10.3 (±15.4) vs. 3.5 (±6.1), p = 0.018, respectively. These mean values were neither correlated with Gleason score, nor PSA, nor risk groups.
Mean number of pathological voxels for patients with a tumor ⩾T2b−c was: 6.31 (±7.43) and 4.09 (±7.64) if ⩽T2a (p = 0.03). The mean number of pathological voxels was significantly correlated with increased PSA: (OR: 1.80 [95% CI 1.016–1.149], p = 0.013). No correlation was found with the Gleason score. Patients with intermediary risk have a significantly higher number of pathological voxels than in the low risk group (OR: 1.075 [95% CI 1.009–1.145], p = 0.025). A similar result was found for high risk pts with respect to the intermediary group.
Conclusion: At 3 T, biomarkers expressing the relative Choline content and number of pathological voxels were powerful parameters for the localisation of PCa and could be helpful for determining the prognosis more accurately.

PP38

Proteomic analysis of plasma from BRCA1/BRCA2 mutation carriers as modifier risk factor of breast cancer

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Background: BRCA1 and BRCA2 mutations were estimated to cause cumulative lifetime risks of breast cancer of 70% by 70 years. However, there is a marked variation of phenotypic expression among BRCA mutation carriers. Identification of markers that can be used to refine estimates of a woman's individual cancer risk appears to be warranted in order to individualizing prevention strategies. The aim of this study was to analyze using proteomics modifications in the plasma protein map from BRCA1/2 mutation carriers with respect to a control group, as well as potential differences between cancer patients (CP) and cancer-free carriers (CFC). Preliminary results were presented at ICACT 2009 (abstract 237).
Materials and Methods: We use two-dimensional gel electrophoresis and mass spectrometry to analyze plasma levels of the following proteins in 40 female BRCA mutation carriers (22 BRCA1 − 10 CP and 12 CFC − and 18 BRCA2 − 12 CP and 6 CFC): fibrinogen (fgg), haptoglobine (hap), serotransferin (ser), convertase (con), alpha1-antitrypsin (AAT), apolipoprotein A-I (APO AI) and A-IV (APO AIV) and C-reactive protein (PCR). The control group included 10 age-matched non-carriers without personal or family history of cancer. We also use the PD-QUEST software to determine the total protein count for each group.
Results: AAT 4 (p = 0.038), 5 (p = 0.023) and 6 (p = 0.037), hap 4 (p = 0.023) and APO AI 2 (p = 0.038) and 5 (p = 0.013) were increased in plasma from BRCA2 mutation carriers with respect to BRCA1 ones. BRCA1 CFC showed increased levels of AAT 6 (p = 0.058) and APO AIV (p = 0.004) when compared to BRCA1 CP. No significant differences were found in the protein map between BRCA2 CP and CFC. BRCA2 CP when compared with BRCA1 showed increased levels of AAT 6 (p = 0.023), APO AI 2 (p = 0.038) and 5 (p = 0.019) and APO AIV (p = 0.010) and decreased levels of con 3 (p = 0.023). Fgg 2 (p = 0.001) and 3 (p = 0.001), ser 4 (p = 0.001) and con 1 (p = 0.041), 2 (p = 0.006), 3 (p = 0.001), 4 (p = 0.001) and 5 (p = 0.001) were found significatively increased in plasma from CFC with respect to the control group. No significant differences were found in the total protein count among groups using PD-QUEST.
Conclusion: BRCA2 mutation carriers showed a favorable plasma protein map when compared with BRCA1 ones, as well as observed in control group with respect to BRCA1/2 carriers. Moreover, BRCA1 CFC had better prognosis proteomic profile than BRCA1 CP. The confirmation of these results in a larger cohort is required before proteomic analysis could be used to guide prevention strategies among BRCA mutation carriers.

