present with Stage D disease (9% versus 26%, p = 0.0009). The median lymph node yield at surgery was significantly higher for MSI CRC (17.5 versus 14 nodes, p = 0.007). There was trend for a higher median body mass index (BMI) in patients with MSI CRC (28.3kg/m^2 versus 26.6kg/m^2, p = 0.07). Neither smoking nor diabetes were associated with MSI.
Conclusions: In this selected patient cohort we found a higher lymph node yield in association with MSI, and a possible association with increased BMI. We could not confirm the previously reported association between smoking and MSI. These findings should be explored further as they may provide insight into the biology underlying the development of MSI CRC.

1320 POSTER

Potential of heat shock protein 90 expression in the nucleus as a useful parameter for neoadjuvant chemotherapy in gastric cancer

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Purpose: Heat shock protein 90 (Hsp90) is a molecular chaperone that plays crucial roles in cellular responses to stressful conditions. Most studies related to cancer treatment have focused on cytoplasmic Hsp90. However, Hsp90 is also found in the nucleus, albeit at considerably smaller levels (Dote et al. Cancer Res. 2006;66:9211−20). Our previous study showed that Hsp90-negative expression correlated with more aggressive disease and could provide useful prognostic information for gastric cancer patients (Dote et al. AACR2009 abstract #1632). Neoadjuvant chemotherapy with S-1/cisplatin has shown some success in the treatment of gastric carcinoma, but objective parameters for measuring its effects are lacking. In this study, we studied the correlation between Hsp90 expression and the histological chemotherapeutic effect in advanced gastric cancer with S-1/cisplatin neoadjuvant chemotherapy.
Material and Methods: Sixteen primary advanced gastric cancer patients were recruited into the study. Two cycles of continuous oral administration of S-1 (100–120 mg/body/day, 21 days) plus drip infusion of cisplatin (60 mg/m^2/day, Day 8) was performed as neoadjuvant chemotherapy. Histological chemotherapeutic responses of the resected specimens were classified into good responders and poor responders. Hsp90 expression on formalin-fixed paraffin-embedded specimens both before and after neoadjuvant chemotherapy was examined immunohistochemically. Chi-square test and Kaplan-Meier analysis were used for statistical analysis.
Results: High expression level of Hsp90 in cytoplasm (defined as stronger staining compared with adjacent normal gastric mucosa) was found in 7 tumors (44%) in pretreatment biopsy and 8 tumors (50%) in surgically resected specimens. There was no significant correlation between Hsp90 expression and pathological response and survival rates in both biopsy and surgical specimens. Interestingly, in 6 patients with recurrence, Hsp90 expression in the nuclei was observed in 3 surgically resected specimens. However, no significant difference was detectable because of the small number of patients.
Conclusions: Our results suggest that Hsp90 expression in the cytoplasm did not correlated with neoadjuvant chemotherapeutic effect and prognosis for advanced gastric cancer patients. However, the potential of Hsp90 expression in the nuclei as prognostic biomarkers for neoadjuvant chemotherapy warrants further validation.

1321 POSTER

A novel epigenetic biomarker panel for early detection of colorectal cancer and adenomas

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Background: Diagnosis of colorectal tumors at an early resectable stage will significantly reduce colorectal cancer mortality. The presence of cancer-specific DNA methylation in epithelial cells shed into the lumen as well as in tumor-derived free DNA in blood serum makes a non-invasive approach to early detection of cancer possible. Biomarkers used in diagnostics or screening must have an optimal sensitivity and specificity. The purpose of the present study was to investigate a panel of novel epigenetic markers for the detection of CRC and adenomas.
Material and Methods: We used methylation-specific polymerase chain reaction to investigate the promoter methylation status of 14 previously identified candidates in colon cancer cell lines (n = 20). Seven were hypermethylated in >80% and were subjected to quantitative methylation analysis in test sets of CRC, adenomas, and normal mucosa. Findings were verified in validation series.
Results: Five of the candidates, *CNRIP1*, *FBN1*, *INA*, *SNCA*, and *SPG20,* harbored frequent promoter hypermethylation in colorectal carcinomas (66−94%) as well as in adenomas (43−92%). In contrast, methylation was rare among normal mucosa samples (0−7%). By combining all five genes in a biomarker panel and require two or more methylation positives, 93% of the colorectal carcinomas and 87% of the adenomas could be detected, with a specificity of 98%. Both benign and malignant tumors could be detected independent of clinical characteristics, such as tumor stage, location in the colon, microsatellite instability and *BRAF* mutation status, as well as the gender and age of the patient.
Conclusions: The novel epigenetic marker panel identified here demonstrates high and diagnostically promising sensitivity and specificity measurements for colorectal carcinomas as well as adenomas. The findings underline that this biomarker panel will be highly suitable for early detection of colorectal cancer and adenomas.

