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p53, p21, p27 and bcl-2 as predictors for clinical outcome in rectal cancer

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Purpose: The purpose of this study was to examine whether molecular markers, including p53, p21, p27 and bcl-2, can be used to predict tumor response to pre-operative radiotherapy/chemoradiotherapy in rectal cancer.

Methods: From January 1998 to June 2002, there were 77 patients with rectal cancer enrolled. The preoperative chemoradiotherapy delivered radiation with 45 Gy in 25 fractions over 5 weeks with continuous infusion 5-flurouracil (300 mg/m^2/day). Surgery, such as LAR or APR, was performed 4 weeks after completion of chemoradiotherapy. Immunohistochemistry of p53, p21, p27 and bcl-2 were performed in the pre-radiation biopsy of specimen of 70 patients and in the post operation specimen of 53 patients. The end-points for evaluation of tumor response were down-staging (DS), tumor-shrinkage (TS), and tumor-shrinkage more than 50% (50%-TS). Besides, fair response (FS), including complete regression and tumor in situ, was also evaluated. Meanwhile, the relationship between those molecular markers and overall survival rate and disease free survival rate were evaluated.

Results: The percentages of patients of DS, TS, 50%TS and FS were 50%, 94.8%, 70.7% and 17.7% respectively. In the pre-radiation biopsy of specimen, the positive rate of p53, p21, p27 and bcl-2 were 63.3%, 16.3%, 37.2% and 16.7%, but in the post operation specimen, the positive rate was increased, 69.4%, 20.4%, 73.5% and 41.7%, accordingly (p=0.58, 0.79, 0.001 and 0.012). Of the pre-radiation biopsy of specimen for DS and TS patients, there was no significant difference in molecular markers p53, p21, p27, and bcl-2. For 50%-TS patients, the significant differences were found in p21 and bcl-2 (p=0.005 and 0.046). As for FR patients, the significant findings were noted in p53, p27 and bcl-2 (p=0.006, 0.012 and 0.027). The 3-year disease-free and overall survival rates in our patients were 75% and 85%. The 3-year overall survival rates for pathologic specimen p27 (+) and p27(-) patients were 87% and 78% respectively (p=0.029). However, there was no significant finding between molecular markers, no matter pre-radiation or post operation, and disease free/overall survival rates, with all p values more than 0.05.

Conclusions: After neoadjuvant therapy, the positive rates of all molecular markers, including p53, p21, p27 and bcl-2, increased. In the pre-radiation biopsy of specimen, the p21 (-) and bcl-2 (+) were good predictors for 50%-TS while p53 (-), p27 (+) and bcl-2 (+) were good for FR. More p27 (+) patients survived over 3 years, but other molecular markers could not predict disease free and overall survival.

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Survivin expression: an independent prognostic factor in rectal cancer patients with and without radiotherapy

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Background: Survivin blocks apoptosis by inhibiting caspase 3 and 7. This protein is undetectable in many normal tissues and is re-expressed in several types of human cancers. The survivin expression in rectal cancer patients who received preoperative radiotherapy alone has not been studied. Our aim was to analyze the relationship of survivin expression with radiotherapy, clinicopathological variables, apoptosis, Ki-67 and p53 in rectal cancer patients who participated in a trial of preoperative radiotherapy.

Methods: We investigated survivin expression by using immunohistochemistry on 98 rectal tumors with 74 cases that had normal mucosa adjacent to the tumors. Fifty-seven patients had curative tumor resection alone and 41 received preoperative radiotherapy, 25 Gy before surgery.

Results: The survival rate of the patients with survivin positive tumors was significantly reduced compared to those with survivin negative tumors independently of clinicopatholgical variables (P = 0.02). The patients with survivin positive tumors tended to have a higher risk of local (P = 0.12) and distant (P = 0.11) recurrence. In subgroups analyzes of non-irradiated or irradiated patients, the positive expression tended to associate with unfavorable survival (P = 0.08, P = 0.19). After radiotherapy, survivin expression in the normal mucosa was increased (33% of the cases vs 67% of the cases, P = 0.057) but not in the tumors (P = 0.71).

Conclusion: Survivin positive expression was independently related to unfavorable survival in rectal cancer patients who participated in a trial of preoperative radiotherapy. Compared to tumor tissue, survivin expression in normal mucosa was greatly up-regulated after radiotherapy. Survivin protein does not predict the response to radiotherapy of rectal tumours.

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Quality of life in patients with rectal carcinomas treated by preoperative radiotherapy: a longitudinal prospective study

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Background: Recently it was shown that preoperative radiotherapy (RT) reduces local recurrences and may improve overall survival. Although RT is believed to increase treatment sequela, data on quality of life (QoL) after such a therapeutic strategy are scarce. The present study aimed to assess prospectively the QoL of patients treated by preoperative RT for locally advanced rectal cancers.

