bevacizumab. Panitumumab is a fully human monoclonal antibody directed against the epidermal growth factor receptor (EGFR). Study 20050181, a randomized phase 3 trial comparing panitumumab + FOLFIRI with FOLFIRI alone, demonstrated a significant improvement in progression-free survival (PFS) with the addition of panitumumab to second-line treatment of patients with WT *KRAS* mCRC.

Methods: Patients were randomized 1:1 to panitumumab 6.0 mg/kg Q2W + FOLFIRI or FOLFIRI alone. Prior treatments with oxaliplatin or bevacizumab were predefined stratification factors for randomization. Patient eligibility criteria included: $\geqslant$ 18 years old, no prior irinotecan or anti-EGFR therapy, and ECOG $\leqslant$ 2. The co-primary endpoints were PFS and overall survival (OS). This subset analysis reports the efficacy of panitumumab + FOLFIRI versus FOLFIRI alone in WT *KRAS* mCRC patients who had progressed on prior oxaliplatin- or bevacizumab-containing regimens.

Results: In study 20050181, 395 patients with WT *KRAS* mCRC had prior oxaliplatin and 115 patients had prior bevacizumab. 76% (87/115) of patients treated with bevacizumab also received oxaliplatin. Patients with WT *KRAS* mCRC who had received oxaliplatin or bevacizumab in first-line therapy had longer PFS and OS with panitumumab + FOLFIRI versus FOLFIRI alone. Results by prior treatment are shown (table).

Conclusions: Second-line therapy with panitumumab + FOLFIRI may benefit patients with WT *KRAS* mCRC who have progressed on prior oxaliplatin- and/or bevacizumab-containing regimens.

	PFS		Overall Survival	
	Pmab+FOLFIRI (n = 303)	FOLFIRI (n = 294)	Pmab+FOLFIRI (n = 303)	FOLFIRI (n = 294)
Prior ox treatment, n (%)	204 (67)	191 (65)	204 (67)	191 (65)
Median, mos (95% CI)	5.6 (4.6–6.7)	3.7 (3.4–3.9)	14.3 (11.8–15.7)	11.2 (8.9–12.8)
Hazard ratio (95% CI)	0.68 (0.53–0.86)		0.79 (0.63–0.99)	
Descriptive p-value	0.001		0.044	
Prior bev treatment, n (%)	55 (18)	60 (20)	55 (18)	60 (20)
Median, mos (95% CI)	5.8 (5.2–6.7)	3.7 (3.5–5.3)	15.7 (12.6–23.8)	12.5 (9.2–16.1)
Hazard ratio (95% CI)	0.71 (0.45–1.13)		0.68 (0.43–1.07)	
Descriptive p-value	0.150		0.093	

pmab = panitumumab; ox = oxaliplatin; bev = bevacizumab; mos = months; CI = confidence interval

6133 POSTER

High Levels of Immature Blood Vessels in Colorectal Tumours and Metastases Correlate With Survival and Are Independent of Oxidative Damage in the Tumour

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Background: Angiogenesis drives cancer growth, tumour progression and metastases. Hypoxic tumours initiate recruitment of their own blood supply and enhance expression of Vascular Endothelial Growth Factor (VEGF). Bevacizumab is a recombinant humanised monoclonal anti-VEGF antibody which prevents VEGF binding to its receptors. It is the first anti-angiogenic treatment licensed and has been used in Ireland since 2004. Bevacizumab improves overall survival in metastatic colorectal cancer patients when combined with cytotoxic chemotherapy. Currently, bevacizumab is indicated as a first line treatment in all metastatic colorectal cancer patients, however only 38−44% of these patients will have a response to treatment. There is no good marker to predict treatment response. Blood vessels mature by the recruitment of pericytes. We hypothesise those blood vessels that lack pericytes will be more susceptible to regression following treatment with bevacizumab.

Materials and Methods: Gross sections from 80 tumours were stained using dual immunofluorescence staining for factor VIII (an endothelial marker) and α-smooth muscle actin (a pericyte marker). Fluorescent microscopy was used and the mean levels of immature and mature blood vessels were scored and correlated with survival using Spearman correlations and multivariate analyses. TMAs were constructed and stained for the markers of oxidative damage; 8oxodg and 4HNE, and the proliferation marker Ki67.

