Results: Characteristics of the patients (pts) were as follows: mean age: 71 years (range: 28−92); sex: 92 male and 102 female; location: 80 right colon and 114 left colon; Follow-up: mean: 9.4 years. Disease free survival: St2A: 77.23%, St3B: 57.89%, St3C: 45.45%.
IHC: 170 cases were positive (87.62%) and were classified as follows: staining was cytoplasmic predominance in 86 cases (44.32%) and 84 (43.29%) with membrane predominance.
Disease free survival according EGFR status was: Negative and Cytoplasmic positivity cases: St2A: 77%, St3B: 73.68%, St3C: 52.9%. Membrane positivity: St2A: 77.5%, St3B: 42.1%, St3C: 37.5%. Only those patients with tumors harboring membrane positivity (intensity 1(+), 2(+) and 3 (+)) had more probability to present metastasis ($p < 0.05$). No significant differences were found between negative and cytoplasmic staining.
Conclusions: EGFR overexpression evaluated as a membrane pattern is related with metastatic development in colonic carcinomas. This worse prognostic is maintained over 9 years after resection, and affects only stage 3.
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6117 POSTER

Circulating endothelial cells (CECs) and FDG-PET for early prediction of response in high-risk locally advanced rectal cancer (HR-LARC) patients (pts) treated with two different schedules of bevacizumab (BEV) in combination with preoperative chemo-radiotherapy (CT-RT)

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Background: Vascular endothelial growth factor (VEGF) has a crucial role in tumor angiogenesis; its inhibition leads to the normalization of tumor vessels, increases tumor oxygenation and drug delivery. However, the clinical benefits of current anti-VEGF treatments have thus far been rather moderate, stimulating interest in developing more effective ways to combine anti-VEGF drugs and CT, and in identification of predictive biomarkers of clinical benefit. We have previously shown that pre-operative oxaliplatin (OXA), raltitrexed (RTX), fluorouracil (5FU), and folinic acid (LFA) during pelvic RT yelded a high rate of complete (TRG1) or subtotal (TRG2) tumor regression in HR-LARC. Therefore, we planned to add BEV, a MoAb against VEGF, to primary CH-RT in two different schedules, to evaluate the relevance of BEV timing during CT and RT. Changes of CECs and glucose metabolism evaluated by flow cytometry and FDG-PET were used as early surrogate markers of tumor response.
Methods: 28 pts (cT4, cN+, cT3$\leqslant$5 cm from the anal verge and/or +ve CRM, M1 resectable/initially unresectable) received 3 biweekly courses of OXA (100 mg/m^2)/RTX (2.5 mg/m^2) on day 1, and 5FU (800 mg/m^2)/LFA 250 mg/m^2) on day 2 during pelvic RT (45 Gy). In schedule A (16 pts) BEV (5 mg/kg) was given biweekly from day −14 for 4 courses, while in schedule B (12 pts) it was given from day −4 for 2 courses. Toxicity was graded with NCI-CTC version3. According to the Simon's two-stage design, assuming a hypothesis of 50% TRG1 (α error = 0.05, β error = 0.20), at least 6/16 TRG1 should be obtained to continue pts accrual.
Results: No death occurred. As in the previous phase II study without BEV, grade 3/4 neutropenia was the most common adverse event with schedule A (7 pts, 44%), but it was lower with schedule B (2 pts, 17%). After the 1st course of CT, a significantly greater reduction of CECs levels (median, −78% *vs* −29%, $p < 0.05$), and of tumor metabolic volume (median, −78% *vs* −50%, $p < 0.05$) was observed with schedule B compared to schedule A. Moreover, we observed different CECs kinetics with schedule B compared with schedule A. So far, all but one pt (because of refusal) in schedule A, and 11 pts in schedule B have proceeded to surgery. In the schedule A, only 2 (12%) pts obtained a TRG1, while the number of TRG1 required by the statistical design has already been reached in schedule B (6 cases, 55%).
Conclusion: Our data suggest the relevance of BEV schedule to optimize the feasibility and efficacy of the combination treatment, and the potential role of CECs and FDG-PET as early predictive markers of tumor response.