Patients and Methods: Forty-three patients accepted to participate in the present study. All patients were enrolled in two successive phase I-II trials and treated preoperatively with 50 Gy in 40 fractions of 1.25 Gy over 4 weeks with or without concomitant chemotherapy (gemcitabine). Rectal surgery was scheduled 6 weeks after completion of RT. QoL was assessed using two self-rating questionnaires developed by the European Organization for Research and Treatment of Cancer: one for cancer-specific QoL (EORTC QLQ-C30) and one for site-specific QoL (EORTC QLQ-CR38). Participants were asked to complete the questionnaires before RT and one-year post-treatment. We hypothesized that at least some scores of the various scales would vary between the 2 analyses. The two-tailed paired t-test was used to compare the mean values of the different scores.

Results: Compared to pre-RT scores, at one year patients reported a significant improvement in the emotional function (median: 92 vs 75, p=0.003), their perspective for the future (median: 100 vs 67, p=0.003) and their global quality of life (p=0.015) as well as a decrease in gastrointestinal symptoms such as constipation (p=0.006) and diarrhea (p=0.035). However, the sexual dysfunction score increased significantly, particularly for males (median: 33 vs 83, p=0.005), and body image score decreased significantly (median: 100 vs 67, p=0.007). The latter score was significantly correlated to the severity of late complications (p=0.02) as well as defecation and stoma problems.

Conclusion: The present prospective study highlights some significant score changes in functions as well as symptoms one year after treatment compared to the pre-RT base line scores. While body image and sexual functioning decreased significantly, global quality of life and the perspective for the future were significantly improved. Any further improvement in QoL outcome may require refinements in the RT and surgical techniques to reduce late sequela, particularly operative procedures allowing preservation of sexual function.

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Preoperative radiochemotherapy with capecitabine in locally advanced rectal cancers - a phase-II-study

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Experimental findings have demonstrated that thymidine phosphorylase, which is necessary for the final conversion of the prodrug capecitabine, is predominantly expressed in tumor cells and is further upregulated by radiotherapy (XRT) in malignant but not in healthy tissue. Based on these findings, the concurrent administration of radiotherapy and capecitabine may improve tumor response and/or reduce toxicity. As shown in our previous phase I dose finding study (J Clin Oncol 2002, 20: 3983-91), the concurrent administration of daily capecitabine with pelvic XRT appears to be feasible and effective in advanced rectal cancer. The objective of the present expanded phase II trial is to establish the use of this combined modality approach in a multicenter setting, focussing on its application as neoadjuvant treatment of locally advanced (cT3-4) or primarily inoperable tumors. A total irradiation dose of 50.4 to 55.8Gy was administered in conventional daily doses of 1.8

Gy over a period of approximately 6 weeks. Capecitabine was given at an oral dosage of 825 mg/m* bid on each day of the radiotherapy period (the recommended dose from the phase-I-study), with the first daily dose applied two hours before irradiation. Up to now, 45 patients (pts) (60% male, 40% female) have been recruited from 6 university clinics in Germany since June 2001. Mean age was 64 years, with an unimpaired performance status (ECOG 0) in 55%. Clinical staging revealed T3 and T4 tumors, respectively, in 50% of the cases, each, and involved lymph nodes (cN+) in 53%. In 81% of the pts, the radiochemotherapy induced a clinical complete or partial remission, leading to a similar rate of R0 resections. The comparison of initial diagnosis and pathological findings showed a downstaging in 72% of pts, mainly from cT4 to pT3 ñ pT0. Only 8% remained inoperable at the end of the irradiation period. Safety findings were concordant to the phase I results with >10% incidence of NCI grade 2/3 only in leukopenia (22%), anemia (13%), skin (15%) and diarrhea (17%). The concurrent combination of radiotherapy and continuous daily capecitabine proved to be well tolerated in a multicenter setting and showed major clinical response in the vast majority of the patients.

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Evaluation of thymidine phosphorylase, dihydropyrimidine dehydrogenase and thymidylate synthase mRNA levels in colorectal cancer reveals significant correlations to tumor histopathology and disease-free survival in 5-FU treated patients

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Purpose: Evaluation of three mediators of pyrimidine metabolism - thymidine phosphorylase (TP), dihydropyrimidine dehydrogenase (DPD) and thymidylate synthase (TS) - in colorectal cancer (CRC) as markers of prognosis and/or response prediction to 5-fluorouracil (5-FU) chemotherapy (CTX).

Materials and Methods: RNA was isolated from microdissected tumor areas of formalin-fixed and paraffin-embedded CRC tissues and subjected to quantitative RT-PCR in the LightCycler® system. RT-PCR data was correlated to tumor histology (n=102), patient prognosis ("no CTX"/ n=40 and "CTX" / n=52) and the clinical response to adjuvant 5-FU CTX (n=52).

