histo negative group. These 5 patients developed recurrent disease.

Conclusion: The correlation between histological and molecular positivity for SLN is high in our material where melanoma specific markers have been used but our data indicates that RT-PCR may identify a subgroup of histo negative patients at risk for disease progression. RT-PCR should be further evaluated in the assessment of SLN for melanoma.

P115

MGMT promoter hypermethylation predicts sensitivity to temozolomide in patients with recurrent glioma

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Introduction: The O6-methylguanine-DNA-methyltransferase (MGMT) DNA-repair protein reverses the effect of the cytotoxic drug temozolomide (TMZ). The MGMT gene promoter is hypermethylated in about half of all high-grade gliomas (HGG) and co-determines the sensitivity of these tumors to alkylating agents. We investigated the correlation between the methylation status of the MGMT gene promoter and the benefit from treatment with TMZ at recurrence in patients with HGG.

Main Message: The degree of MGMT-promoter methylation was determined with a quantitative methylation sensitive polymerase chain reaction (QMSP) on the DNA extracted from archival glioma tissue samples from 63 HGG patients who had been treated with TMZ at recurrence. FISH served to characterize the glioma samples for possible loss of heterozygosity (LOH) of chromosome fragments 1p and 19q. These molecular-genetic test data were correlated with the clinical outcome of the patients. An informative QMSP-test result could be obtained for 56 tumor samples from 52 patients (in 29% of cases MGMT gene promoter was hypermethylated and 71% unmethylated). None of these gliomas had a combined LOH for chromosomes 1p and 19q. Among the 38 patients (4 anaplastic oligo-astrocytoma (AOA), 12 anaplastic astrocytoma (AA) and 22 glioblastoma multiforme (GBM)) who were chemo-naïve at the time of recurrence, none of the 10 patients with a hypermethylated MGMT promoter experienced disease progression within the first two TMZ treatment cycles compared with 11 of 27 (41%) patients with a non-methylated promoter (2-sided Fischer exact test, p .016). WHO differentiation grade and MGMT gene promoter methylation were the only two independent baseline variables that correlated with time to progression (TTP) ($p < .05$ in Cox multivariate analysis). In the subgroup of patients with AA/AOA, but not GBM, MGMT gene promoter hypermethylation also predicted for superior overall survival following TMZ.

Conclusion: MGMT promoter methylation was an independent predictor for sensitivity to TMZ at recurrence in HGG patients and may predict a survival benefit in patients with anaplastic glioma.

P117

Telomerase evaluation in cancer patients and in individuals with symptomatic benign diseases

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Introduction: In previous case-control studies we showed that telomerase activity in voided urine represents an important non-invasive tool for bladder cancer diagnosis. However, little data is available on its accuracy in detecting tumors in individuals with symptomatic urinary tract diseases.

Main Message: The study was performed on 515 individuals, 197 with urinary tract symptomatology and 318 patients with a first diagnosis of bladder cancer. Telomerase activity was evaluated in cell pellets obtained from voided urine using a

quantitative TRAP assay and expressed as Arbitrary Enzymatic Units (AEU). Sensitivity ranged from 61% to 93% and specificity from 42% to 88% on the basis of the different AEU cut-off values considered. At the best cut-off of 50 AEU, sensitivity was 90%, specificity 70% and the overall accuracy, in terms of true positives and true negatives, was 80%. Sensitivity did not vary in relation to patient age or tumur grade at diagnosis, and was similar in women and men.

Conclusion: This is the first study to analyze the diagnostic relevance of telomerase activity in a large series including symptomatic individuals as controls. Our results suggest that urine telomerase activity is a good marker for the early diagnosis of bladder tumors in symptomatic individuals, as well as in high-risk subsets. In fact, having to do with a tumour type at low incidence it is unthinkable to activate screening programs on general populations.

P121

In Vitro Drug Response in Primary versus Metastatic Colorectal Tumors

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Introduction: Approximately 20 % of colorectal carcinoma (CRC) patients are initially diagnosed with liver metastases, with objective response rates of only 50% and recurrent disease in the responding patients. Current protocols, based on 5-fluouracil (5FU) and folinic acid (FA) combined with irinotecan (IR) or oxaliplatin (OX), result in similar response rates. New predictive tests are needed to guide the optimal treatment selection. If predictive marker testing on the primary tissue will be also predictive for metastatic tumors, the need for biopsy material from metastatic sites will diminish. We therefore analyzed in vitro drug response in primary vs liver metastatic tumors in a large cohort of CRC patients.