PP17

The predictive value of HLA Class I tumor cell expression and tumor infiltration by regulatory T cells for chemotherapy in patients with early breast cancer

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Background: Additional prognostic and predictive factors may direct tailored treatment in early breast cancer and thus decrease morbidity and mortality rates. We hypothesized that T cell immune interactions affect tumor development and therefore clinical outcome. Therefore, we examined the clinical impact of human leukocyte antigen (HLA) class I tumor cell expression and regulatory T cell (Treg) infiltration in breast cancer.
Materials and Methods: Our study population (n = 677) consisted of all early breast cancer patients primarily treated with surgery in our center between 1985 and 1994. Formalin-fixed paraffin-embedded tumor tissue was used for immunohistochemical staining with HCA2 and HC10 (recognizing HLA Class I) and Foxp3 (recognizing Treg) antibodies. HLA class I expression was evaluated by combining results from HCA2 and HC10 antibodies and was classified into three groups: loss, downregulation and expression. Treg infiltration was defined as complete absence or presence of Treg.
Results: Remarkably, only in patients treated with chemotherapy after surgery, both presence of Treg (p = 0.01; Hazard Ratio (HR): 2.04) and expression of HLA class I (p = 0.002; downregulation HR: 2.11; loss HR: 3.34) resulted in less relapses over time, independently of other clinicopathological parameters. Treg and HLA class I were not of influence on outcome in patients who did not receive chemotherapy. An interaction between chemotherapy and HLA class I (p < 0.001) and Treg (p < 0.001) was found in cox regression analysis.
Conclusion: We showed that HLA class I and Treg both affect prognosis, exclusively in chemotherapy-treated patients. In mouse studies it has been shown that chemotherapy can selectively eliminate Treg, therefore possibly enabling Cytotoxic T-lymphocytes to kill tumor cells that have retained HLA class I expression. Therefore, we conclude that HLA class I and Treg could be applied in response prediction to chemotherapy in early breast cancer patients.

PP28

Prediction of response to neo-adjuvant radiotherapy in patients with locally advanced rectal cancer by means of sequential 18F-FDG-PET

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Background: The current approach to curative treatment of locally advanced rectal cancer is comprised of surgery combined with neo-adjuvant (chemo)radiotherapy. Radiosensitivity of rectal cancer however is variable. Morphological imaging techniques are performing poorly in assessing the response because of their inability to differentiate residual neoplastic from scar tissue. The aim of this study was to investigate the potential of sequential 18F-FDG-PET(CT) in assessing the response of rectal cancer to neo-adjuvant radiotherapy (RT), and to determine which parameter(s) can be used as useful metabolic response indice(s).
Materials and Methods: Patients (pts) were treated by Tomotherapy: dose of 46 Gy to the presacral space and integrated boost to 55.2 Gy on the tumor, if circumferential resection margin was <2 mm on magnetic resonance imaging. Pts underwent total mesorectal excision within 6 w after completion of RT, and tumor regression was graded histologically.
18F-FDG-PET or PET/CT scans were acquired prior to and in the 5 w after the end of RT. Tumoral uptake of 18F-FDG was assessed semi-quantitatively using standardized uptake values (SUV). The percentage difference (Δ) between pre- and post-RT scans in SUVmax, SUVmean (average SUV of tumoral pixels with SUV ⩾2.5) metabolic volume (MV, sum of tumor pixels with SUV ⩾2.5) and the total glycolytic volume (tGV, MV × SUVmean) was investigated as possible metabolic response indices.
Results: 45 consecutive pts (34 male and 11 female; age 65.4±12.5) with histologically confirmed rectal adenocarcinoma (cT3/T4) were enrolled.
Following neo-adjuvant RT, of the 45 pts 20 (44.4%) were classified as responders, while the remaining 25 (55.6%) were non-responders. Intense 18F-FDG uptake was seen in all tumors prior to neo-adjuvant RT: average SUVmax 12.9±6.0 (no significant different beween responders and non-responders). When classifying pts according to histology significant differences in average ΔSUVmax (55.8% vs 37.4% decline, p = 0.023) and ΔSUVmean (40.1% vs 21.0%, p = 0.001) between responders and non-responders respectively were observed. For MV and tGV decreases were more prominent in responders (72.9% vs 62.7% and 80.2% vs 68.1%, respectively), but not significantly different from non-responders.
Conclusion: Sequential FDG-PET(CT) is a useful method to evaluate response of rectal cancer to neo-adjuvant RT. ΔSUVmax and ΔSUVmean are parameters that can be used as indices of metabolic response.

