

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<sup>2</sup> versus 26.6kg/m<sup>2</sup>,  $p=0.07$ ). 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.

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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<sup>2</sup>/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.

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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.

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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 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 PyroMark™ Q24 system (Qiagen). After PCR amplification 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.

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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