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

all proteolytic factors except uPA, PAI-1 and KLK13; there were substantial upward trends in uPA, PAI-1, and KLK5 from primary tumor to omentum. In univariate analysis, larger level differentials of uPA, KLK5, 6, 7, 10 were significantly associated with disease progression in the cohort as a whole, as was residual tumor mass. Higher level differentials of uPA, KLK5, 6, 7, 8, 10, 11 were strongly associated with residual tumor mass >10 mm. In the subcohort of patients with residual tumor mass <10 mm, level differentials of KLK5, 6, 7, 8, 10, 11 had a significant impact on tumor progression, whereas those of uPA, PAI-1, and KLK13 did not. Hence, the observed impact of level differentials in KLK5, 6, 7, 10 on tumor progression was not simply attributable to their association with surgical success. Positive level differentials of uPA and KLK10 were also associated with poorer overall survival, as was presence of residual tumor mass. No significant association of level differentials of the proteolytic factors assessed with response to first-line chemotherapy was observed.
Conclusion: Since protein level differentials between primary tumor and omentum metastasis of the assessed proteolytic factors measured at initial presentation impact both surgical outcome and survival in advanced ovarian cancer, these measurements could support clinical decisions on surgical and systemic therapy or help in patient selection for novel targeted therapies.

PP29

Tissue factor expression in colorectal cancer: a surrogate for KRAS and p53?

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Background: Tissue factor (TF), a glycoprotein involved in physiologic and cancer-related thrombosis, has been shown to be associated with mutated (mt) KRAS in colorectal cancer (our unpublished data). Interestingly, recent data indicate that p53 mutations confer sensitivity to EGFR-inhibitors in metastatic colorectal cancer. In the present study we address the question of a potential relation of TF expression to the mutational status of both KRAS and p53. Since overexpression of amphiregulin and epiregulin, both EGFR-ligands, are known to be associated with sensitivity to EGFR inhibitors, we were also analyzing whether these ligands might modulate TF.
Materials and Methods: An established microarray database (AFFYMETRIX; U133) of colorectal cancer genotyped for KRAS, BRAF and p53 was analyzed for expression of TF, amphiregulin (AREG) and epiregulin (EREG). Expression analysis was correlated to the underlying mutation of the tumor sample using a two-sample t-test. Correlation between expression levels of two genes was evaluated using Pearson correlation.
Results: A total of 161 primary colorectal cancer cases were analyzed. 93 cases were wild-KRAS, 52 mutated-KRAS and 17 mutated-BRAF; p53 status was available in 121 tumors (43 wt-p53; 78 mut-p53). Tumor samples with the genotype mt-KRAS/wt-p53 ($n = 10$) had higher levels of TF than those with wt-KRAS/mt-p53 ($n = 56$) ($p = 0.008$). TF expression between mt-KRAS/mt-p53 ($n = 22$) and wt-KRAS/wt-p53 ($n = 33$) was not significantly different. When comparing TF expression between wt-KRAS/mut-p53 with wt-KRAS/wt-p53, TF expression was trending towards lower levels in wt-KRAS/mut-p53 ($p = 0.284$). Studying expression of AREG as well as EREG between wt-KRAS and mt-KRAS patients, AREG and EREG were significantly downregulated in mt-KRAS ($p = 0.001$). Correlation of TF expression to AREG and EREG expression restricted to samples with wt-KRAS showed an inverse association with EREG ($p = 0.023$) and AREG(215564_at) ($p = 0.039$), but not AREG(205239_at) ($p = 0.42$).
Conclusion: We report a stepwise increase of Tissue Factor in colorectal tumors with lowest TF levels in wt-KRAS/mt-p53 and highest levels in mt-KRAS/wt-p53; no direct correlation with EGFR-ligands AREG and EREG was observed. Immunohistochemical studies to evaluate the role of TF as surrogate marker for KRAS/p53 status are ongoing.