PP20

Clinical significance of the epidermal growth factor receptors (EGFR and HER-2) overexpression in esophageal squamous cell cancer

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Background: The present study was aimed to assess EGFR and HER-2 expression in esophageal squamous cell carcinoma (ESCC) and to correlate the results with clinic-pathological findings and prognosis.
Materials and Methods: The immunohistochemical staining of EGFR and HER-2 receptors were retrospectively investigated in biopsy specimens from 31 patients with T (2−3), N-any, and M-0 ESCC. The results were classified according to the Herceptest criteria (Dako): negative (0/1+) and positive (2+/3+).
Results: The EGFR expression was negative in 7/31 (22.6%) and positive in 24/31 (77.4%), of which 12 (38.7%) were 2+ and 12 (38.7%) were 3+.

EGFR diffuse strong positivity (2+/3+) was significantly related to vascular invasion ($p = 0.038$) in a subgroup of ESCC patients. Statistical trend towards poor outcome was observed in ESCC patients overexpressing EGFR (3+). The HER-2 expression was negative in 14/31 (45.2%) and positive in 17/31 (54.8%), of which 12 (38.7%) were 2+ and 5 (16.1%) were 3+. No significant associations were found among protein expression and clinic-pathological data. Our results revealed a high rate of HER-2 overexpression in the group of ESCC patient with poor disease outcome ($p = 0.019$).
Conclusion: Our data demonstrate the great potential prognostic interest of evaluation EGFR and HER-2 overexpression in ESCC. Protein overexpression of HER-2 (3+) is an indicator of poor prognosis in ESCC patients, although the results should be confirmed in a larger series.

PP73

Differential staining of SPARC across 3 different tumor types treated with nab-paclitaxel

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Background: Overexpression of albumin-binding protein SPARC (Secreted Protein Acidic and Rich in Cysteine) in tumors is generally associated with poor prognosis. SPARC expression in the tumor is complex with many SPARC-expressing components including stroma, fibroblasts, tumor cells, inflammatory cells, normal tissues, nerve, and blood vessels. To define the component responsible for SPARC being a negative prognostic factor, we conducted a comprehensive analysis of SPARC expression across 3 tumor types (breast, melanoma, and pancreas).
Materials and Methods: A series of antibodies were evaluated against SPARC. SPARC IHC was performed in a CLIA approved central laboratory using 2 antibodies with different affinities against SPARC. Detailed pathological evaluation was performed by a board certified pathologist. Score was assigned on scale of 0−3, with 3 being positive. Breakdown of the various components was performed to include: tumor, blood vessels, fibroblast, acellular stroma, inflammatory cells, and normal anatomy. Clinical samples for these analyses came from three clinical trials with nab-paclitaxel: 1) metastatic pancreatic cancer, 2) unresectable stage IV melanoma, and 3) neoadjuvant breast cancer.
Results: Two epitopes were defined during the evaluation of all anti-SPARC antibodies, with one preferentially expressed on fibroblasts (antibody 1) and one preferentially expressed on tumor cells (antibody 2). The profile of SPARC staining was distinct for each tumor type. For pancreatic cancer, SPARC positive staining by antibody 1 and antibody 2 respectively was 10/36 vs 7/36 for tumor cells, and 18/29 vs 5/29 for fibroblasts. For melanoma, SPARC positive staining by antibody 1 and antibody 2 respectively was 30/41 vs 20/41 for tumor cells, and 19/33 vs 14/33 for fibroblasts. For breast cancer, SPARC positive staining by antibody 1 and antibody 2 respectively was 22/76 vs 27/76 for tumor cells, and 60/77 vs 20/77 for fibroblasts. This same epitope on fibroblasts was found on blood vessel endothelial cells. Preliminary data from 3 clinical trials including pancreatic, melanoma, and neoadjuvant breast cancer suggest that positive SPARC expression may correlate with response to nab-paclitaxel.
Conclusion: SPARC expression profiles across the various components in patient tumors were examined for 3 tumor types: pancreas, breast, and melanoma. The distinctive SPARC expression profiles suggest that the role of SPARC in each tumor type may be contextually different.