6118 POSTER

The prognostic value of tissue inhibitor of metalloproteinases-1 (TIMP-1) in metastatic colorectal cancer treated with third-line cetuximab-irinotecan

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Background: KRAS wildtype (wt) status is essential to the effect of treatment with EGFR inhibitor cetuximab, but still the overall response rate is less than 40%. Consequently the majority of patients will suffer from substantial side effects and no apparent benefit. TIMP-1 is a glycoprotein, which regulates activity of matrix metalloproteinases and may consequently play a prominent role in tumour behaviour. TIMP-1 has shown a promising potential as biomarker in colorectal cancer (CRC), and recent results have demonstrated a relation between TIMP-1 and EGFR. The aim of the present study was to investigate the clinical value of plasma TIMP-1 in patients with KRAS wt metastatic CRC treated with cetuximab and irinotecan.
Materials and Methods: Patients with KRASwt chemotherapy resistant mCRC submitted for third-line therapy with cetuximab (initial 400 mg/m^2 followed by weekly 250 mg/m^2)/irinotecan (350 mg/m^2 q3w) were prospectively included in a biomarker study. Pre-treatment blood samples were collected and plasma TIMP-1 was measured by a validated in-house ELISA assay. Response was classified according to RECIST. Survival data were analysed by the Kaplan-Meier method and log-rank testing.
Results: 51 patients were included and the overall response rate was 35%. The median plasma TIMP-1 level was 282 ng/ml (range 75–948) and used as cut-off level for statistical analysis. There was no correlation between pre-treatment patient characteristics and TIMP-1 levels. However, the median baseline plasma TIMP-1 levels were significantly higher in patients with early progression compared to patients who achieved disease control, 349 ng/ml (233–398 95%CI) and 215 ng/ml (155–289 95%CI), respectively, $p = 0.03$. This difference translated into a longer PFS in patients with low plasma TIMP-1 levels;7.7 months (5.3−9.2 95%CI) compared to 2.8 months (2.3−6.5 95%CI), respectively, ($p = 0.056$). Furthermore, a significantly different median OS of 11.5 months (8.6−17.3 95%CI) versus 5.3 months (3.9−10.4 95%CI) was demonstrated, and the HR was 1.83 (1.02−3.28), p = 0−03.
Conclusion: Despite the obvious limitations of a small sample size the present results suggest that KRASwt patients with a low pre-treatment plasma TIMP-1 level are more likely to benefit from third-line treatment with cetuximab/irinotecan. Further analyses in larger studies are warranted.

6119 POSTER

Triple mutational testing for response to EGFR inhibitor treatment with Cetuximab and Irinotecan in metastatic colorectal cancer

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Background: A major fraction of KRAS wild-type patients are non-responders to EGFR inhibitor treatment. Investigations of supplementary predictive factors are therefore highly relevant. Mutations in the other genes coding for the RAS-RAF-MAPK pathway have been identified and may also determine primary resistance to EGFR inhibition. As supplement to KRAS analysis, BRAF and PIK3CA mutations may account for additional non-responders. A few previously published studies have combined data from patients treated with different EGFR containing regimes and different treatment lines. We investigated the predictive and prognostic value of these mutations in a uniform material of third-line treatment with combination therapy CETIRI.
Materials and Methods: 88 patients with mCRC were prospectively included. All patients were previously exposed to 5FU, irinotecan and oxaliplatin and progressed on treatment. Treatment consisted of CETIRI (irinotecan (350 mg/m^2 q3w) and cetuximab (400 mg/m^2 loading dose followed by weekly 250 mg/m^2)). Response was evaluated according to RECIST. Following tumour DNA purification, mutational analyses were performed on tumour tissue by commercially available KRAS, BRAF and PIK3CA kits. Survival analysis was performed by Kaplan Mayer plot with log rank testing.
Results: A total of 88 patients had DNA available for triple mutational testing. The median number of cycles was 6 (range 1−18), and 21% achieved a partial response. Forty patients (45%) harboured KRAS mutations, 3 (3.4%) BRAF and 14 (16%) PIK3CA. All were non-responders,