PP53

Hakai − a novel tissue based tumour progression marker

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Background: The transition from adenoma to carcinoma has been linked to the disruption of cell-cell contacts and with the loss of E-cadherin, a membrane protein with a critical function in the formation of adherens junctions. Although many proteins are involved in the establishment of cell-cell contacts, E-cadherin is perhaps the most important protein for the formation of tight, compact intercellular adhesions. E-cadherin is regarded as a tumour suppressor gene and its loss as a predictor of poor prognosis in colon cancer.
In 2002, Hakai was identified as a novel E3 ubiquitin-ligase, related to Cbl-family ubiquitin ligases, that acts on the E-cadherin complex and mediates its ubiquitination, endocytosis and degradation via lysosomes, thereby altering cell-cell adhesions.
The loss of E-cadherin at the surface of cancerous cells correlates with the process of invasion and metastasis. The excessive internalization or degradation of E-cadherin, such as when Hakai is over-expressed, may achieve similar results, raising the possibility that Hakai may participate in tumour progression and metastasis. Indeed, Hakai can regulate the proliferation of cell cultured cells in an E-cadherin-independent manner. Moreover, Hakai induces anchorage-independent cell growth, further underscoring its oncogenic potential and preliminary results show that the expression is often enhanced in human colon and gastric adenocarcinomas. The main objective of this study is to determine the potential of Hakai as a tumour progression marker in human colon tissues.
Materials and Methods: We have analyzed the expression of Hakai in different normal human and colon cancer tissues by immunohistochemistry using Hakai (2498) antibody. We have analyzed 15 normal human mucosa and the same number of grade I, grade II and grade III of tumour differentiation stage. We have correlated the tumour differentiation stage with the expression level of Hakai protein by immunohistochemistry
Results: Preliminary results indicate that there is an enhanced expression of protein level in tumour samples than in normal mucosa. We have obtained more than 3-folds increase of enrichment of Hakai protein in tumour tissues versus normal mucosa analyzed. We haven't found any significative correlation between every differentiation stage and Hakai expression level.
Conclusion: These results suggest that Hakai can be used as a novel tumour marker in colon tissues and the overexpression detected in tumour samples is not related to tumour differentiation stage.

PP119

VEGF polymorphisms as predictors of bevacizumab efficacy in metastatic colorectal cancer

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Background: During cancer processes, VEGF sustains cancer neoangiogenesis and is largely released by normal cells under cancer mediator stimulation. The anti-VEGF antibody bevacizumab (B) has demonstrated relevant efficacy in metastatic colorectal cancer patients (MCRC pts) when given in combination with the chemotherapeutic regimen FOLFIRI (F). The aim of this study was to evaluate potential influence of germline VEGF gene polymorphisms (VGPs) on B efficacy.
Materials and Methods: 55 MCRC pts (40 treated with F+B, 15 with F alone; M:F 31:24, median [m] age 62 years) with available blood sample for genotyping entered the study between November 2005 and July 2008. 9 VGPs (7 of which single nucleotide polymorphisms) within the 5′UTR/promoter region spanning from nt −2713 to nt −585 were evaluated. Primary endpoint was median progression free survival (mPFS), secondary endpoints were radiological response rate (RR) and overall survival (OS).
Results: 5 VGPs were strongly linked to each other and, among pts receiving F+B, the 17 pts with the homozygotic haplotype for these 5 VGPs: −2578 C/C, −1512 18base pairs deletion/deletion, −1451 C/C, −1411 repeats 5G/5G, −460 T/T, had significantly longer mPFS compared to the other 23 F+B treated pts, 15.4 v 9.0 months (mo), respectively, HR 0.38, p 0.02. Also the VGPs −152 (G/G v G/A + A/A) and −1154 (G/G v G/A + A/A) were significantly associated with mPFS in F+B pts: 15.4 v 8.9 mo, HR 0.28, p 0.01 and 16 v 9.8 mo, HR 0.43, p 0.03, respectively. OS was not significantly influenced by any of the investigated VGPs in patients receiving F+B. With regard to RR, among all the VGPs only the −634 VGP was significantly associated with response to F+B, RR for G/G vs. G/C + C/C = 64% vs. 14%, Fisher's test $p = 0.03$. All the VGPs did not significantly influence the outcomes of F alone treated patients.
Conclusion: Germline VGPs of the 5′UTR/promoter region may help identify patients more sensitive to anti-VEGF agents.