34-month RFS was 95%. All pts achieving a TRG1, and all but one with TRG2 (pCRM +ve), were recurrence-free.
Conclusions: These data confirm the feasibility and activity of the whole treatment. A slight reduction of FU dosage appeared to improve the safety of this combination. Currently, we are now evaluating the addition of bevacizumab, 5 mg/kg every 2 weeks, before and during this concurrent

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Induction of dihydropyrimidine dehydrogenase expression by Mitomycin C in colorectal cancer

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Background: Since thymidine phosphorylase (TP) is an essential enzyme for the activation of capecitabine to 5-fluorouracil (5-FU) in tumors, TP up-regulators should enhance the efficacy of capecitabine. Dihydropyrimidine dehydrogenase (DPD), on the other hand, is considered to be a key enzyme in the catabolism of 5-FU, and its high expression in a tumor is thought to reduce the efficacy of 5-FU against tumors. The aim of this study was to confirm whether or not mitomycin C (MMC) is a TP and/or DPD regulator.
Materials and Methods: Biopsy specimens were obtained from 62 colorectal cancer patients preoperatively by colonoscopy. After a biopsy, 33 patients received neoadjuvant chemotherapy with MMC and underwent operations after 1−13 days. Using biopsy and operative specimens, TP and DPD levels in the tumors were examined. Patients were divided into three groups; an MMC(−) group (no MMC), a Short group (operation within four days after MMC) and a Long group (operation over six days after MMC).
Results: In the MMC(−) and Short groups, no significant differences in DPD levels before and after MMC were observed. In the Long group, on the other hand, DPD levels were elevated ($p = 0.026$). As for TP, MMC did not raise the levels of TP in the MMC(−) and Short groups, but it tended to do so in the Long group ($p = 0.13$).
Conclusions: Although MMC appears to be a TP up-regulator, it is also a DPD up-regulator at appropriate intervals.

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A Swiss multicentre phase II study of capecitabine plus oxaliplatin (CAPOX) in combination with preoperative pelvic radiotherapy in patients (pts) with locally advanced rectal cancer

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Background: This study evaluated the addition of capecitabine and oxaliplatin (CAPOX) to preoperative radiotherapy (RT) in patients with locally advanced rectal cancer (LARC).
Materials and Methods: Patients (pts) with T3/T4 rectal adenocarcinoma with or without nodal involvement staged by endorectal ultrasound were recruited. Treatment consisted of a full dose cycle with CAPOX (capecitabine 1000 mg/m^2 bid on days 1–14 and oxaliplatin 130 mg/m^2/d on day 1), followed by RT as 25 daily fractions of 1.8 Gy on 5 consecutive weeks in combination with capecitabine 825 mg/m^2 bid on days 22–35 and 43–56 and oxaliplatin 50 mg/m^2/d on days 22, 29, 43 and 50. Surgery was scheduled 5 weeks after completion of CAPOX-RT. Primary endpoint was pathological complete tumour response (pCR) prospectively defined as grade 3 or 4 in the histological grading of regression according to Dworak classification (DC). Secondary endpoints were rate of sphincter preservation, R0 resection in pts with T4 tumours, downstaging, pathological incomplete tumour response rate and safety. Second-opinion pathology review was performed in all tumours categorised as DC grade 2 or 3.
Results: 60 pts were enrolled from 6 cancer centres. Median tumour size was 50 mm. Nodal infiltration was diagnosed in 47 pts. Tumour location (from anal verge): $\leqslant$5 cm in 21 pts, 5–10 cm 22 pts, >10 cm 17 pts. 58 pts received CAPOX-RT and underwent surgery (49 TME, 9 abdominoperineal resection), 1 pt withdrew consent and refused further treatment, and 1 died during neoadjuvant CAPOX. R0 resection was achieved in 57 pts, including all 5 pts with T4 tumours. The pCR rate was 23% (95% CI, 13–36; DC 3: 7 pts, DC 4: 7 pts). Sphincter preservation was achieved in 84% of pts. Tumour downstaging (T and/or N) was observed in 65% of pts. Pathological incomplete response (DC 0/1/2) was observed in 1/20/23 pts. Main grade 3 adverse events were: diarrhoea 20%; thrombosis 3%; nausea, vomiting, proctitis, fatigue, hand–foot syndrome 2% each. No grade 3/4 haematological adverse events, except lymphocytopenia (43%), were observed.
Conclusions: Preoperative combined treatment with CAPOX and RT is feasible and resulted in encouraging high rates of pCR, R0 resection, sphincter preservation, and tumour downstaging in this group of pts with LARC.

