of nuclear Bag-1 was significantly associated with longer TTR ($p \leqslant 0.001$) and improved OS ($p = 0.001$) in patients treated for adenocarcinoma of the pancreatic head. Moreover, twenty-four percent and 19% of patients with nuclear Bag-1 were recurrence free and alive respectively 5 years following surgery compared with none of the patients lacking expression. In fact, for this tumor type, nuclear Bag-1 proved the only independent prognostic factor for outcome in multivariate analysis after adjustment for conventional prognostic factors, such as tumor extension, nodal involvement and differentiation. In periampullary tumors however Bag-1 failed to demonstrate an association with outcome. This observation is suggestive for different pathways in both types of pancreatic cancer.
Conclusion: The present study shows that patients treated for adenocarcinoma of the pancreatic head, whose tumors fail to express nuclear Bag-1, are more likely to develop recurrent disease and experience decreased survival than those with tumors expressing this biomarker. Nuclear Bag-1 thus seems to hold promise as a prognostic marker in this type of pancreatic cancer and could provide new leads in therapy.

6652 POSTER

Comparison of fluorescence in situ hybridization and dual colour chromogenic in situ hybridization for the assessment of HER2 status on gastric cancer biopsies

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Background: HER2 over-expression and/or amplification are present in 9−38% of gastric or gastro-oesophageal junction (GEJ) cancers and correlate with poor outcome and more aggressive disease. It is well known that immunohistochemistry can give conflictive results, especially on small histological samples. Therefore, it has been recently proposed in breast carcinomas that up-front *in situ* hybridization may be the best option for assessing HER2 status. The aim of this study is to evaluate the concordance between *HER2* gene amplification determined by fluorescence *in situ* hybridization (FISH) and a new dual colour cromogenic *in situ* hybridization (CISH) in a series of gastric cancer patients. The results of a pilot study are presented herein.
Material and Methods: 30 gastric adenocarcinoma diagnosed by either endoscopically or surgically obtained biopsies were selected from our files. Dual colour FISH (Dako, Glostrup) and dual colour CISH (Dako, Glostrup) were performed in each case. Scoring of the FISH and CISH slides was identical, counting *HER*2 and CEN-17 signals from 30 tumour nuclei per case. All cases were evaluated in a blinded manner by 2 different physicians. Finally, the gene to CEN-17 ratio was calculated using the cut-off value of *HER*2/CEN-17 ratio >2 as amplified.
Results: All 30 specimens were analyzed successfully by CISH and FISH. A high concordance was found between FISH and CISH in the assessment of HER2 status. 9 cases were amplified and counted easily with both techniques, showing similar ratios. No Polysomy was detected with any technique in these 30 cases.
Discussion: Given the previous experience with the quality of HER2 testing in breast carcinoma in situ hibridation may be an accurate alternative for HER2 testing in gastric carcinomas. CISH allows for a better concurrent analysis of morphology, which is particularly important when studying small samples. A final report on >100 samples will be available at presentation.

6653 POSTER

Exploratory study of the subcutaneous fat gene expression profile in patients with metastatic pancreatic carcinoma treated with standard gemcitabine chemotherapy regimen

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Background: Most clinical trials are designed to assess the antitumor effect of the chemoterapeutic intervention. There are few examples where the endpoint is to assess the biology of the host response to the treatment of the tumor. A large number of patients with pancreatic cancer present features of the cachexia syndrome and specially a marked weight loss. It has been postulated that a "cytokine storm" is the cause of the profound effect that this cancer has on distant tissues. This trial analyzed changes in the subcutaneous fat gene expression profile in relation with the clinical benefit variable with standard gemcitabine (G) treatment.
Methods: Patients with histology confirmed advanced pancreatic cancer, adequate organ function and written informed consent. Eligible pts were intended for a subcutaneous fat biopsy pretreatment and after 7 weeks of gemcitabine 1000 mg/m^2 together with response assessment. Clinical benefit (CB) (pain, analgesic consumption, Karnofsky and weight), QLQ-C30, serum cytokines and tumor markers were evaluated pretreatment, at 4 and 8 weeks. Fat gene expression profile was analyzed using Affimetrix U133Plus2.0 with the corresponding bioinformatic software. Serum cytokines where analyzed with xMAP technology with the Luminex 200 platform.
Results: 16 pts [8 m, 8 f, median age 62 yrs (range 47−72)]. Median weight change −0.75 kg (range −4.5 to 2). Nine pts had pre and post treatment biopsies and 7 only pretreatment. Three pts achieved CB at 8 weeks. Objective responses: 0 CR, 0 PR, 31% SD and 68%PD. Toxicity was similar to the one reported in gemcitabine's label. It was possible to extract quality RNA for microarray from subcutaneous fat use from all samples but 1. The limited number of samples precluded to obtain genes clearly involved in cachexia, however the IL-8 expression ($p = 0.03$) was significantly correlated with CB response either to gene and serum profile.
Conclusions: It is feasible to study prospectively the impact of cancer treatment on different tissue biomarkers and correlated with standard antitumor evaluation system. The reduced number of samples in this exploratory trial precludes producing significant biological conclusions.

