Results: I.p. application of catumaxomab was well tolerated. MTD was defined at 10–20–50–200 μg for the 1st, 2nd, 3rd and 4th i.p. infusion. Most frequent adverse events of the first 17 patients >CTC grade 2 were nausea/vomiting (14), abdominal pain (12), fever (6), exanthema (4), elevation of liver enzymes (4) and cholangitis (2), which could all be successfully treated by conventional medication. Analysis of the peritoneal lavages showed a decrease/complete disappearance of tumor cells after trAb treatment in 7/8 patients. After a follow-up period of 15 months, 7/17 patients (41.2%) are alive. At present, the median survival is 9 months (mean 8.6) after treatment and 12 months (mean 12.2) after diagnosis of PC. The updated results at presentation will contain new information about safety (shorter infusion time, dose escalation with premedication, pk data) and survival.
Conclusion: I.p. application of the trifunctional antibody catumaxomab is safe and technically feasible and may represent a new concept for treatment of PC due to gastrointestinal cancer.

660 POSTER

Toward a circulating tumour cell analysis as an early marker for relapse in stage II and III colorectal cancer patients

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Introduction: Different technological approaches have recently used to evaluate the presence of circulating tumour cells (CTC) as a prognostic marker in cancer patients. Contradictory results can be easily found in the literature. However, CTC analysis in metastatic prostate or breast cancer patients resulted in a valuable tool for predicting progression free survival and overall survival.
Material and methods: A two-step design was made: (i) A comparative study was performed to assess the efficiency in the number of tumour cells obtained with four different molecular and cellular methods. We used two systems for tumour cell enrichment (immunomagnetic beads with anti-EpCAM and gradient of density), combined with two different methods to quantify tumour cells (flow cytometry with anti-CD45, anti-CK7 and anti-CK8 antibodies; and quantitative RT-PCR for CK20 gene expression). These experiments were performed in a model system using serial dilutions of HT29 tumour cell-line cells with lymphocytes (from 1 to 10000 HT29 cells in $5X10^6$ lymphocytes). The euclidean distance of the test curve to the perfect one was measured in order to determine the most efficient method along the different tumour cell dilutions.
(ii) CTC analysis using the technical approach selected in the first objective is being performed prospectively every four months, in blood samples (20 ml) from stage II and III colorectal cancer patients, after surgical resection of the primary tumour and informed consent.
Results: (i) Statistical analysis results showed that the immunomagnetic beads, as tumour cell enrichment method, followed by flow cytometry to quantify cells, was the most efficient combination (ED = 60.53); no significant difference was observed when compared to the perfect curve (p = 0.5).
(ii) The follow up of the patients recruited in the study is ranging from 12 to 34 months. Up to date, there are 20 patients with a minimum of six blood samples analysed in our study. In only two cases tumour relapse has been clinically documented. In both patients, we were able to detect a significant increase in the CTC number, five and six months earlier, respectively, to the date that relapse was clinically evidenced. An increase of CTC is also being observed in two other cases but there is not yet any clinical evidence of metastatic disease. Up to now, the rest of cases have a very low, or no detectable, number of CTC and no clinical evidence of relapse.
Conclusions: These preliminary results show that colorectal cancer CTC analysis is a promising tool to detect earlier tumour relapse when compared to conventional methods. More work needs to be done in order to confirm, and definitively conclude, the usefulness of CTC analysis for an early detection of tumour relapse in patients with stage II and III colorectal cancer.