1322 POSTER

Diagnostic of KRAS gene mutations in colorectal cancer: evaluation of direct sequencing, pyrosequencing and allele specific amplification

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Background: Mutations of the KRAS gene are are predictive of response to anti-EGFR drugs in colorectal cancer. Direct sequencing could be considered as the gold standard to determine single base substitution. Recently, different rapid techniques showing high sensitivity have been developed. The aim of this study is to compare different KRAS mutation detection methods suitable for use in clinical routine.
Materials and Methods: DNA extracted from cell lines with wild type (LNCaP) or mutated KRAS codon 12 sequence (SW620) and from 5 µm paraffin embedded sections of colo-rectal tumors was used to perform detection of KRAS mutations. Three different methods were compared.
(1) Direct sequencing of amplification products were performed in both the forward and reverse directions using automated fluorescence dideoxy sequencing (ABI 3130 genetic analyser). (2) Pyrosequencing was performed by using the KRAS v2.0 assay on the PyroMarkTM Q24 system (Qiagen). After PCR amplication of a DNA segment spanning codons 12 and 13, the "sequencing by synthesis" methodology quantifies mutations in these codons. (3) TheraScreen® KRAS Mutation was the first diagnostic test to obtain CE Mark certification for the detection of KRAS mutations in colorectal cancer. The test use real-time PCR and combine allele specific PCR (Amplification Refractory Mutation System®) with the Scorpions® technology to detect the 7 most common mutations founded in colorectal cancer
Results: Both methods, sequencing and pyrosequencing, were able to detect up to 5% of mutated alleles present in wild type genomic DNA. With the Therascreen test the c.35G>T, p.G12V mutation was reproducibly and unambiguously detected even when the mutated DNA represented 1% of the total DNA in reaction. The concordance rate between direct sequencing and pyrosequencing (n = 89 patients, 57 wild-type and 32 mutated) was 100%. The concordance rate between sequencing and Therascreen (n = 34 patients) was 85%. The discrepancies (n = 5 patients) were due to mutations detected by Therascreen but not by direct sequencing
Conclusions: The three methods used are able to detect mutations present at a rate of, at least, 5% in the sample. Direct sequencing allows an exhaustive detection of the different type of mutations. Pyrosequencing can be used to perform quantitative detection of the mutations. Therascreen test shows the highest sensitivity.

1323 POSTER

mTOR expression in gastrointestinal (GI) tract poorly differentiated endocrine carcinoma (PDEC)

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Background: Neuroendocrine tumours (NETs) arise from the cells of the disseminated neuroendocrine system, which is widely distributed in the body. These are a relatively rare and heterogeneous group of neoplasms characterized by differences in embryologic, biologic, histopathologic aspects and also in their aggressiveness and prognosis. In particular GI tract PDEC are rare tumours accounting 0.1%-1% of all GI malignancies. mTOR signalling pathway has emerged as a promising target for well-differentiated endocrine carcinoma therapy. Because the biologic behaviour

of these tumours, PDEC are usually excluded from clinical trials employing the mTOR inhibitor RAD001 and actually their activity in PDEC is tested only in cell lines.
Objective: We conducted a retrospective analyses of PDEC in a mono-institutional series revised according to WHO classification and validated with immunohistochemistry (IHC) for endocrine markers. We also aimed to testing if mTOR is expressed in the human PDEC.
Methods: Between 1984 until 2007, 640 NETs referred to our Institution for diagnosis, treatment and follow-up, of whom 36 (5.6%) were diagnosed as PDEC (excluding SCLC). The staining for mTOR was optimised employing slides of normal kidney as positive control. To ensure antibody specificity, consecutive sections were incubated in the absence of primary antibody. The immunoreactivity was evaluated on a semiquantitative scale considering the extent (score: 0−4) and the intensity (score: 0−3) of staining. The product was used to obtain an immunostaining score (total score 0−12).
Results: At diagnosis gender distribution was 19 males and 17 females and median age 59 years (range, 17−75). The primary site was: pancreas 12 (34%), colon 6 (19%), lung 6 (19%), unknown 5 (14%), small bowel 4 (11%), others 3 (10%) in particular 30 pts (83%) had Stage IV disease while 6 (17%) underwent surgery. The overall survival was 18 months (range, 4−61+). mTOR expression is maintained at similar levels in 80% of samples, with no relationship with tumour origin, function, proliferation rate valued through MIB-1.
Conclusions: In our series PDEC are more frequent and have a longer survival than in the literature. Our biological findings demonstrate expression of mTOR in human PDEC and support an extended analysis in order to understand the role of mTOR and the real activity of RAD001 in PDEC.
Partially supported by Fondazione Giacinto Facchetti O.N.L.U.S.

