these were enrolled in registered clinical trials (n = 61). KRAS analysis was successful in 125 cases. Main reasons for no KRAS analysis were clinical deterioration (n = 13), inadequate or insufficient tumour material (n = 6) or other/not specified (n = 4).
All patients but one were Caucasian, most were women (n = 91) and the median age was 68 years (range 26−83). Nineteen patients had KRAS mutant tumours, and all mutations were found in exon 12. The median overall survival was 6.6 months. It was independent of KRAS status (wild type 7.0 months and mutant 8.3 months, p = 0.75). Median survival for patients without KRAS analysis was 2.3 months.
In the 109 patients receiving chemotherapy, 16 (14.7%) had tumours with KRAS mutations.
Conclusion: KRAS mutations were found in 14.7% of a cholangiocarcinoma population eligible for chemotherapy. The number differs from surgical cohorts and this must be taken into account when designing studies testing the influence of KRAS status on treatment effect.

6129 POSTER

Analysis of Surfactant on Primary(COLO-320) and Metastatic(COLO-741) Human Colon Cancer Cells Treated With A-Lactalbumin or Sulindac

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Background: Colon cancer is the second most frequent reason in the cancer-related deaths in the world. During cancer therapy, the correct time and correct medicine is crucial for different patients. In addition, the primary and metastatic colon cancer therapy may also be different because of cancer cell behavior. Our aim is investigating the surfactant has efficiency on the medicine during treatment of cancer cells.
Materials and Methods: Colo-320 and Colo-741 lines were used in this study. The cells were cultured in RPMI-1640 media including %10 FCS, %1 L-glutamine and %1 penicillin-streptomycine. The cells were cultured in 24 wells of tissue culture plate. After subculturing of cells, they were cultured 24 hours. After 24 hours of culture, the cells will be treated with either α-lactalbumin or sulindac or α-lactalbumin+surfactants or sulindac +surfactants. After 48 hours of treatment, culture mediums from all groups were collected for cytotoxicity analysis, the cells from all groups were fixed in %4 paraformaldehyde for 30 minutes for histochemical analysis. Cell cytotoxicity were evaluated with ELISA. Cell death was investigated using TUNNEL assay.
Results: The Colo-320 cells were semi-adhesive cells; the Colo-741 cells were attachment cells. After treatment with sulindac of Colo-320 cells, the number of alive cells was less when compared with other groups. It was also observed that the number cells in Colo-741 cells which were treated with only α-lactalbumin or only sulindac groups had less cell amount than the other groups. We are still evaluating the affect of surfactants during treatments of colon cancer.
Conclusions: Our hypothesis suggests that both primary and metastatic cells will be affected when surfactants during treatment. However, the addition of surfactants during treatment protocols may cause differences in drug interactions with cells. In farther researchs. Simultaneously, Surfactants might be used to treat with different medicines of other cancer types. Researchs are still going on about this issue in our faculty.

6130 POSTER

Survivin Expression in Rectal Cancer During Preoperative Radiochemotherapy and Its Impact on Metastasis and Patients' Survival

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Background: Valid molecular markers need to be implemented in clinical trials to fulfill the demand of a risk-adapted and more individualized multimodal therapy of locally advanced primary rectal cancer. In the present study the expression of the inhibitor-of-apoptosis (IAP) protein Survivin was evaluated in pre-treatment biopsies and corresponding post-treatment resection specimens, and was correlated to histo- pathological tumour characteristics and clinical follow-up.
Material and Methods: 116 patients with stage II/III rectal cancer treated with 5-FU/Oxaliplatin based neoadjuvant radiochemotherapy (RCT) at a single university medical centre within the German Rectal Cancer Trials were investigated. Survivin expression in pre-treatment biopsies and surgical resection specimens were determined by immunohistochemistry by two independent institutions and correlated with histopathologic parameters, tumour recurrences, disease-free and cancer-specific overall survival.
Results: In pre-treatment biopsies, a higher Survivin expression correlated with advanced ypT (p = 0.026) and ypUICC (p = 0.05) stage as well as decreased disease- free survival (p = 0.038) after preoperative RCT. High post-treatment Survivin levels were associated with advanced ypT stage (p = 0.03) and residual lymph node metastases (p = 0.04). Moreover, neoadjuvant RCT resulted in a significant down-regulation of Survivin expression (p < 0.0001). A failure of RCT-induced down-regulation was associated with development of distant metastases (p = 0.0056) and cancer-related death (p = 0.026), and was significantly correlated with disease-free (p = 0.011*/0.02**) and cancer- specific survival (p = 0.0017*/0.01**) in uni* and multivariate** analyses.
Conclusions: Survivin expression in rectal cancer displays a marker with prognostic validity. These results underline the usefulness of Survivin to monitor individual response to RCT in rectal cancer, and encourage anti-Survivin strategies in multimodal rectal cancer therapy within future randomised clinical trials.

6131 POSTER

Evaluation of BRAF Mutational Status in Wild Type (WT) KRAS Metastatic Colon-Rectal Cancer (MCRC) Patients (pts) Treated With Cetuximab (C) − a Single Institution Experience

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Background: KRAS mutations in pts with mCRC have since emerged as the major negative predictor of efficacy in pts receiving anti-EGFR therapies such as C. Nevertheless, the occurrence of KRAS mutation only accounts for approximately 35−45% of nonresponsive pts. Mutations in BRAF have been recently shown to impair responsiveness to these agents, with no response observed in BRAF mutated pts.
Materials and Methods: From 11/2008 to 01/2011, 95 mCRC pts were tested for KRAS mutations and 30 pts resulted KRAS WT, all of them were treated with C+ chemotherapy (CHT). We now retrospectively analyze the clinical outcome of 20 pts according to their BRAF mutational status, in order to evaluate if BRAF mutation influence the clinical outcome. fivepts were excluded from the analysis due to the impossibility to evaluate BRAF status for little DNA. Genomic DNA was extracted from formalin-fixed, paraffin embedded (FFPE) tumour samples using a commercial kit (Invisorb Spin Tissue Mini Kit, Invitek, Berlin, Germany). V600E mutation of the BRAF gene was evaluated through an allele specific multiplex PCR (CRC Kit 2, Experteam, Venezia, Italy). Median age was 69 years (42−81), all pts underwent surgery for colon (16/20, 75%) or rectal (5/20) cancer, 8/20 (40%) received adjuvant CHT, with FOLFOX4 (50%) or De Gramont regimen.
Results: All pts received C with Irinotecan16/20, 80%) or FOLFIRI (4/20) as 2nd or 3rd line treatment. Five pts (25%) resulted as BRAF mutated (BRAF+). No response has been observed among BRAF+ pts, whereas PR or SD was obtained in 6/15 (40%) BRAF− ones. Median TTP was significantly longer in BRAF− vs BRAF+ (12 vs 8 months).
Conclusion: Our single institution experience confirms the recent data about the hypothesis that BRAF mutational status could be a predictive factor for response to anti-EGFR therapy in KRAS WT mCRC pts. Further evaluation of the RAS/RAF pathway or the analysis of polymorphism of the EGFR in BRAF− pts who didn't obtained a response to anti-EGFR therapy is needed to better understand the lack of response in KRAS WT pts.

6132 POSTER

Efficacy of Panitumumab Plus FOLFIRI Versus FOLFIRI Alone in Patients With Wild-Type (WT) KRas Metastatic Colorectal Cancer (mCRC) Treated With Prior Oxaliplatin or Bevacizumab Regimens: Results From 20050181

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Background: First-line treatment regimens for mCRC often contain oxaliplatin and the anti-vascular endothelial growth factor antibody,

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