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Randomized strategical trial of chemotherapy in metastatic colorectal cancer (FFCD 2000−05): preliminary results

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Background: The survival benefit of using a combination therapy instead of keeping it for a second line (L2) has not been demonstrated in metastatic colorectal cancer. The purpose of this trial was to compare the efficacy of simplified LV5FU2 (s) followed by FOLFOX6 (arm A) to FOLFOX6 followed by FOLFIRI (arm B) on progression-free survival after two lines of chemotherapy. We present here preliminary results relating to toxicity, observance and overall survival.
Materials and Methods: Inclusion criteria: a) non resectable metastases of histologically proven colorectal adenocarcinoma, b) evaluable disease (WHO criteria), c) absence of previous chemotherapy other than adjuvant. Treatment was as follows: LV5FU2s = at day 1, folinic acid 400 mg/m^2, 5-FU bolus 400 mg/m^2 and continuous infusion over 46 hours 2400 mg/m^2/2 weeks; FOLFOX6 = LV5FU2s + oxaliplatin 100 mg/m^2 at day 1; FOLFIRI = LV5FU2s + irinotecan 180 mg/m^2 at day 1.
Results: 410 pts out of 570 initially planned (early stopping due to slow accrual and new treatments) were included from 02/2002 to 02/2006 (205 in each arm). Median follow-up was 25 months. The median number (range) of cycles (28 days) in first line (L1) was respectively 5 (1−24) and 6 (1−24) in the arms A and B ($p = 0.01$), and for L2 (152 and 144 pts in the arms A/B): 5 (1−17) and 3 (1−33) (NS). In the arms A and B, 74% and 70% of pts had L2. L1 was stopped for toxicity for 1% and 16% of the pts in arms A and B ($p < 0.0001$); L2 respectively for 15% and 2% pts ($p < 0.0001$). The percentages of pts presenting at least a grade 3−4 hematologic toxicity (mainly neutropenia) by arm were: 6% versus 37% ($p < 0.0001$) for L1 and 30% versus 27% (NS) for L2; grade 3−4 non hematological toxicity (grade 2−4 neurotoxicity): 26% (1%) versus 56% (64%)($p < 0.0001$; $p < 0.0001$) for L1 and 54% (60%) versus 46% (40%) of the pts for L2 (NS; $p < 0.01$). No toxic death was observed in the arm A against 5 in the arm B: 3 in L1 and 2 in L2. Overall survival medians were 17 and 16 months in arms A/B (logrank $p = 0.64$) (preliminary results, 291 observed deaths).
Conclusions: This trial does not show any substantial difference in treatment duration and overall survival between both arms and shows a more important toxicity in the arm with first line combined chemotherapy.

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Significance of polymorphisms in biotransformation enzymes for colorectal carcinogenesis

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Background: Biotransformation enzymes play important role in metabolism of xenobiotics. Genetic polymorphisms in biotransformation enzymes may result in variations in detoxification capacity. Interaction

between environmental exposure and individual susceptibility towards activation and detoxification of procarcinogens may influence levels of genotoxic agents and subsequently modify the risk of carcinogenesis. One of the most common forms of cancer is colorectal cancer (CRC). CRC affects approximately 5% of worldwide population. The majority of CRC is sporadic with unknown etiology. Exposure to procarcinogens through alimentary chain and smoking are considered as environmental factors contributing to CRC incidence. We followed associations of genetic variability in GSTM1, GSTT1, GSTP1, NQO1, CYP1B1 and EPHX1 genes with CRC risk in a case-control study.
Materials and Methods: Polymorphisms in GSTM1 (deletion), GSTT1 (deletion), GSTP1 (Ile105Val), NQO1 (Pro187Ser), CYP1B1 (Asn453Ser and Leu432Val) and EPHX1 (Tyr113His and His139Arg) were assessed by PCR RFLP based methods in groups of 649 CRC patients and 745 unrelated hospital-based controls of Czech Caucasian origin. EPHX1 (Tyr113His) variants were verified by sequencing analysis.
Results: Statistical analysis showed that variant genotype in GSTP1 (Val105Val) significantly increases the risk of CRC (crude OR = 1.48, CI = 1.02−2.15, P = 0.037). No significant association among other investigated single polymorphisms and susceptibility to CRC was found. Individuals carrying at least one variant allele in GSTP1 in combination with GSTM1 or GSTT1 deletion were under significantly increased risk of CRC in comparison with those carrying wild-type genotypes (P < 0.001 and P = 0.011, respectively). Combination of variant alleles in all three GSTs genes also significantly increased the CRC risk (P = 0.020). Combination of variant alleles in GSTP1 with altered EPHX1 also conferred increased CRC risk. Age and sex did not play a role as confounding factors.
Conclusions: Our study suggests that combinations of polymorphisms in xenobiotic-metabolizing enzymes may confer increased risk of CRC and should be further followed by larger study on related populations. Polymorphisms confirmed as risk factors may then be used for identification of subpopulations under increased CRC risk and subsequent targeting of preventive strategies.
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Cannabinoids induce apoptosis through CB1 and CB2 receptor activation in human colon cancer cells