Results: 37 patients were metastatic at diagnosis and 43 were initially Dukes' A, B or C at diagnosis (early stage) and subsequently developed metastases. 10 patients had matched liver metastases. Patients with higher levels of immature blood vessels had longer survival following treatment with bevacizumab (p value = 0.026). This remained significant following multivariate analyses correcting for gender, stage at diagnosis, whether patients received chemotherapy before or after treatment with bevacizumab and whether or not bevacizumab was first line or not. There was no difference in levels of immature blood vessels between primary and liver metastases. Levels of immature blood vessels did not correlate with levels of oxidative damage in the tumour samples.

Conclusion: We have shown for the first time that the maturity levels of blood vessels in tumours significantly correlates to survival following treatment with bevacizumab. There is no difference in levels between primary tumours and metastases and these levels are independent of oxidative damage in the tumour.

6134 POSTER

The Role of Circulating Levels of Hepatocyte Growth Factor and Vascular Endothelial Growth Factor Receptor 2 in Colon Cancer Patients With Liver Metastases Who Have Responded to Neoadjuvant Chemotherapy Plus Bevacizumab

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Background: Hepatocyte growth factor (HGF) is reported to play important role in angiogenesis and in liver regeneration, while Vascular endothelial growth factor receptor 2 (VEGFR2) is more significant for tumour angiogenesis. In patients (pts) with colon cancer (CC) with liver metastases (LM) an increased circulating levels of HSF have been reported. The study aimed to evaluate the interplay between HGF and VEGFR2 levels in pts with LM from CC who have responded to neoadjuvant Bevacizumab and chemotherapy.

Materials and Methods: We examined 40 plasma and serum samples in duplicate from 4 patients with sigmoid CC and synchronous primary unresectable LM using quantitative sandwich enzyme immunoassay technique (R&D System). Blood samples were drawn at baseline, at 2nd and at 4th cycle of neoadjuvant therapy (XELIRI plus Bevacizumab), on day 7th and one month after operation (radical liver resection), and every two cycles thereafter during postoperative treatment. Control group consisted of 4 healthy women. Immunochistochemical (ICH) staining of LM was performed with polyclonal rabbit antibodies to Flk-1/VEGFR2 (Diagnostic BioSystems) and to HGF (Santa Cruz Biotechnology, Inc). Clinical and biochemical parameters of pts were also collected. The scoring system consisted of intensity of staining and % of tumour cells involved.

Results: The difference between mean values of serum and plasma HGF and VEGFR2 levels in colon cancer pts and healthy women was statistically significant. The Pearson's strong correlation was found between plasma and serum HGF ($r = 0.82$, $p < 0.0001$),while plasma and serum VEGFR2 levels correlation was week and did nod reached statistical significance. Positive correlations between plasma and serum HGF and tumour markers CA19-9 and CEA were noticed ($p < 0.05$), while negative moderate correlation between serum VEGFR2 and CEA ($r = 0.43$, $p < 0.017$) was found. Negative correlation between VEGFR2 and HGF levels was also established ($r = 0.50$, $p < 0.001$). ICH evaluation of LM revealed that strong intensive staining of HGF (score 3) corresponded with weak or missing VEGFR2 staining (score 1 or 0).

Conclusion: In responders to neoadjuvant chemotherapy and Bevacizumab, VEGFR2 circulating and tissue levels correlate with angiogenesis suppression and chemotherapy response. HGF levels correlate with clinical course of disease, at the diagnosis the HGF levels corresponded to angiogenesis activation, while thereafter they reflect liver regeneration.

6135 POSTER

Relationship Between ABO and RH Blood Groups and K-Ras Phenotype in Patients With Colorectal Adenocarcinoma

