We are sure that type of mutation significantly influenced GIST prognosis and mutations should be analyzed before adjuvant therapy.

6607 POSTER
Description of Mutation in CNR1 Gene and VEGF Expression in Esophageal Cancer

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Aims and Background: Cannabinoid receptors have an impact on gastrointestinal function, but it remains unknown whether mutations may affect tumour susceptibility in patients with esophageal carcinoma. The aim of this study was to determine mutation in the cannabinoid receptor-1 (CNR1) gene and its relation to vascular endothelial growth factor (VEGF) expression as an angiogenic and poor prognostic factor.
Methods: 179 esophageal tissue samples from 69 patients (29 with esophageal cancer and 40 controls) were studied. CNR1 gene mutation (1359 G $\rightarrow$ A in codon 453) was detected with PCR, using the MspI restriction enzyme. VEGF was determined by immunoassay.
Results: Genotyping in control patients' samples revealed that 24/40 were G/G wild type and 16/40 were G/A; no samples were A/A. Of the 139 tissue samples from the 29 esophageal cancer patients, 15 were G/G homozygous, 85 G/A heterozygous, 11 had an A/A genotype and 28 were without amplification. In the normal tissue adjacent to tumour, some mutations were observed. The overall survival time was reduced in patients with the A/A type in all their 5 samples, in comparison to G/G type (P = 0.04, chi-square: 4.26). VEGF expression was higher in tumour than nontumour areas (P < 0.025). VEGF expression was not correlated with survival time.
Conclusions: Our preliminary findings in esophageal tissue showed a high frequency of G $\rightarrow$ A mutation in the CNR1 gene. No correlation between VEGF expression and gene receptor mutation was found. Patients with mutation in all their samples had a reduced survival time.

6608 POSTER
Bax and p53 as Outcome Predictive in Metastatic Gastric Cancer (MGC) Patients Treated With First-Line COI (Capecitabine, Oxaliplatin, and Irinotecan) Regimen

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Background: Bax plays a central role in apoptosis signalling and might be a chemosensitivity biomarker. P53 is a gene regulator of Bax, but its significativity as independent biomarker is controversial. Poorer survival for localised tumours harbouring double alteration of Bax and p53 was previously reported, although only Bax was an independent prognosticator (Mrózek, 2003). The aim of this retrospective study was to investigate the predictive/prognostic value of Bax/p53 in mGC pts homogeneously treated with a oxaliplatin-containing regimen.
Methods: First-line treatment with COI (capecitabine 1000 mg/m^2 twice daily d2−6; oxaliplatin 85 mg/m^2 d2; irinotecan 180 mg/m^2 d1; biweekly schedule) was administered to a consecutive series of mGC pts for up to 8 cycles, or until progressive disease (PD)/unacceptable toxicity. Performance status (PS) $\leqslant$1: eligibility criteria to triplet chemotherapy. Tissue blocks available for 23 pts who provided written consent. Bax/p53 expression assessed by immunohistochemistry, with dicotomic discrimination. Association of both biomarkers with RECIST response by two tailed Fisher's exact test. Correlation of Bax/p53 and PS with progression-free (PFS) and overall (OS) survival by univariate and multivariate Cox's proportional hazard model.
Results: Two pts not evaluable by RECIST criteria. Overall, 71% response rate (15/21, 11 PR/4 CR). Bax-positive was documented in 74% (17/23) samples and negative in 26% (6/23); p53 negative in 61% (14/23) and overexpressed in 39% (9/23). Response rate was 87% (13/15) in Bax-positive and 33% (2/6) in BAX-negative (p = 0.03). By Cox univariate analysis, Bax negative tumours showed a statistically significant shorter PFS (3.9 vs 7.4 mos; HR = 3.40, CI 1.17–9.93; p = 0.02) and OS (p = 0.04). In multivariate analysis for Bax and PS, Bax-negative tumours showed a significantly higher risk for progression (HR 4.51, CI 1.30–15.6; p = 0.02) and death (HR 6.69, CI 1.30–15.6; p = 0.01); and sub-optimal PS (ECOG 1) was associated with a trend for worst overall survival (p = 0.08). p53 evaluation failed to show any significant correlation with outcome.