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Background: Recent experimetal studies have pointed to cannabinoids as potential anticancer agents. One of the possible molecular mechanisms underlying the antitumor effect of these compounds is the ability to induce tumor cell apoptosis through activation of their cellular receptors, namely CB_1 and CB_2. The aim of this study was to determine the effect of the CB_1 receptor agonist arachinodyl-2′-chloroethylamide (ACEA) and the newly synthesized 1,8-naphthyridin-4(1H)-on-3-carboxamide derivative (compound 3g) CB_2 receptor agonist on inducing apoptosis and decreasing cell proliferation in the human colon cancer cell lines HT29 and DLD1.
Methods: mRNA and protein expression of the CB_1 and CB_2 receptors in human colorectal cancer specimens and in the HT29 and DLD1 cells were investigated by RT-PCR and Western blot analysis, respectively. The proapoptotic effect of ACEA and 3g on the two colon cancer cell lines was evaluated by means of caspase 3 activity determination and flow cytometry analysis (Annexin V test) of apoptotic cells. Tumor cell proliferation was determined by the [^{3}H]thymidine incorporation assay. The effects of the CB_1 and CB_2 agonists on ceramide and TNF-alfa production were also assessed. The HT29 and DLD1 cells were treated with 100 nmol/l ACEA and 3g.
Results: Both CB_1 and CB_2 receptors were expressed in the human colorectal cancer specimens and in the colon cancer cell lines. Treatment of the HT29 and DLD1 cells with either ACEA or 3g induced a significant increase in caspase-3 activity and number of apoptotic cells. The same treatment determined a significant decrease in tumor cell proliferation after their stimulation with 100 nmol/l epidermal growth factor. All these effects were prevented by the administration of 10μM fumonisin B1, a ceramide synthase inhibitor. Moreover, treatment of the HT29 and DLD1 cells with ACEA or 3g significantly increased the production of both ceramide and TNF-alfa.
Conclusions: Our data showed that the cannabinoid receptors CB_1 and CB_2 are expressed in human colorectal cancer. Moreover, cannabinoids can induce apoptosis in human colon cancer cells and reduce their proliferation through activation of both CB_1 and CB_2 receptors. These effects seem to be mediated by an increase in ceramide production, a known mediator of apoptosis. We also hypothesized that ceramide production is in turn stimulated by an increase in TNF-alfa production through activation of both CB_1 and CB_2.

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Identification and validation of novel serum tumour markers for colorectal cancer applying proteomics approaches

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Background: The goal of this study was to identify and validate novel serum markers of human colorectal cancer as potential candidates for non-invasive detection of early colorectal neoplasm.
Materials and Methods: Two-dimensional gel electrophoresis (2-DE) and matrix assisted laser desorption/ionization-mass spectrometry (MALDI-MS) as well as a complementary shotgun proteomics approach applying nano flow two-dimensional liquid chromatography coupled to electrospray ionization MS (2-D-LC-ESI-MS) were used to analyze 16 matched colorectal cancer and adjacent normal tissue samples. Antibodies against selected proteins found to be elevated in cancer tissue, were generated and used for further validation by immunoblotting of tissue samples and immunohistochemistry. Highly sensitive immunoassays were developed for assessment of serum levels of selected proteins.
Results: In total, 735 distinct proteins were identified in colon tissue with the 2-DE/MALDI-MS approach. For a small number of these identified proteins, among them nicotinamide N-methyltransferase (NNMT) and proteasome activator complex subunit 3 (PSME3), strong elevation in colorectal cancer was confirmed by immunoblot analysis and immunohistochemistry, respectively. Highly sensitive immunoassays revealed that elevated levels of NNMT and PSME3 are found in serum from colorectal cancer patients. Employing a receiver operating characteristic curve based on the measurement of 109 colorectal cancer patients and 317 healthy controls, we obtained an area under the curve (AUC) of 0.84 for NNMT and of 0.79 for PSME3, respectively, which was superior to the established tumor marker carcinoembryogenic antigen (CEA) with an AUC of 0.77.
The 2-D-LC-ESI-MS approach led to the identification of further proteins, which were partly not identified in the 2-DE/MALDI-MS approach. Further analysis of the 2-D-LC-ESI-MS data and validation of thereof derived proteins elevated in cancer tissue is currently ongoing and the results will be presented at the conference.
Conclusions: The results of the presented study indicate that it is essential to combine different, complementary proteomics approaches to obtain a most comprehensive description of the proteome of a given tissue. It is proposed that the serum levels of NNMT and PSME3, respectively, may have a value in the early detection and in the management of colorectal cancer patients.

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Antiangiogenetic-based therapy for advanced colorectal cancer patients seems to enhance the antitumor cellular immunoresponse

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Background: Multiple links have been found between angiogenesis and immunoresponse in human tumors. Vascular endothelial growth factor (VEGF) is a key agent in promoting and sustaining the immune tolerance during the cancer growth, particularly because of the indirect impairment on the functional maturation of dendritic cells (DCs) (Johnson BF, Expert Opin Biol Ther, 2007). Preclinical murine models have been shown that the block of VEGF could enhance the efficacy of cancer immunotherapy in colorectal carcinoma (Li B, Clin Cancer Res, 2006). Bevacizumab, the humanized monoclonal antibody against VEGF, is largely employed in the treatment of metastatic colorectal cancer (mCRC) pts in addition to chemotherapy (CT), and its in vivo impact on pts immune system has not been clarified.
Material and Methods: During our ongoing studies on the immunosuppressive effect of cancer treatments, we have now focused on the impact of first-line Bevacizumab-based combination therapy on 27 pts with mCRC (M/F: 20/7, median age: 55 yrs), in absence of clinically relevant infections.