3038 POSTER

Left-sided microsatellite unstable colorectal cancers show less frequent methylation of hMLH1 and CpG island methylator phenotype than right-sided ones

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Background: MSI colorectal cancer occurs in 10 to 20% of unselected series of patients with colorectal cancer. Somatic hMLH1 promoter methylation is reported to cause MSI in sporadic cases. Many researchers report that MSI colorectal cancers are more frequently located in the right-side colon than MSS colorectal cancers. Though the number is very small, some MSI colorectal cancers are located in the left-side colorectum. We focused on the existence of left-sided MSI colorectal cancers and investigated whether they arise through hMLH1 methylation as they do in right-sided ones.
Materials and Methods: Thirty-eight sporadic MSI colorectal cancers were included in the study. The methylation status of hMLH1, p16, MINT1, 2 and 31 were examined and the proportions of methylated samples for each locus were compared.
Results: The left-sided group showed significantly less frequent methylation in hMLH1, p16, MINT1, 2 and 31. The frequency of CIMP+ samples in the left-sided group was significantly lower than the right-sided group.
Conclusions: Left-sided MSI colorectal cancers show significantly less frequent methylation of hMLH1. They also showed significantly less frequent occurrence of CIMP+ than right-sided ones. It is possible that left-sided MSI colorectal cancers differ from the right-sided ones in the way of acquiring MSI.

3039 POSTER

Flow cytometric determination of circulating endothelial cells in advanced colorectal cancer patients treated with bevacizumab-based combination therapy

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Background: Antiangiogenetic therapy is a promising approach to cancer treatment but, to date, no pharmacodynamic marker for its efficacy, easy to detect in clinical setting, has been validated. The flow cytometric (FCM) evaluation of circulating endothelial cells (CECs) and their progenitors (CEPs) has been proposed as a surrogate biological marker of angiogenesis because the well-known correlation with tumor angiogenetic activity and growth. CECs and CEPs blood concentrations in untreated cancer pts are significantly increased in comparison to healthy subjects, correlating with the tumor progression. Recent data suggest that their modification during antiangiogenetic therapy have a potential role as prognostic markers in breast cancer pts (Mancuso P, Blood 2006). No data are available on the effect of Bevacizumab-based first-line therapy on blood concentration of distinct population of CECs and CEPs in metastatic colorectal cancer (mCRC).
Material and Methods: We analyzed blood levels of CECs (resting and activated) and CEPs by a 4-colour FCM in 15 normal donors (M/F: 10/5, median age 37 yrs), in 5 mCRC pts treated with first-line chemotherapy (CT) (M/F: 1/4, median age 67 yrs) and in 8 mCRC pts receiving a fist-line therapy including Bevacizumab (M/F: 4/4, median age 56 yrs). Resting CECs were defined as negative for CD45 and CD106 and positive for CD34 and CD146. Activated CECs were defined as CD45−, CD34+, CD146+ and CD 106+ cell. CEPs were depicted by the expression of the stem cell marker CD133.
Results: With respect to normal donors, mCRC pts treated with CT alone in first-line setting show a decrease of absolute number of the two CEC subsets and of the CEPs. At the same time, Bevacizumab-based therapy correlates with a trend toward the increase of CEPs and CECs, especially in activated subsets, in comparison to mCRC pts treated with CT alone. No statistically significant correlation was found between this trend and the number of antiangiogenetic treatment courses administered.
Conclusions: We suggest that the determination of CECs and CEPs by FCM could be an effective and rapid method for monitoring the clinical impact of antiangiogenetic therapies in mCRC pts.