whereas the response rate in triple negative patients was 44% ($p < 0.1^{-6}$). KRAS and BRAF mutations were mutually exclusive, but 6 of the 14 PIK3CA mutations were also KRAS mutated. PFS was 7.7 month (6.0−8.7 95%CI) in triple negative patients compared to 2.5 month (2.1−3.5 95%CI) in the group of patients who harboured any mutation. (HR = 0.43, 0.28−0.67 95%CI, p = 0.0001). The triple-negative patients achieved a median overall survival of 10.4 months compared to 4.8 months in patients harbouring any mutation (HR = 0.7, 0.46−1.08, p = 0.1).
Conclusion: Although the low frequency of these mutations implies a need for larger studies, the present results suggest that also BRAF and PIK3CA mutations are significantly associated with clinical resistance to third-line cetuximab/irinotecan in metastatic colorectal cancer. Consequently, these mutations may contribute as additional selection criteria when added to KRAS status.

6120 POSTER

Ursodeoxycholic acid inhibits proliferation of intestinal epithelial cells: role of EGF and ERK pathway

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Introduction: Ursodeoxycholic acid (UDCA) prevents colitis-related colon cancer which potentially could be attributed to enhanced proliferation during tissue regeneration. One of the pathways regulating epithelial cell proliferation is the EGF-MEK-ERK pathway. We investigated therefore the effects of UDCA on the growth of rodent intestinal epithelial cells *in vitro* and *in vivo* in relation to this pathway.
Materials and Methods: Two groups of six C57BL/6J mice were fed with standard diet with and without 0.4% UDCA for 3 weeks. Sections of the colon were stained with antibodies against Ki-67 protein and phosphorylated ERK protein. The normal rat intestinal epithelial cell line IEC-6 was used for *in vitro* experiments. MTT test was performed on cells treated with UDCA (0 to 800 μM for 3 days) with and without EGF addition. Western blots were made to check the effect on ERK phosphorylation. Cells were treated with different concentrations of U0126, an inhibitor of MEK kinase, for 3 days and ERK phosphorylation was monitored.
Results and Discussion: Treatment of IEC-6 cells with EGF (50 ng/ml) significantly increased the cell number (160% of control after 2 days). This increase of proliferation was abrogated in the presence of 400 μM UDCA. After 3 days of treatment with UDCA (600 μM) alone, proliferation decreased by 60%. This inhibition was concomitant with the inhibition of ERK phosphorylation by about 70%. 10 μM U0126 inhibited both proliferation and ERK phosphorylation by about 70%. Feeding UDCA to mice reduced the number of Ki-67 expressing cells by 40% in relation to the non-treated group. The treatment also decreased the amount of phosphorylated ERK in the proliferating compartment of the crypt.
Conclusion: Our results show that UDCA decreases proliferation of normal colonic epithelial cells both *in vivo* and *in vitro*. The inhibition of the EGF signalling pathway and/or decrease of ERK phosphorylation might be responsible for the proliferation inhibition caused by UDCA.

6121 POSTER

Gene expression profile related to oxaliplatin (OXA) intrinsic resistance in a panel of 14 human colorectal cancer (CRC) cell lines

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Background: Platinum drugs resistance is a complex process based in the alteration of genes that belong to several pathways related to drug metabolism. To clarify these multifactorial mechanisms, we analyzed gene expression profile in fourteen CCR cell lines with different sensitivity to oxaliplatin. The aim of this work was to compare gene expression profile between high IC50 (IC50 > 1 mM) and low IC50 (IC50 < 1 mM) cells to determine genes that could play a role as a marker in oxaliplatin intrinsic resistance.
Methods: Gene expression profile was analyzed through microarray technology (Human 19K oligo; labeled with Genisphere; data analysis by Genesis 1.5.0). We analyzed changes in gene expression comparing high (LOW OXA sensitivity) versus low IC50 (HIGH OXA sensitivity) groups., the set of genes was analyzed by using two Array-tools t-test based statistical methods (NCI: class-comparisons and SAM) in order to determine their probability to be false positive markers.
Results: We obtained a gene expression profile of 198 candidate genes by using hierarchical clustering and ANOVA function (p < 0.01). According to Array-tools analysis, 16 genes were selected because they did not show any probability to be false positive markers.
Conclusions: In our model, an expression profile of 16 genes showed to be related to oxaliplatin intrinsic resistance without probability to be false positive markers. These set of genes should be validated in patients to establish their potential role in CRC treatment selection.