6654 POSTER

Immune response to gastrin-17 is an independent covariate for survival in colorectal, gastric and pancreatic cancers

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Many gastrointestinal (GI) cancers are sensitive to the mitogenic effects of autocrine/endocrine gastrin-17 (G17). The novel autologous immune stimulator, Polyclonal Antibody Stimulator (PAS), elicits antibodies that neutralize G17, thereby blocking its proliferative activity. Early research suggested clinical benefit for patients who mounted an immune response. We analyzed the data from more than 1200 patients from 5 monotherapy and combination chemotherapy studies in three GI cancers to define the relationship between immune-response and clinical efficacy and determine the dependence of this effect to baseline characteristics related to patient's health status.
PAS immune responders were defined by enzyme-linked immunosorbent assay. Relationships between demographics and baseline disease characteristics and immune response were examined by using a logistic regression analysis; relationships between immune response and survival were analyzed using Cox regression analysis.
In Stage II-IV pancreatic cancer patients, overall median survival (MS) was 111 days; MS was 176 days for immune responders and 63 days for non-responders; patients who received placebo had MS of 83 days ($p = 0.028$, log-rank). Stage IV pancreatic responder patients had higher MS (167 days versus 104 days). Similarly, Stage I-III pancreatic responders had higher MS (179 days versus 146 days in non-responders). For advanced gastric cancer patients who received PAS in combination with cisplatin and 5-FU, overall MS was 265 days. Those considered anti-G17 immune responders had a MS of 303 days compared to 70 days for non-responders ($p < 0.001$, log-rank). Under monotherapeutic conditions in colorectal studies, patients who were considered responders showed better survival (267 days) than non-responders (192 days). In metastatic colorectal cancer patients who had progressed after an irinotecan-based chemotherapy regimen, overall MS was 227 days; MS of PAS responders was 249 days versus 119 days for non-responders ($p < 0.001$, log rank).
Overall, patients who generated antibodies following immunization with PAS (between 57% and 100% of patients receiving 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 highly favorable safety profile indicate that PAS has exciting prospects for an improved anti cancer treatment regimen for various GI cancers.

6655 POSTER

The emerging role of the novel serum marker GOLPH2 in detecting and monitoring of hepatocellular carcinoma

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In patients with HCC, surveillance strategies during the course of the disease are necessary, especially when testing the efficacy of novel

therapies. Current strategies include imaging techniques and serum markers such as alpha-fetoprotein (AFP). Recently, we discribed the value of GOLPH2, a Golgi-phosphoprotein associated with development and progression of HCC (1).
Using tissue microarrays and immunohistochemistry we semiquantitatively analysed GOLPH2 protein expression in patients with HCC (n = 170), benign liver tumours (n = 22) BDC (n = 114) and normal liver tissue (n = 105). The newly designed sandwich ELISA was used to analyse GOLPH2 levels in the sera of patients with HCC (n = 18), HCV (n = 10), BDC (n = 5) and healthy control persons (n = 12).
GOLPH2 protein is highly expressed in HCC. Significant serum GOLPH2 levels are detectable and quantifiable in the sera of patients by our novel ELISA. In Hepatitis C genotype 1b, serial ELISA measurements in the course of the disease appear to be a promising complementary serum marker in the surveillance of HCC. Meanwhile by expanding our analysis we and others find AFP and sGOLPH2 to be at least equally discriminative in detecting early HCCs and conclude that the complementary use of both markers improves the detection and surveillance of HCCs.

6656 POSTER

Evaluation of sE-Selectin and circulating Vascular Endothelial Growth Factor (VEGF) in patients with advanced gastric or gastroesophageal junction (GEJ) adenocarcinoma treated with cetuximab in combination with cisplatin and docetaxel (Italian phase II DOCETUX Study)