3040 POSTER

Optimal dose for an every 2 week (q2w) cetuximab (C) regimen in patients (pts) with metastatic colorectal cancer (mCRC): a phase I safety, pharmacokinetics (PK) and pharmacodynamics (PD) study of weekly (q1w) and q2w schedules

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Background: This study compared the safety, PK and PD of a q2w schedule of cetuximab (Erbitux®) with the approved q1w regimen in pts with EGFR-expressing mCRC.
Methods: C was given first-line to 13 pts in Group A (control; 400 mg/m^2 initial dose then 250 mg/m^2/week). In Group B (experimental), 38 pts received C q2w: 10 pts each at 400, 500, 600; 8 pts at 700 mg/m^2). Doses were escalated in the absence of dose-limiting toxicities (DLTs: Grade [Gr] 3/4 toxicities, except for hypersensitivity reactions, Gr3 skin toxicity, and/or <66% of the assigned dose due to toxicity). Complete PK profiles for 6w treatment with single-agent C were obtained for all groups. FOLFIRI (irinotecan/5-FU/FA) was then added. Skin and tumor biopsies obtained before therapy and on day 28 were analyzed for EGFR signaling, proliferation and apoptosis by IHC. Plasma samples and tumor biopsies were analyzed for protein and gene expression.
Preliminary results: 51 pts have been included. One DLT (Gr4 dyspnea) was reported at 700 mg/m^2. The safety profiles from all groups were similar. PK parameters ($t_{1/2}$, CL_{ss}) from the q2w (400–600 mg/m^2) regimens are in the range of data from the weekly group. Trough levels for 500 and 600 mg/m^2 q2w regimens and the weekly regimen are comparable, whereas levels for 700 mg/m^2 are considerably higher. PD data in skin show significant changes in pEGFR, pMAPK, Ki67, p27 and pSTAT3 with no major differences between the different C schedules. Biomarker analysis in tumor is ongoing.

Response

Cetuximab dose	Group A, control: weekly	Group B, experimental: every 2 weeks	
	250 mg/m^2 (n = 13)	400 mg/m^2 (n = 10)	500 mg/m^2 (n = 10)
Best overall response, n (%)			
Partial response (PR)	4 (31)	5 (50)	4 (40)
Stable disease (SD)	7 (54)	5 (50)	6 (60)
Progressive disease (PD)	2 (15)	0	0
Overall response rate (CR+PR), % [95% CI]	31 [9.1, 61.4]	50 [18.7, 81.3]	40 [12.2, 73.8]
Disease control rate (CR+PR+SD), % [95% CI]	85 [54.5, 98.1]	100 [69.2, 100]	100 [69.2, 100]

Response data for 600 mg/m^2 and 700 mg/m^2 groups are not yet available
Conclusions: Cetuximab can be safely administered in a q2w regimen from 400–700 mg/m^2. The MTD of q2w cetuximab has not been reached. Available overall response rates in the q2w regimens (400 and 500) and weekly are comparable. Trough level PK data for 500 and 600 mg/m^2 q2w regimens and the weekly regimen are in the same range, whereas levels for 700 mg/m^2 are considerably higher. Based on these data, cetuximab 500 mg/m^2 q2w may be an alternative and convenient dose and schedule of administration.

3041 POSTER

Alpha(1,6)fucosyltransferase immunohistochemical expression and the survival of patients with colorectal cancer

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Increased levels of fucose residues and changes in fucosylation patterns, as a result of the different expression of various fucosyltransferases, act as specific markers in several tumour processes. For example, in human ovarian serous adenocarcinomas, both α(1,6)fucosyltransferase [α(1,6)FT]