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Background: Colorectal cancers (CRC) are the most third cancer in both women and men and responsible for approximately 10% of all cancers. Also CRC are third most common cause of cancer-related mortality for both sexes. For the year 2008 about 1.2 million cases and 600 thousand deaths to be estimated worldwide. Age, adenomatous polyps, smoking, inflammatory bowel disease and dietary factors are some of the risk factors. Some familial cases of colon cancer identified in the etiology of various genetic factors. Familial adenomatous polyposes (FAP), hereditary non-polyposis colorectal cancer (HNPCC) or Lynch syndrome are hereditary factors. ABO blood group genes are mapped at the chromosome 9, in which the genetic alteration is common in many cancers. Aird and his colleagues reported that the relationship between stomach cancer and the A blood group in 1953. Association between ABO blood groups and cancer of the pancreas was recently described. Such a relationship isn't identified for CRC. K-ras (Kirsten rat sarcoma) is a proto-oncogene which located on chromosome 12 and encoded protein that involved in normal cell proliferation and signal transduction. The K-ras becoming oncogene by

mutations in the DNA sequence of K-ras gene, especially in codon 12 or 13. Nowadays, EGFR targeted therapies, such as cetuximab and panitumumab were used for CRC and K-ras mutation analysis has gained importance. In this study, possible relationship between ABO-Rh blood groups factor and K-ras status was investigated.
Material and Methods: In 94 patients with CRC, blood group and Rh factor were examined. The relationship of blood groups with wild type K-ras status was evaluated and compared with the healthy volunteer donors control group of 22,821 people which admitted to Ankara University Medical School Blood Center at 2010.
Results: Information on ABO blood type and Rh factor were available for 94 patients. Of patients 34% (32 patients) was female and 66% (62 patients) was male. The most of patients had (40.4%) blood group A. Overall, the ABO blood group distribution of the 94 patients with CRC was similar to that of the general population. There wasn't statistically significant difference ($p = 0.83$) between groups (see Table 1).
Conclusion: This study is the first study done on this issue. In our study, we didn't find any relationship between K-ras status and ABO blood group and Rh factor. However further studies with larger number of patients are needed to establish the role of blood groups in this population.

Table 1: The blood group distribution of patients and control group

Blood groups	K-ras WT patients		Control group	
	n	%	n	%
A Rh (+)	33	35.1	8795	38,54
A Rh (−)	5	5.3	1130	4,95
B Rh (+)	13	13.8	3185	13,96
B Rh (−)	2	2.1	425	1,86
AB Rh (+)	3	3.2	1581	6,93
AB Rh (−)	1	1.1	205	0,90
0 Rh (+)	32	34.0	6550	28,70
0 Rh (−)	5	5.3	950	4,16
Total	94	100	22821	100

6136 POSTER

Relatively High Discordant Rate of KRas Mutation Between Primary and Metastatic Sites, and a Different Pattern of KRas Mutational Status According to Metastatic Sites in Korean Patients With Colorectal Cancer

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Background: KRAS mutations predict resistance to cetuximab or panitumumab therapy in patients with metastatic or recurred colorectal cancer (MRCRC). The aim of this study was to determine the concordance rate of KRAS mutational status between primary tumours and related metastases, and to find out the association between KRAS mutational status and clinicopathologic features in Korean patients with MRCRC.
Material and Methods: KRAS mutational status in codon 12, 13, and 61 from formalin-fixed sections of primary tumours and related metastases was analyzed. In addition, the association between KRAS mutational status and clinicopathologic features was evaluated.
Results: Of 128 patients whose primary and matched metastatic tissues were analyzed for the KRAS mutation status, 5 patients were excluded because of a failure in the process of KRAS mutation analysis and 123 patients were finally included in this study. Matched metastatic sites for KRAS analysis were liver (n = 46), lung (n = 27), peritoneum (n = 26), distant lymph node (LN; n = 13), ovary (n = 10) and pancreas (n = 1), respectively. KRAS mutation was observed in 52 (42.3%) of primary tumours, and in 54 (43.9%) of related metastatic sites. Discordance of KRAS status between primary and metastatic sites was observed in 18 patients (14.6%; kappa = 0.702) and KRAS mutation rate considering both primary and metastatic sites was 50.4% (62/123). When the association between KRAS status and initial metastatic sites at the time of diagnosis of stage 4 cancer or recurrence (in the cases of initial stages 1−3) was analyzed, a high percentage of wild type KRAS was observed in patients with initial liver or distant LN metastases compared with patients without liver or LN metastases ($P = 0.001$ in liver; $P = 0.037$ in LN). However, patients with initial lung metastases had a higher rate of KRAS mutation than patients without lung metastasis ($P = 0.002$). Other clinicopathologic features including age, gender, histologic grade, primary tumour site, obstruction or perforation of primary site, and microsatellite instability were not related to the KRAS mutational status.
Conclusions: The discordant rate of KRAS mutation status between primary and metastatic sites was relatively high (14.6%) in Korean MRCRC patients compared with the rate (<10%) previously reported in Western patients. The frequency of KRAS mutation was different according to the initial metastatic or recurred sites.