Conclusions: In mGC pts selected for good-intermediate PS, Bax expression is associated with higher responses to first-line COI regimen. Bax negative pts showed poorer outcome, while p53 overexpression did not have an impact on disease prognosis. Prospective confirmation of predictive/prognostic role of Bax in mGC treated with specific chemotherapeutic drugs is warranted.

6609 POSTER
RON (MST1R) is a Novel Therapeutic Target for Gastroesophageal Adenocarcinoma

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Background: RON (MST1R) is a member of the MET receptor tyrosine kinase family with a putative role in cancer, and we have recently described its expression and function in human tissues and in vitro in gastroesophageal cancer (GEC) (AACR 2011, LB-124, Catenacci et al; in press); here we further describe the role of RON expression and therapeutic potential in a murine model, with focus on RON and MET signaling synergy and redundancy as a mechanism of resistance to individual receptor inhibition.
Methods: To confirm our in vitro findings, we assessed the function of RON in a GEC cell line, AGS, in a subcutaneous nude mouse model. GEC cell line growth inhibition was evaluated using RON specific novel monoclonal blocking antibodies, small molecule tyrosine kinase inhibitors and a RON shRNA AGS line. We used immunohistochemistry (IHC), immunoblotting (IB), and fluorescence in situ hybridization (FISH) to evaluate RON and MET expression, activation, and copy number in harvested treated tumours and controls to evaluate for mechanisms of resistance. We assessed RON and/or MET inhibition in order to evaluate for inhibitory synergism as a result of RON and MET functional reciprocity and signaling redundancy as we recently described in vitro.
Results: Tumour take rate was significantly inhibited with RON knockdown in the shRON AGS line versus scrambled control (p < 0.01). Those shRON tumours that did take occurred significantly later than control − with some revealing increased MET expression and others through selection of a RON re-expressing clone. shRON tumours were significantly less vascular as assessed grossly and by anti-CD31 IHC. shRON tumour growth rate was significantly less than the scrambled AGS control (p < 0.01). RON and MET simultaneous inhibition with monoclonal antibodies or small molecules resulted in a lower tumour take rate and growth rate than with inhibition of either receptor alone or negative controls.
Conclusions: RON protein knockdown and inhibition with antibodies and small molecules significantly decreased the ability of tumours to take, with less tumour vasculature providing a possible downstream mechanism of action. This suggests a role for neoadjuvant/adjuvant anti-RON treatment of GEC in the peri-operative setting to improve disease-free survival, as well as in the advanced metastatic setting given the observed tumour growth inhibition. RON and MET co-inhibition led to optimal results, confirming our previous in vitro observations. These studies further define RON as an important novel therapeutic target for GEC, and supports continued investigation of its role and development of RON specific inhibitors for this deadly disease.

6610 POSTER
The Significance of the Changing of Serum M30 and M65 Values After Chemotherapy and Relationship Between These Values and Clinicopathological Factors in Patients With Advanced Gastric Cancer

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Background: M30 and M65 are intact forms of cytokeratin 18 and, they release during apoptotic cell death from the epithelial cells. In some studies, prognostic importance and predictive significance to detect response to chemotherapy of M30 and M65 values have been reported. In the present study, we aimed to determine the changing of serum M30 and M65 values after chemotherapy and the impact of these values on treatment response and progression-free (PFS) and overall survival (OS) of patients with advanced gastric cancer.
Material and Methods: A total of thirty-one patients with advanced gastric cancer were included. M30 and M65 values were measured by quantitative ELISA method in serum samples before and 48 hours after first chemotherapy cycle. Pre- and postchemotherapy values of M30 and

M65 were compared with Wilcoxon test. Means of the difference between M30 and M65 values before and after chemotherapy were calculated then prognostic significance of these increments for survival was evaluated by univariate and multivariate. Logistic regression analysis was performed to predict response to chemotherapy.