Results: Significant correlations were found for tumor 1) T and N category and UICC stage (TP mRNA, TP:DPD ratio), 2) T category (TS:DPD ratio), and 3) differentiation grade (TS mRNA and TS:DPD ratio). Moreover, whereas overall survival was correlated to the TS:DPD ratio in the "no CTX" group (p=0.032), neither TP, DPD and TS mRNA nor the TP:DPD ratio had any prognostic impact in this group. However, both DPD and the TP/DPD ratio were correlated to disease-free survival in 5-FU treated patients, with p=0.05 and p=0.002, respectively.

Conclusion: We present a novel, high throughput approach for TP, DPD and TS mRNA quantification in archival, microdissected tissue specimens. Besides a significant correlation of TP, DPD and TS mRNA expression with tumor histology and stage, the TS:DPD ratio may represent a prognostic marker in colorectal cancer patients treated with resection alone. Moreover, DPD mRNA expression and the TP:DPD ratio were identified as potential predictive markers for disease-free survival in adjuvant 5-FU treated colorectal cancer patients.

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DCC protein expression in colorectal cancer (CRC) metastases and lack of response to biochemically-modulated 5-fluorouracil (FU) among patients (pts) with low level of thymidylate synthase (TS) protein expression

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TS protein expression in CRC metastases has been shown to predict for the clinical response to FU in multiple studies. However, this correlation is not absolute as 1/3 of the pts with low TS levels fail to respond to FU-based chemotherapy (CT). Since the absence of the DCC protein has been shown to convey a poor prognosis to pts with resected CRC and DCC status may affect drug sensitivity, we have investigated whether assessment of DCC expression in CRC metastases could improve response prediction identifying pts that will not respond to FU despite low levels of TS expression. DCC and TS protein were retrospectively analysed by immunohistochemistry on archival, formalin-fixed, paraffin-embedded tumor samples from 41 pts with unresectable metastatic CRC homogeneously treated with a regimen alternating bolus and infused 5-FU with schedule-specific biochemical modulators. Positive immunostaining was detected in 19 out of 41 pts (46%). DCC status was not related to the level of TS expression (high TS: 10/22, 45% versus 9/19, 47%, in DCC negative and positive tumors, p=0.90). Consistently, the mean/median TS score was 1.95/2 and 2.21/2, in DCC negative and positive tumors (p=0.54). The proportion of pts responding to CT was not significantly different between tumors with DCC deletion and those expressing DCC (35% vs 52%, p=0.24). However, among the 22 pts with low TS, 8 out of 10 expressing DCC responded to CT as compared to 5/12 pts with DCC negative tumors (response rate 80% vs. 42%, p=0.06). 7 out of 9 pts with low TS that failed to achieve an objective response had a defective DCC protein expression in their tumors. Although statistical significance was not reached, the improvement in response prediction associated with DCC assessment was specific for the subgroup of pts with low TS expression. DCC status was not related to tumor response, time-to-progression (TTP) or survival (OS) among pts with high TS. Expression of DCC was also associated with a longer TTP and OS in the whole cohort of pts (8.3 vs 7.2 months, p=0.06 and 21.4 vs 14.3, p=0.05, respectively) but the rates of objective responses were similar in the two groups (35% vs. 52%, p=0.24). These data indicate that DCC maintains its prognostic value in advanced CRC and suggest that the poor prognostic features of tumors without DCC expression may overcome the favorable condition of a low level of TS expression accounting, at least in part, for the therapeutic failures observed in this group.

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Capecitabine and Mitomycin C (MMC) is an active well-tolerated regimen as first line treatment for metastatic colorectal cancer (MCRC).

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The combination of MMC and protracted venous infusion (PVI) 5FU has demonstrated superior efficacy compared to PVI 5FU alone as initial therapy in MCRC. Due to potential synergy based on upregulation of thymidine phosphorylase by MMC coupled with non-overlapping toxicity profiles capecitabine and MMC could enhance these results further. This phase II study was designed to evaluate the safety and efficacy of capecitabine in combination with MMC as first line treatment in MCRC.

Methods: Eligible patients were required to have WHO performance status (PS) 0-2, measurable disease, written consent, received no prior lines of palliative chemotherapy or adjuvant chemotherapy within the previous 6 months. Capecitabine (1250 mg/m2) was given twice daily for 14 days followed by 7 days rest, every 3 weeks, and MMC (7mg/m2 IV bolus) was given once every 6 weeks, maximum of 4 injections. CT response assessment according to RECIST criteria took place at 12 and 24 weeks.

Results: 64 patients have been accrued and complete toxicity and response data are available for 61 and 62 patients respectively. Median age 69 (range 29-82) years, PS was 0, 1, 2 in 36%, 58%, and 6% of patients. 55% of patients had more than 2 sites of disease. Overall response rate was 36% [95% CI: 24.3- 48.9] and 28% of patients had stable disease. Grade 3-4 toxicities were diarrhoea 12.5%, hand foot syndrome 22%, nausea and vomiting 3%, neutropenia 5%, and stomatitis 0%. Median failure free survival was 7 months.

Conclusion: Capecitabine and MMC has significant activity and a relatively favourable toxicity profile for previously untreated patients with MCRC.