mRNA and protein levels show important and specific elevations. Moreover, in papillary carcinoma the increase of α (1,6)FT content has been related with the biological aggressiveness and anaplastic transformation of the primary tumour. In previous studies we have demonstrated a significant increase of α(1,6)FT immunohistochemical expression in colorectal cancer (CRC) tissues. This enzyme expression was also higher in advanced stages of the tumour than in early ones. In the present study, we have evaluated the relation between α(1,6)FT expression and the patient survival with the aim to determinate the prognostic value of α(1,6)FT expression in CRC.
With this purpose, 101 colorectal tumour tissues were analysed by immunohistochemistry. The semiquantitative staining analysis was performed by expert pathologists. The statistical analysis was performed by the Kaplan-Meier method, the log-rank test and the Cox's multivariate analysis.
The difference between the overall survival and the disease-free survival rates from patients with weak (82.6% and 79.7%, respectively) and strong (79.4% and 69.3%, respectively) α(1,6)FT expression was not statistically significant ($p = 0.61$ and $p = 0.22$, respectively). Nevertheless, we found that patients with weak α(1,6)FT expression present relapse-free survival rates (94.9%) significantly higher ($p = 0.012$) than patients with strong expression (77.8%). Besides, the strong α(1,6)FT expression in patients with advanced stages and lymph node invasion was associated with a worse rate of overall and disease-free survival. On the other hand, in multivariate analysis the lymph node metastasis was found to be an independent prognostic factor for disease-free survival ($p = 0.004$), as well as the intensity of α(1,6)FT expression was the only prognostic factor for relapse-free survival ($p = 0.014$).
In summary, it seems that α(1,6)FT immunohistochemical expression is really associated with the capacity of the primary tumour to form new tumour focus in the intestinal wall. Thus, α(1,6)FT expression could be a clinically useful information for patient survival.

3042 POSTER

Impact of the number of examined lymph nodes on prognosis in colon cancer: a population based study in North Netherlands

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Background: Lymph node metastasis is a determinant factor in the adjuvant treatment and an important predictor of survival in colon cancer patients. Adequate surgical resection and punctual pathological examination of the resected specimen is desirable. We studied whether the number of reported lymph nodes examined in colon specimens has an effect on stage migration and survival.
Patients and Methods: Between 1998 and 2002 a total of 2751 resections of colon cancer were performed in 17 hospitals and analyzed in 7 pathology laboratories in the northern part of The Netherlands. The Comprehensive Cancer Centre North-Netherlands, which covers a population of 2.1 million inhabitants, superintends the quality of guidelines and follow-up of cancer patients. Factors associated with the number of examined lymph nodes were studied as well as the effect of tumor characteristics and number of lymph nodes on nodal status and survival. The influence of possible determinants was tested in a general linear model after transformation for continuous variables and binary logistic regression analysis for nominal variables. Survival was calculated from the date of diagnosis until the date of death, the date of most recent linkage with the municipal population registries or the date of last contact.
Results: The number of harvested and examined nodes increased with higher T-stage ($p < 0.001$) and a mucinous morphology ($p = 0.002$), but decreased with increasing age ($p < 0.001$). Localization was also of influence on the number of examined nodes. The proportion of node-positive patients increased with a larger number of nodes examined. T-stage and the number of examined nodes were of significant influence on nodal status. Based on co-morbidity and age, adjuvant chemotherapy was given to 52% of node-positive patients. The 5-year overall survival rate was 49.6% for node-positive patients versus 67.8% for node-negative patients. Survival increased with more nodes examined in node positive as well as node negative patients.
Conclusions: In this study T-stage, localization and patient age are of significant importance for the number of nodes examined by the pathologist. A higher number of examined nodes was associated with an increase in node-positivity and an improved accuracy of the pathological status. Conscientious pathological sampling with more harvested nodes seems to improve nodal staging and through this, more patients can be offered adjuvant treatment leading to a better survival.