661 POSTER

The effect of dihydropyrimidine dehydrogenase (DPD) activity and germline thymidylate synthase (TS) gene polymorphisms on the survival of colorectal cancer patients treated by adjuvant 5-fluorouracil

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Background: The antitumor activity of 5-fluorouracil (5-FU) is limited by various factors: i.e. the expression of its molecular target TS and the catabolic activity of the DPD. The TS gene is polymorphic, contains 5'-TSER and 3'-TSUTR polymorphisms, which influence its expression. In the present study we investigated the DPD activity and TS gene polymorphisms in the PBMCs of colorectal cancer (CRC) patients treated with adjuvant 5-FU and the relationship between the disease free (DFS) and overall (OS) survival of the patients and the studied prognostic factors.
Material and methods: 166 CRC patients receiving adjuvant 5-FU chemotherapy were involved in this study. Patients were followed-up for 19±14 (median±SD) months. DPD activity from the PBMCs was analysed by radioenzymological and TS polymorphisms by PCR-PAGE and RFLP methods on the DNA samples isolated from the PBMCs.
Results: Based on the DPD activity, patients were divided in four groups: ⩽10; 10–20; 20–30 and >30 pmol/min/10^6 PBMCs. The Kaplan-Meier survival analysis showed significant difference for both DFS and OS (p = 0.0197 and 0.0046, respectively) between the lowest (<10) and highest (>30) activity-groups indicating a significantly longer survival of patients with the lowest DPD activity. 5'-TSER 3R/3R homozygotes showed significantly longer DFS and OS (p = 0.048 and 0.009, respectively). At the same time the 3'-TSUTR genotypes were not significantly associated with DFS or OS although 0bp/0bp genotype-group showed higher hazard ratio compared to that of patients containing at least one 6bp allele. Combining the two TS polymorphisms eight groups were obtained. Evaluating the hazard ratios of the relapse, obtained by applying Cox regression analysis for the eight genotype combination patients were divided in two prognostic groups: "A" (3R/3R with any 3'-TSUTR genotype and 2R/3R with 6bp/6bp) with low (HR ⩽ 1) and "B" (all other genotype-combinations) with high (HR > 1) relapse risk, respectively. Multivariate Cox regression analysis demonstrated the following parameters as significant independent prognostic factors for DFS: tumor localisation, Dukes' stage, treatment type (bolus vs continuous infusion), DPD activity and TS polymorphism combination (p = 0.043, 0.028, 0.003, 0.044, 0.004, respectively).
Conclusion: DPD activity and TS gene-polymorphism combination of PBMCs are independent prognostic factors for DFS in adjuvant-treated CRC patients.
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662 POSTER

Modification of the circadian rest/activity rhythm by 1st line oxaliplatin (l-OHP), 5-fluorouracil (5FU) and leucovorin (LV) in patients (pts) with metastatic colorectal cancer (MCC). An international study (EORTC 05963)

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Background: Circadian rest/activity rhythm correlates with several Quality of Life items and is an independent and strong prognostic factor of survival both in chemotherapy-naïve and in pre-treated MCC pts (ASCO 2005 Clin Cancer Res, 2000: 6; 3038). In this international study, we prospectively evaluated the effect of 4 courses of 1st-line chemotherapy (CHT) with biweekly infusional l-OHP, 5FU and LV on circadian rest/activity rhythm in MCC pts.
Methods: 77 MCC pts had rest/activity rhythm assessed for 3 days using a small wrist-watch (actigraph), which records the number of arm movements per minute, both before the beginning of the first course (C0) and after 4 courses (C4) of CHT with l-OHP (100 mg/m^2/course), 5FU (3000–3600 mg/m^2/course) and LV (600 mg/m^2/course). Three validated circadian rhythm parameters were calculated: mean activity (mAct), autocorrelation coefficient at 24 h (r24), indicating the robustness of the activity pattern over