6122 POSTER

MicroRNA expression profile in stage II colorectal cancer

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Background: Mi(cro)RNAs are non-coding molecules which regulate gene expression by translational repression or mRNA degradation. Aberrant miRNA expression has been demonstrated in many malignancies, including colorectal cancer (CRC). Moreover, miRNAs have potential roles as diagnostic and prognostic biomarkers, and therapeutic targets. Stage II CRC has posed a significant challenge to manage due to high risk of recurrence and lack of consensus to guide adjuvant therapy. We aimed to characterise miRNA expression profiles of patients with stage II CRC and to investigate their association with clinicopathological variables.
Materials and Methods: Following ethical approval and patient informed consent, high throughput miRNA microarray was performed on a cohort of 20 tissue samples from patients with stage II CRC to profile the expression of 380 miRNAs. Differentially expressed miRNAs were validated by real-time quantitative (RQ)-PCR in an expanded cohort of 106 tissue specimens from 58 patients.
Results: On array analysis, 20 miRNAs were identified as upregulated and 13 downregulated in tumours. Five miRNAs were selected for validation in a wider cohort of CRCs by RQ-PCR; their differential expressions were confirmed: *miR-10b* ($p < 0.001$), *miR-143* ($p = 0.003$), *miR-145* ($p = 0.001$), *miR-21* ($p = 0.002$) and *miR-31* ($p < 0.001$). *Mir-31* was the most dysregulated miRNA with a fold change of over 5. Furthermore, increased *miR-31* and reduced *miR-143* expression levels were associated with disease aggressiveness.
Conclusion: This study demonstrates dysregulated miRNA expression in stage II CRC tumours. Moreover *miR-31* and *miR-143* were associated with disease aggressiveness. Dysregulation of these miRNAs is consistent with their oncogenic and tumour suppressor regulation of gene targets known to be dysregulated in CRC (*miR-21 – PTEN*, *PDCD4*; *miR-143 – KRAS*, *BCL2*). This could represent a novel means of prognostication and to guide adjuvant chemotherapy in CRC.

6123 POSTER

Circulating cytokeratin 18 fragments – M30 and M65 – as marker of postoperative residual tumour load in colorectal cancer patients

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Background: Despite radical surgery up to 50% of colorectal cancer patients subsequently develop distant metastases. Appropriate detection systems for the routine clinical use to determine the extend of pre- and prioperative haematogenic tumor cell dissemination are still missing. Soluble cytokeratin 18 (CK18; M65) and a caspase-cleaved fragment of CK18 (M30) have been used as biomarkers, corresponding to tumor cell death and apoptosis respectively. Aim of this study was to evaluate the significance of pre- and postoperative cell death measurements in serum of patients operated for colorectal cancer.
Material and Methods: M30 and M65 were quantified in serum samples pre- and postoperatively. Disseminated tumor cells in bone marrow of colorectal cancer patients, as negative prognostic factor were assessed by staining with the pan-cytokeratin antibody A45-B/B3 in bone marrow aspirates. A total of 64 colorectal cancer patients and 22 people without cancer were included into the study.
Results: Patients with colon tumors of stages UICC I and IV had significantly elevated M30 serum concentrations compared to controls. M65 measurements showed elevated levels in UICC I and IIA, compared to normal controls ($p < 0.05$). In 31 colon cancer patients, M30 and M65 determinations were performed prior to and seven days after tumor surgery. A group of 24 patients exhibited a significant decrease of M30 in response to tumor removal, in contrast to seven patients with either persistent or higher M30 levels postoperatively. M30 correlated significantly with the