3043 POSTER

Anti-tumour effect of L-arginine on gastric and colorectal cancers

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Background: L-arginine has been shown to have anti-atherogenic, anti-oxidant and immunomodulatory actions. The effect of L-arginine on gastrointestinal tumours has not been studied. This study aimed to evaluate the effect of L-Arginine on gastric and colorectal cancer cell lines in terms of effects on cell growth and the cell cycle.
Materials and Methods: AGS (gastric adenocarcinoma) and WiDr (colorectal adenocarcinoma) were used. L-arginine was added at various concentrations and time points. Cell proliferation was assessed using MTT assay. Flow cytometry was undertaken to analyse effects on the cell cycle. Three experiments were performed with six replicates undertaken for the MTT assay. Statistical analyses were undertaken using the Student t test.
Results: There was inhibition of growth in both AGS and WiDr cells in a dose-dependent manner. Growth inhibition was 22.7% ($p < 0.01$) for AGS cells and 32.5% ($p < 0.05$) for WiDr cells when incubated with 8 mM L-arginine after 48 hours compared with untreated cells. When incubated with 32mM L-arginine the inhibition was 61.5% ($p < 0.01$) for AGS cells. With WiDr cells the inhibition was 60.8% ($p < 0.01$) at 32 mM L-arginine. Flow cytometric analysis of the cell cycle did not show any differences between L-arginine-supplemented and non-supplemented AGS and WiDr cells.
Conclusion: L-Arginine inhibits cell growth of gastric and colorectal cancer cells in a dose-dependent manner. These results suggest that alteration of cell cycle kinetics is not the mechanism of this inhibition and further studies are required to understand how this inhibition occurs.

3044 POSTER

Capecitabine or Folinic acid/Fluorouracil i.v. bolus plus Eloxatin evaluation (COFFE trial) in metastatic colorectal carcinoma (MCRC): final results of the Southern Italy Cooperative Oncology Group (SICOG) phase III trial 0401

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Background: Preliminary safety analysis of the biweekly OXXEL regimen compared with the biweekly OXAFAFU regimen has previously been reported. Here we report on the response rate (RR), which was the primary end-point of this phase III trial.
Methods: Patients (pts) with MCRC, previously untreated for their metastatic disease, and with at least one bidimensionally measurable lesion, were randomized, after stratification for performance status (0 vs 1−2), and previous adjuvant treatment (yes vs not), to receive either OXAFAFU: oxaliplatin 85 mg/sqm iv on day (D) 1, levo-folinic acid 250 mg/sqm plus 5-fluorouracil 850 mg/sqm iv bolus on D 2; or OXXEL: oxaliplatin 100 mg/sqm iv on D 1, capecitabine 1,000 mg/sqm twice daily orally from D 1 (evening) to D 11 (morning). Cycles were delivered q 2 weeks up to progressive disease (PD), or for a maximum of 12 cycles. A total of 242 pts and 257 PDs have an 80% power to demonstrate, with an alpha error = 0.05, a 15% difference in RR (30% vs 45%), and a 50% prolongation of progression-free survival (PFS). Response and toxicity were assessed according to WHO criteria.
Results: From May 2004 to Jan. 2007, 306 (OXAFAFU, 156; OXXEL, 150) eligible pts were recruited from 23 SICOG centers. Characteristics (OXAFAFU vs OXXEL arm) were: males, 54% vs 66% ($P = 0.029$); median age (range), 65 (37−83) vs 64 (39−84) yrs; age $\geqslant$70 yrs, 42% vs 32% ($P = 0.062$); primary colon, 70% vs 72%; adjuvant CT, 24% vs 23%; PS 0, 59% vs 61%; liver mets, 76% vs 84%; $\geqslant$2 sites, 57% vs 49%; basal CEA $\geqslant$5 ng/mL: 78% vs 77%. Median number of delivered cycles was 8 (range, 2−12) in both arms. CRs+PRs were 6+45, RR=33% (95% CI, 0.25−0.41) in the OXAFAFU arm, and 11+39, RR=33% (95% CI, 0.26−0.42) in the OXXEL arm. So far, a PD was registered in 211 pts; median PFS was 6.4 (95% CI, 5.8−7.0) mo. for OXXEL arm, and 6.1 (95% CI, 5.5−6.7) mo. for OXAFAFU arm(HR = 0.92; 95% CI, 0.70−1.21, $P = 0.550$). Severe neutropenia (12% vs 27%) and febrile neutropenia (6% vs 13%) favored the OXXEL regimen, but G $\geqslant$ 3 emesis (7% vs 2%), diarrhea (14% vs 8%), and skin toxicity (4% vs <1%) were less frequent with the OXAFAFU regimen.