6137 POSTER

Biological Analysis of Phase II Study Evaluating the Activity of Cetuximab Combined to Oxaliplatin and Fluoropirimidine (TEGAFOX-E) as First Line Treatment in Metastatic Colorectal Cancer (mCRC) Pts by the Italian Trials in Medical Oncology (I.T.M.O.) Group

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Background: There is some evidence that *p53* inactivating mutant (mut) confer oxaliplatin resistance, but improve cetuximab benefit in KRAS wt pts *(Oden-Gangloff, 2009)*. On the other hand, KRAS mut predict cetuximab resistance, but increase oxaliplatin sensitivity in a p53-dependent manner (de Bruijn, 2010). We conducted a prospective Phase II evaluating the activity of the cetuximab plus an oxaliplatin-based regimen as first line treatment in mCRC, and the correlation with predictive biomarkers such as *p53* and *KRAS* status.
Methods: Twenty-eight elderly mCRC pts (age $\geqslant$70 yrs) were enrolled in a multicenter, prospective study and treated with TEGAFOX-E regimen (oxaliplatin 120 mg/m^2 d1, UFT 250 mg/m^2 d1−14, cetuximab 400 mg/m^2 initial dose, then 250 mg/m^2 weekly) up to 8 cycles, followed with one-year maintenance cetuximab, or until progressive disease (PD)/unaccectable toxicity. *KRAS, p53*, *BRAF* and *PI3KCA* mut were successfully analyzed by genomic sequencing in 23 samples. A mutant-enriched PCR was performed for codons 12 and 13 of *K-Ras*.
Result: TEGAFOX-E regimen produced a 44% objective response rate. *KRAS* mut were detected in 12/23 (52%) and *p53* mut in 5/21 (24%) samples. Two samples were not evaluable for *p53* status. Except for one case, *PI3KCA* mut (26%) were always coupled with *KRAS* mut. No statistically significant difference could be found between responders and non responders in terms of *KRAS* or *p53* mut. On the basis of their *KRAS* and *p53* status, the samples (21 cases) were molecularly classified in three groups and associated to response and mPFS. Group 1 (*KRAS* mt/*p53* mt): all 2 pts harbouring double mutation showed PD at 9-weeks, i.e., the first reassessment; Groups 2−3 (*KRAS* mt/*p53* wt or *K-ras* wt/*p53* mt): in this cross-interference groups 7/12 pts (58%) showed disease control [5 PR, 2 SD and 5 PD], with mPFS of 11 weeks; Group 4 (*KRAS* wt/*p53* wt group): in this double drug-sensitivity group 86% (5/7) pts benefited from treatment (5 PR, 1 long-lasting SD and 1 PD), with a mPFS of 44 weeks.
Conclusions: TEGAFOX-E combination displayed promising efficacy in pts with both wt *KRAS* and *p53* tumours and these hypothesis-generating results should be verified in larger, prospective and randomised phase III trials.

6138 POSTER

Prognostic Role of KRas and BRAF Mutation in Colorectal Cancer

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Background: In numerous studies on the development of cancer induced by K-Ras mutation, raf kinase has been shown to be an important signal transducer in the activation of Ras oncogene. To see whether the mutation of K-Ras gene and BRAF gene is associated with colorectal cancer, in colorectal cancer patients, we compared the presence or absence of the mutation of K-Ras gene and BRAF gene with the clinicopathological characteristics of colorectal cancer patients, and examined the effect of the mutations on survival rate.
Material and Methods: DNA was extracted from 162 cases of colorectal cancer tissues of patients performed surgery for colorectal cancer from 2002 to 2007 and the presence or absence of the mutation of K-Ras and BRAF was assessed by the use of the K-Ras and BRAF detection test kits applying PNA PCR claming method developed recently. The presence or absence of the mutation of K-Ras as well as BRAF was compared with various clinicopathological factors. 5-year survival rate was analyzed by the Kaplan–Meier survival analysis.