Results: Serum M30 and M65 levels were significantly found to be increased after chemotherapy (M30, pre- 582.7 ± 111.5 U/L vs. postchemotherapy 983.3 ± 214.1 U/L, p = 0.01; M65, pre- 2061.7 ± 431.2 U/L vs. postchemotherapy 2646.3 ± 433.1 U/L, p = 0.003). Means of the difference M30 and M65 levels before and 48 hours after chemotherapy were 400.5 ± 190 U/L [(M30-difference) M30-D)] and 584.6 ± 335.4 U/L (M65-D), respectively. Patients whose serum M30-D $\leqslant 400.5$ U/L had better median PFS and OS times than patients with M30-D >400.5 U/L (PFS, 7 vs. 3.1 months, p = 0.004 and OS, 8.2 vs. 4.1 months, p = 0.002). In addition, median PFS and OS intervals in patients with serum M65-D >584.6 U/L were significantly worse than those of patients whose M65-D was lower than or equal to 584.6 U/L (PFS, 7 vs. 4.7 months, p = 0.004 and OS, 8.2 vs. 4.7 months, p = 0.002). Patients with increased M30-D and M65-D had better tumour response compared to patients with low M30- and M65-D (p = 0.02 and p = 0.006, respectively). In the logistic regression analysis, only M65-D was significantly found to be an independent factor in predicting response to chemotherapy (p = 0.018, OR:1.4). However, prognostic significance of M30 and M65 levels before and after chemotherapy could not be proved in the multivariate analysis.

Conclusions: These results showed for the first time that both M30 and M65 in serum samples of patients with advanced gastric cancer were elevated 48 hours after chemotherapy and these were poor prognostic factor for PFS and OS of patients. Moreover, increased plasma M65 level after chemotherapy can be predict tumour response.

6611   POSTER

MUC4 Expression as a Prognostic Factor in Gastric Cancer − Clinicopathologically Significant Only in the Intestinal Phenotype

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Background: Gastric cancer is histologically classified into two types, intestinal and diffuse type, based on the gland formation tendency. Mucins are high molecular weight glycoproteins that play important roles in carcinogenesis and tumour invasion. Claudins are the proteins that participate in the formation of tight junctions. Tight junction proteins are believed to be involved in the regulation of proliferation, differentiation, and other cellular functions. Expression of mucin and claudin family in gastric cancers has been studied by many laboratories, but the results are conflicting. Therefore, we investigated the potential of MUC and claudin family to be used as a prognostic marker in gastric cancer according to histologic subtype.

Methods: Three-hundred sixty-five gastric adenocarcinoma patients who underwent surgical resection and had not received any pre- or -post surgery therapy, were selected for this study. Among the 365 gastric cancer samples tested here, 124 (34%) were early gastric cancer, and 241 (66%) were advanced. Intestinal type was 68.7% and diffuse type was 30.7%. We made tissue microarrays with paraffin-embedded formalin fixed blocks of gastric cancer and these microarrays were evaluated for phenotypic expression of MUC1, MUC4, Claudin 1, 3, 4, 5, 7 and 10 using anti-human rabbit or mouse polyclonal antibody. The expression levels were correlated with key clinicopathologic features and patient outcomes.

Results: There was no significant difference of MUC and claudin expression between early and advanced gastric cancer. Gastric cancer patients with increased MUC4 and claudin 10 expression were significantly associated with better overall survival (p = 0.049, 0.012). When we evaluated accoding to the histologic type, only significant in the intestinal type (p = 0.032). High expression of claudin 1 was associated with better disease free survival. In early gastric carcinoma, high expression of claudin 5 was significantly correlated with poor disease free survival (p = 0.045), but in advanced gastric carcinoma, high expression of claudin 5 was not correlated with poor disease free survival. Low expression of claudin 10 was significantly correlated with poor overall survival (p = 0.021).