1324 POSTER

Detection of circulating tumour cells in locally advanced colorectal cancer: preliminary data

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Background: Circulating tumor cell (CTC) number at baseline and follow-up has been recently shown as an independent prognostic factor in metastatic colorectal cancer. We seek to investigate the prognostic and predictive role of CTCs in rectal cancer patients undergoing neo-adjuvant chemo-radiotherapy (CX-RT) before curative surgery.
Patients and Methods: In a prospective single-institution study, patients with cT3−4 or N+ rectal cancer staged by transrectal ultrasound and/or pelvic MRI and chest-abdomen CT scan, are submitted to capecitabine (825 mg/mq, orally, twice daily continuous) with concomitant radiotherapy (50.4 Gy/fractions to the primary tumor and perirectal nodes), followed by two cycles of capecitabine (1250 mg/mq, orally, tid 14/21 days). Primary endpoint is evaluation of CTCs at baseline (t0), after neoadjuvant therapy and before surgery (t1), after surgery (t2), and at 6-month follow-up (t3) and its correlation with survival parameters. CTCs counts with immunomagnetic separation in 7.5 ml peripheral blood were performed at the time-points mentioned above (CellSearch System, Veridex Inc).
Results: Twenty-six patients (16 male; 10 female; median age: 63±13 yrs; range: 44−83 years) underwent t0 sampling, 8 pts completed CX-RT and therefore underwent t1 and t2 sampling. At baseline (t0) three patients presented 1 CTC (12%), one 2 CTCs (3.5%), one 27 CTCs (3.5%) while in twenty-one (81%) no CTCs were detected. At t1 and t2 none of the eight pts analyzed showed CTCs. No significant correlation between uTNM at baseline and number of CTCs was found; in addition, among uN0 patients only one resulted to have CTCs.
Conclusions: A CTCs count $\geqslant$1 is found in 15% of our patients, but the sample is too small for statistical analysis. However, furtherdata will allow to determine prognostic and predictive significance of CTCs during treatment in this setting.

1325 POSTER

Regulation of Tissue factor (TF) in colorectal cancer: association with KRAS

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Background: Tumor progression in colorectal cancer is determined by genes such as KRAS, BRAF and p53, but the responsible effector pathways involved are incompletely understood. Tissue factor (TF), a glycoprotein involved in hemostasis, is considered a key driver in cancer-related thrombosis and regulates targets such as prothrombin (T) via protease-activated receptor (PAR). *In vitro* studies in colorectal cancer cell lines have shown that mutated KRAS enhances TF expression and concomitant p53 mutations lead to further TF increase. Moreover serum TF level and activity were correlated with KRAS status of colorectal tumors in an animal model. However whether this mechanism is relevant in patients with colorectal cancer is not known.
Material and Methods: Expression of TF, PAR-1 and T were determined with Affymetrix gene (U133A) chip analysis on a microarray database of colorectal cancer genotyped for KRAS, BRAF and p53. Serum and plasma samples will be prospectively collected in patients with liver-confined metastatic colorectal cancer treated within protocols. Expression analysis was correlated to the underlying mutation of the tumor sample using a modified t-test.
Results: Data on 165 primary colorectal cancer (n: 96 wildtype (wt)-KRAS; n = 52 mutated (mut)-KRAS; n = 17 mut-BRAF) was available. Mut-KRAS was significantly correlated with increase in Tissue factor ($p = 0.027$) and decrease in PAR-1 ($p = 0.037$); prothrombin expression was borderline increased ($p = 0.054$). The information on p53 mutation was only available in 104 primary tumors (34 wt-p53; 70 mut-p53). No increase in TF was observed in patients with mut-p53/wt-KRAS (n = 46); increase in TF was observed when both mut-p53 and mut-KRAS (n = 24) were jointly present but corresponded to the level of TF with k-ras mutation alone.
Conclusion: The correlation of TF and PAR-1 with mutated KRAS may be a new effector pathway of KRAS in humans which could serve as new diagnostic test as well as a new target for future drug development. Correlative studies as well as serum analysis of TF are ongoing to better characterize this association.

1326 POSTER

Immune-regulatory (FoxP3+)-T-cell tumor infiltration status is predictive of benefit from chemo-immunotherapy with gemcitabine, oxaliplatin, 5-FU/FA plus GM-CSF and aldesleukine (GOLFIG) in metastatic colon cancer patients

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Background: GOLFIG is a novel chemo-immunotherapy regimen, combining gemcitabine, oxaliplatin, 5-FU/FA with immunoadjuvant GM-CSF and aldesleukine, which resulted safe and very active in colon cancer patients. Anti-tumor activity and immunity feedback to the treatment resulted strictly correlated. The best outcome was observed in patients showing autoimmunity signs, rise in central-memory-T cells, and decline in peripheral and tumor infiltrating immuno-regulatory T (T_{reg}) cells. On these bases, we investigated a possible correlation between T_{reg} tumor infiltration at diagnosis and clinical outcome of these patients.
Methods: An immunohistochemistry study was carried out to quantify the infiltration of T_{reg} ($FoxP3^+$) lymphocytes in tumor samples of 41 colon cancer patients who received FOLFOX-4 chemotherapy or GOLFIG chemo-immunotherapy as enrolled in the ongoing phase III GOLFIG-2 trial. T_{reg} tumor infiltration score (range 0 to 5) was then correlated with survival (OS) and time to progression (TTP).
Results: A higher T_{reg} tumor infiltration score (score 3−5) was associated to a longer OS and TTP in the whole patient population (high vs low score; TTP = 18 vs 9.4 months; $P = 0.002$; OS = 55.7 vs 28.9 months; $P = 0.001$),