PP48

A proliferation measure integrates the outcome-related information contained in the breast cancer transcriptome

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Background: A number of completely distinct gene expression signatures predict disease-free survival in breast cancer patients. However, the biological variables underlying these signatures remain unclear.
Materials and Methods: We established a signature, called 'super PCNA', composed of genes whose expression follows closely that of proliferation marker PCNA in a compendium of gene expression in normal tissues. We then proposed a method to adjust any microarray data set for the signal embedded in the super PCNA signature. This decovolution procedure removes proliferation-related signals without excluding proliferation genes from the data sets. Next, the prognostic abilities of 32 signatures published in the literature and of 10,000 randomly generated signatures were evaluated in the original and in the super PCNA-deconvolved versions of three breast cancer data sets of 295, 380, and 412 patients, respectively.
Results: Although most published signatures were significant predictors of disease-free survival, 36−64% were not significantly better predictors than random signatures in the original data sets. Deconvolving the proliferation-associated signals out of the data drastically reduced or completely cancelled the predictive abilities of both literature and random signatures. By contrast, substituting PCNA by unrelated genes in the deconvolution process had limited influence on predictors' significance.
Conclusion: Because programs related to proliferation affect ubiquitously the breast cancer transcriptome, most signatures − biologically motivated or random − assess the same proliferation-associated phenotypes and are therefore significant, but equivalent predictors. The study suggests new evaluation standards for cancer outcome predictors.

PP26

Immune response to gastrin-17 is an independent covariate for improved survival in gastrointestinal cancers

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Background: The trophic activity of gastrin has generated significant interest in gastrin as a potential growth factor for tumors arising within the gastrointestinal tract. Polyclonal Antibody Stimulator (PAS), is a novel immune stimulator that elicits antibodies that neutralize and block the proliferative activity of gastrin-17 (G17) and its precursor, glycine extended G17 (gly-G17). Early research with PAS suggested a clinical benefit in patients who mounted an immune response. Data from over 1200 patients with pancreatic, gastric, and colorectal (CRC) cancers were analyzed to define the relationship between immune response and efficacy and to determine the dependence of this effect on several baseline characteristics related to patients' health status.
Materials and Methods: PAS was administered intramuscularly as a monotherapy or in combination therapy as three initial doses, with a booster in some studies. PAS responders were defined by ELISA. The relationships between demographics and baseline disease characteristics and immune response and between immune response and survival were analyzed.
Results: In these studies, PAS responders varied between 52 and 89%. In Stage II−IV pancreatic responder patients, median survival (MS) was 176d and 63d for non responders ($p < 0.002$, log rank). Stage IV pancreatic responder patients had higher MS compared with non-responders (167d vs 104d). Similarly, Stage I−III pancreatic responders had higher MS (179d vs 146d in non-responders). For advanced gastric responder patients who received PAS with cisplatin and 5-FU, MS was 303d compared to 70d for non-responders ($p < 0.001$, log-rank). In Stage IV CRC with PAS alone, PAS responder patients showed better survival (267d) than non-responders (192d). In metastatic CRC responder patients who received PAS with irinotecan, MS was 249d versus 119d for non-responders ($p < 0.001$, log rank). Additional analysis showed that this immune responder survival correlation was independent of any covariates.
Conclusion: Overall, patients who generated antibodies to PAS had a significantly prolonged survival rate compared to those who did not. This effect was independent of various covariates that predicted the health status of the patients at baseline. The survival benefit for antibody responders and the favorable safety profile, indicate that PAS has exciting prospects for an improved anti cancer treatment for various GI cancers.

PP11

Expression differences of proteolytic factors uPA, PAI-1, and seven kallikrein-related peptidases (KLK5, 6, 7, 8, 10, 11, 13) between primary tumor and omentum metastasis impact outcome in advanced ovarian cancer

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Background: Primary tumor levels of serine proteases of the KLK family (kallikrein-related peptidases), as well as the serine protease uPA (urokinase-type plasminogen activator) and its inhibitor PAI-1, are related to disease course in ovarian cancer. Level differentials of these factors between primary and tumor omentum metastasis could thus be associated with the aggressiveness of metastastic processes typical for ovarian cancer.
Materials and Methods: Protein levels of uPA, PAI-1, and seven tissue kallikrein-related peptidases (KLK5, 6, 7, 8, 10, 11, 13) were determined in extracts of primary tumor tissues and corresponding omentum metastases of 54 FIGO stage III/IV ovarian cancer patients. Following radical surgery, 31/54 patients had minimal residual tumor (<10 mm), of whom 18 were optimally debulked (0 mm). Median follow-up in patients still alive at time of analysis was 24.5 months. All patients received postoperative platinum-containing chemotherapy.
Results: Collectively speaking, moderate correlations in protein levels between primary tumor tissues and omentum metastases were seen for