a period of 24-h, and dichotomy index (I < O), quantifying the differences in activity distribution between the rest span and the active phase. Paired rhythm parameters at C0 and C4 were compared by Wilcoxon signed rank test.
Results: Median values and quartile distribution [1st-3rd] of the 3 circadian rest/activity rhythm parameters were not significantly different at baseline (C0) and after 4 courses of CHT (C4) (Table 1). After CHT, however, the number of pts remaining in the same tercile ranged from 44 to 53%, according to the parameter (34 pts for r24, 40 for I < O, and 41 for mAct). Among the remaining pts, after 4 courses of CHT, the rhythm parameters significantly improved or deteriorated in nearly half of the pts each (20 and 23 for r24, 19 and 18 for I < O, 17 and 19 for mAct, respectively).
Conclusions: The main chemotherapy regimen for colorectal cancer modified the rest/activity circadian rhythm in nearly half of the patients in opposite directions. This supports large interpatient variability in response of the circadian timing system to chemotherapy. Understanding the relations between circadian system status and treatment-related toxicity and efficacy will lead to improve the therapeutic index through tailoring delivery schedule to the individual features of the patient.

Table 1

	Range of variation	C0	C4	p
r24	−1.0 to 1.0	0.41 [0.25–0.55]	0.45 [0.25–0.57]	0.34
I < O	0 to 100	97.5 [92.2–99.2]	98.2 [95.6–99.3]	0.15
mAct	0 to ∞	112 [90–127]	112 [86–132]	0.88

Publication
GI – colorectal cancer

663 PUBLICATION

Prognostic index for adjuvant treatment in locally advanced rectal cancer after preoperative chemoradiotherapy and radiotherapy

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Background: Preoperative radiotherapy and chemoradiotherapy lower the risk of local recurrence and improve survival in stage II and III rectal cancer. After preoperative treatment is this initially homogeneous group stratified and patients have different relapse rate and survival.
Methods: A total of 174 patients (34% women and 66% men) with locally advanced rectal adenocarcinoma were treated with preoperative radiotherapy or chemoradiotherapy and retrospectively evaluated. The median follow up is 24 months (range 3–74 months). All patients received preoperative external beam radiation (40–50 Gy/20–25 fractions/4–5 weeks) using linear accelerator and 3D planning. Concomitant CHRT with 5-FU was carried out in 25% of patients. The data were analysed with statistical software SPSS version 10.0.
Results: Radical resection underwent 86% of patients, non-radical tumor resection 2% and inoperable tumor persisted in 7% (at the beginning it was 13% of patients). Distant metastases were detected peroperatively in 5%. Statistically significant factors that influence both overall and disease free survival are postradiotherapy stage ($p = 0.005$), postradiotherapy grading ($p < 0.001$), angioinvasion or perineural spread ($p = 0.023$), radicality of surgery ($p < 0.001$) and gender ($p = 0.036$). Local recurrence was associated in preradiotherapy T4 tumors ($p = 0.048$) and angioinvasion or perineural spread (0.049). Two-year OS was 85% and 5-year OS was 60%. Prognostic index is calculated from prognostic factors (stage, radicality of surgical procedure, grade, angionvasion and distance from the anal verge) and overall score devides patients into 4 groups with different relapse risk and OS. Excellent prognosis is achieved in patients of low risk group (radical surgery, pT1-pT2 good differentiated tumors with negative lymphnodes, no angioinvasion, no perineural spread, or complete remision after preoperative treatment), this group counts for 14% of all patients and 2-year DFS and OS and 5-year DFS and OS are 100% (all patients are alive without recurrence). Patients with intermediate risk have a 5-year OS 80%, patients with high risk of relapse have 5-year OS 55% and in group of very high risk no patient survived 5 years.
Conclusion: Patient with low risk relapse have relapse risk less than 5% and in our institution adjuvant chemotherapy in this low risk group after preoperative radiotherapy is omitted.