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Background: The Vascular Endothelial growth factor (VEGF) and the adhesion molecule E-selectin expressed on activated endothelial cells are reported to play an important role in tumour angiogenesis. The aim of this study was to evaluate the predictive significance of sE-Selectin and circulating VEGF modifications in advanced gastric or GEJ cancer pts treated with cetuximab in combination with cisplatin and docetaxel as first-line therapy (DOCETUX STUDY).
Materials and Methods: Pts received cetuximab 400 mg/m^2 *iv* followed by 250 mg/m^2 *iv* weekly, cisplatin 75 mg/m^2 *iv* and docetaxel 75 mg/m^2 *iv* d1 every 3 weeks, for a maximum of 6 cycles; cetuximab alone was continued in pts with CR/PR/SD. Anti-tumor activity was assessed by CT-scan every 6 weeks. sE-Selectin and VEGF serum and plasma levels were determined on day 1 (baseline) and on days 4, 8, 22, 43 during treatment. Biomarkers levels were assessed using commercial quantitative sandwich enzyme immunoassays (Quantikine Human VEGF Immunoassay, R&D Systems; Human sE-selectin ELISA, Bender MedSystems) according to the manufacturer's instructions.
Results: Forty-five out of 72 pts (72.6%) enrolled in the DOCETUX Study were evaluated. Pt characteristics were: 33 (73.3%) males, 12 (26.7%) females; primary site: 37 (82.2%) stomach, 8 GEJ (17.8%). Forty-two (93.3%) pts were evaluable for response. The objective responses (RECIST) were: 1 complete response, 16 partial response, 16 stable disease (33 pts with disease control) and 9 disease progression. The median time to progression (TTP) was 4 months. The median basal values of biomarkers were: serum sE-selectin = 30.2 ng/ml; serum VEGF = 461.6 pg/ml; plasma VEGF = 64.3 pg/ml. On the day 22 after start of therapy pts who will have disease control presented higher levels of both biomarkers as compared with those who will present disease progression; mean increase of sE-Selectin +21.0% (p = 0.010), VEGF +44.8% (p = 0.014). The biomarker increase was also significantly correlated with TTP $\geqslant$ 4 months: sE-selectin +16.3% (p = 0.041), and VEGF +50.4% (p = 0.003).
Conclusions: These data suggest that cetuximab in combination with cisplatin/docetaxel chemotherapy regimen may induce a modulation in sE-selectin and VEGF circulating levels. An increase in these biomarkers levels would seem to correlate with treatment activity.

Genitourinary malignancies − Prostate cancer

Oral presentations (Mon, 21 Sep, 11:00−13:00)
Genitourinary malignancies − Prostate cancer

7000 ORAL

A multi-institutional analysis comparing adjuvant and salvage postoperative radiation therapy for prostate cancer patients with undetectable PSA and high-risk features in the prostatectomy specimen

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Background: Two prospective RCT showed that adjuvant radiotherapy (ART) to the prostatic fossa after radical prostatectomy (RP) improves all clinical endpoints compared to an observational policy in patients with extracapsular extension (ECE), seminal vesicle invasion or positive surgical margins (SM). It is unclear whether early salvage radiotherapy (SRT) upon PSA relapse might offer the same ultimate benefit as ART in patients with negative SM. This study aims at comparing ART with early SRT after biochemical failure.
Materials and Methods: Using a multi-institutional database, 233 patients receiving ART or early SRT were identified. None of them received hormonal therapy (neo)adjuvantly and all had an undetectable PSA after RP. In total, 93 patients had early SRT and 140 ART (see table for patient characteristics). The patient group was tested for heterogeneity of patient age and tumour parameters (preoperative PSA, Gleason score, T-stage, ECE, SM, capsular, lymphatic and vascular invasion) and then divided into four homogeneous subgroups based on the status (+/−) of lymphatic invasion (LI) and SM to permit a comparison of ART and SRT. There existed marked heterogeneities in one patient group (SM−/LI−) in favour of SRT (lower T-stages, less ECE and less capsular invasion). bDFS was calculated from the date of surgery and from the end of RT for every subgroup.

	ART (n = 140)	Early SRT (n = 93)
Median PSA preRP (ng/mL)	9.7	8.7
SM +	89 (63.6%)	40 (43.0%)
ECE +	99 (70.7%)	58 (62.4%)
Gleason score > 7	10 (7.1%)	13 (14%)
pT		
pT2	37 (26.4%)	32 (34.4%)
pT3a	90 (64.3%)	49 (52.7%)
pT3b	13 (9.3%)	11 (11.8%)
pT4	0 (0%)	1 (1.1%)
Median PSA preRT (ng/mL)	0	0.3
Median FUP (months)		
From RP	105	122
From end of RT	101	79

Results: In one patient group (SM−/LI+), there were no significant predictors of bDFS, probably due to the small patient number in this group. In the three other patient groups, SRT was a significant predictor of a decreased bDFS from the date of surgery and from the end of RT on Cox regression analysis. This was most striking in the SM−/LI− cohort, despite the more favourable prognostic factors in the SRT group. The only other significant predictor in multivariate analysis was Gleason score >7 (in SM−/LI− and SM+/LI− group).
Conclusions: Immediate ART for prostate cancer with high risk features in the prostatectomy specimen significantly reduces the risk of long-term biochemical progression after RP compared with SRT. Gleason score > 7 was the only other factor on multivariate analysis associated with decreased bDFS.