Main Message: Freshly resected tumors were tested in the Extreme Drug Resistance (EDR) assay to 5FU, 5FU+FA, OX, and SN38, the active form of IR, using the Percent of Cell Growth Inhibition (PCI) and EDR/IDR/LDR ratio: Extreme (EDR, one standard deviation more resistant than the median), Intermediate (IDR, between the median and EDR), and Low (LDR, higher than the median), as endpoints. In 4854 primary and 847 unmatched liver metastatic specimens, PCI and EDR endpoints were not statistically different in primary vs metastatic sites for 5FU, OX, and IR (PCI p values: 0.086, >0.9999, and 0.3741, Student's t-test; EDR: 0.0754, 0.9199, and 0.7287, Chi-squared test). Primary tumors were less resistant to 5FU+FA by PCI (p=0.0005) and EDR/IDR/LDR (p=0.005). Primary and metastatic tumors that were resistant to 5FU+FA, OX and IR demonstrated the lowest drug cross-resistance among all the tested drugs (10% and 15%, respectively). OX showed the lowest drug resistance (EDR rate of 16%) in tumors that were resistant to both the 5FU+FA combo and IR.

Conclusion: Primary and metastatic CRC showed similar patterns of in vitro resistance to CRC chemotherapeutic drugs, suggesting that testing results obtained at initial diagnosis may be useful in guiding therapy selection in liver metastatic disease. Although OX and IR showed the highest in vitro cytotoxic activity in combination with 5FY+FA, a distinction should be made between the use of OX and IR in the individual patient. Prospective EDR clinical trials are in progress to substantiate these data.

P122

EGFR and STAT3 Expression Levels Are Associated With Clinical Response to EGFR Antisense Gene Therapy in HNSCC

Stephen Lai..

Introduction: Head and neck squamous cell carcinoma (HNSCC) is characterized by upregulation of the epidermal growth factor receptor (EGFR). EGFR expression levels in these tumors are correlated with decreased survival. We assessed a novel strategy to target EGFR using an EGFR antisense (AS) gene sequence under the control of the U6 promoter in a phase I clinical trial.

Main Message: Advanced SCCHN patients refractory to standard therapies with at least one evaluable and accessible lesion were enrolled. EGFR AS dose was escalated in successive cohorts (classic "3+3" design; 6 dose levels from 60 to 1920 µg per injection). Patients received four weekly intratumoral EGFR AS injections. Tumors biopsies were performed prior to and after completion of therapy. We assessed the level of relevant biomarkers of EGFR AS activity (e.g., EGFR and STAT3) by immunohistochemistry. 17 evaluable patients were treated and no grade 3/4 or dose-limiting toxicities were noted. A maximum tolerated dose was not reached. Five patients (29%) achieved a clinical response (RECIST), including 2 complete responses (CR) and 3 partial responses (PR); 2 additional patients had stable disease (SD) as best response (disease-control rate 41%; 95% CI = 18-65%). There was no apparent correlation between EGFR AS gene dose and the probability of clinical response, however, smaller tumors were more likely to respond ($p = 0.0592$). Immunohistochemical studies demonstrated higher baseline EGFR expression in patients with disease control (CR+PR+SD) as compared to patients with progressive disease (PD; $p = 0.0271$). A comparison of EGFR immunohistochemical staining before and after EGFR AS treatment suggests a larger decrease in EGFR levels in patients with clinical response (CR+PR), as compared to SD+PD patients ($p = 0.0583$). Thus, the decrease in EGFR expression may be associated with clinical response. Additionally, there was a trend towards improved disease control with lower baseline STAT3 expression ($p = 0.0381$).

Conclusion: Intratumoral EGFR AS was safe and resulted in substantial antitumor activity in HNSCC patients. Expression levels of EGFR and STAT3 may be associated with the antitumor effects of EGFR AS gene therapy.