664 PUBLICATION

Cetuximab in combination with irinotecan/5-fluorouracil (5-FU)/folinic acid (FA) (FOLFIRI) in the first-line treatment of metastatic colorectal cancer (mCRC)

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Background: FOLFIRI is a standard option in the first-line treatment of mCRC. Cetuximab (Erbitux®) is an IgG1 monoclonal antibody targeting the epidermal growth factor receptor (EGFR), which is commonly expressed in mCRC. Cetuximab is active in mCRC patients failing on irinotecan-based therapy. This phase I/II trial investigated the safety and efficacy of cetuximab+FOLFIRI in the first-line treatment of EGFR-expressing mCRC.
Materials and Methods: Patients with immunohistochemistry-determined EGFR-expressing mCRC, who had not been treated for metastatic disease, received cetuximab (initial dose 400 mg/m^2 followed by 250 mg/m^2/week). FOLFIRI was given every 2 weeks: irinotecan 180 mg/m^2, FA 400 mg/m^2 and 5-FU 300 mg/m^{2}bolus plus 2,000 mg/m^2/46-h infusion (low-dose, LD) or 400 mg/m^{2}bolus plus 2,400 mg/m^2/46-h infusion (high-dose, HD). The use of LD 5-FU was part of the early dose-finding phase of the study.
Results: This analysis was performed on the per-protocol HD population of 42 patients: 64.3%/35.7% male/female, mean age 60.0 years, median KPS 100, 79% colon primary tumour. There were 19 confirmed objective responses (all partial responses [PR]) (45.2%) and 16 patients with stable disease (SD) (38.1%), giving a disease control rate (complete response+PR+SD) of 83.3%. The median response duration was 306 days (10 months), and median survival was 699 days (23 months). 10 patients (23.8%) were able to undergo resection of metastases for curative intent, 9 of whom had liver metastases. There were 8 R0 resections. Treatment was well tolerated. 66.7% of the 42 patients experienced grade 3/4 adverse events, the most frequent of which were leucopenia (16.7%), diarrhoea (14.3%), vomiting and intestinal obstruction (11.9% each), skin rash and abdominal pain (9.5% each), and asthenia and dyspnoea (7.1% each).
Conclusions: Cetuximab+FOLFIRI, incorporating high-dose 5-FU, is a feasible and active combination for the first-line treatment of EGFR-expressing mCRC. 45.2% of patients achieved an objective response. The median survival was 23 months and 23.8% patients were able to undergo resection of initially unresectable metastases. Based on these results, a new phase III trial was started.

665 PUBLICATION

Phase I/II study of 24-hour infusion of irinotecan (CPT-11) in combination with sequential oral leucovorin (LV) and uracil/tegafur (UFT) for patients with metastatic colorectal cancer

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Background and Objective: A combined therapy using irinotecan (CPT-11), 5-fluorouracil (5-FU) and leucovorin (LV) is one of the standard chemotherapies (CT) for metastatic colorectal cancer (MCRC). The cytocidal effect of CPT-11 is specific to the S phase of the cell cycle. Therefore, its antineoplastic effect may be greater when administered in small dosages over an extended period, rather than when given at larger dosages for a shorter period. It has been reported that when a combination of 5-FU and CPT-11 is given sequentially it is more effective than when given concurrently. The effects of an oral administration of a combination of uracil/tegafur (UFT) and LV and an intravenous infusion of 5-FU combined with LV are comparable but the former is more convenient. Therefore, for a Phase I/II study, a schedule in which 24-hour continuous infusion of CPT-11 followed by sequential oral administration of UFT/LV was selected.
Methods: The subjects were patients (pts) who had MCRC with measurable lesions. Prior CT or adjuvant CT was allowed when they were interrupted at least 4 weeks before beginning this study. Each course was composed of the following: 24-hour infusion of CPT-11 on days 1 and 15: and oral UFT and LV divided into 3 parts were given on days 3–7, 10–14, 17–21 and 24–28. This regimen was repeated every 4 weeks. The dosages given during the Phase I study are shown in the table below. The maximum tolerated dose (MTD) was based on the dose-limiting toxicity (DLT) of the first course: the dosages one level below the MTD was adopted for the recommended dosage (RD) in the Phase II study.
Results: Three pts each were assigned to levels 1 and 2. No DLT was recognized in any of them. At level 3, 3 of the 6 pts developed a DLT, i.e.,