Conclusion: Our present findings show that the increased expression of MUC4 could be used as a good prognostic marker in intestinal type gastric cancer. Overexpression of claudin-10 is a prognostic indicator of prolonged survival of patients with early and advanced gastric cancer.

6612   POSTER

The Role of Vascular Endothelial Growth Factor (VEGF) and VEGF-receptors Genotyping in Guiding the Metastatic Process in Radically Resected Gastric Cancer Patients

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Background: In radically resected gastric cancer the possibility to predict the site of relapse could be clinically relevant for the selection of post-surgical management. We previously demonstrated that tumour integrins genotyping is involved in determining the metastatic sites. Preclinical studies suggested that tumour angiogenesis may also be crucial for the metastatic process of gastric cancer cells. We then investigated the role of VEGFs and VEGF receptors genotyping in determining either peritoneal carcinosis or hematogenous metastases in radically resected gastric cancer patients.

Patients and Methods: Genotyping for VEGF-A, VEGF-C and VEGFR-1,2,3 was carried out on pT4a radically resected gastric tumours recurring with either peritoneal-only carcinosis or hematogenous metastases. Tumour genotyping for integrins was also performed according to our previous findings.

Results: 101 patients fulfilled the inclusion criteria: 57 with peritoneal carcinomatosis only and 44 with hematogenous spread only. At multivariate analysis, intestinal histology and the AC genotype of rs699947 (VEGFA) showed to independently correlate with hematogenous metastases, whereas diffuse histology and the AA genotype of rs2269772 (ITGA) independently correlated with peritoneal-only diffusion (p = 0.001) (Table 1).

Conclusions: Our results seem to indicate that combining information from genotyping of rs699947 (VEGFA, AC), rs2269772 (ITGA, AA) and tumour histology could allow clinicians to individuate gastric cancer at high risk for recurrence either with peritoneal or hematogenous metastases. The selection tool deriving from this analysis may allow an optimal use of the available treatment strategies in these patients.

Table 1

	GG	GA	AA	ND
rs10434 (VEGFA, G > A)				
Peritoneal carcinosis, n (%)	16 (28)	30 (53)	5 (9)	6 (10)
Hematogenous metastases, n (%)	11 (25)	20 (46)	12 (27)	1 (2)
p	n.s.	n.s.	0.0282	
rs699947 (VEGFA, A > C)	AA	AC	CC	ND
Peritoneal carcinosis, n (%)	9 (16)	17 (30)	26 (45)	5 (9)
Hematogenous metastases, n (%)	5 (11)	26 (59)	11 (25)	2 (5)
p	n.s.	0.006	n.s.	
rs7993418 (FLT1, A > G)	AA	AG	GG	ND
Peritoneal carcinosis, n (%)	34 (60)	16 (28)	1 (2)	6 (10)
Hematogenous metastases, n (%)	21 (48)	13 (29)	7 (16)	3 (7)
p	n.s.	n.s.	0.0259	

6613   POSTER

Genetic Polymorphism of Biotransforming Enzymes and Risk of Pancreatic Cancer

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Background: The incidence of pancreatic cancer (PC) in the Czech Republic is highest in the world. Both genetic and enviromental factors may be involved in pancreatic carcinogenesis. Genetic and enviromental factors may also interact, e.g. when polymorphism of biotransforming enzymes is associated with increased susceptibility enviromental chemical mutagens. PC remains an incurable disease in most patients. Preventive strategies aim to identify the population at risk that could be followed more closely in screening programs. In addition, elimination of enviromental risk factors or chemopreventive strategie could be studied in high risk population. The present study was focused on gene polymorphisms in biotransforming enzymes in PC patients.

Materials and Methods: 278 PC patients and 403 healthy controls were studied. Gene polymorphisms of following biotransforming enzymes was investigated: CYP1B1, EPHX, NQO1 GSTP1, GSTT1 and GSTM1. DNA was amplified by PCR, subsequently split by restriction enzymes, and the