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Influence of smoking on the clinico-pathological features of colorectal cancer: review of a prospective database

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Background: Smoking is a risk factor for colorectal cancer (CRC) development. The impact of smoking on clinico-pathological features of CRC is not well defined. A prospective cancer database was used to review the effect of smoking status on the clinico-pathological features of CRC.
Material and Methods: Data from 5 hospitals in Victoria, Australia, was collected prospectively between January 2003 and December 2008. All stages of CRC were included. Patients were classified as never smokers, ex-smokers (no cigarettes for at least one month) or current smokers. Demographic factors, histopathology and post-surgical complications were compared when stratified by smoking status. Data was analysed using a repository (BioGrid Australia) allowing linkage of de-identified data across institutions.
Results: In total 1805 patients were identified with known smoking status and CRC. 13.7% (n = 245) were current smokers, 30% (n = 541) ex-smokers and 56.5% (n = 1019) never smokers. The median age of current smokers was significantly younger (61) than for ex-smokers (70.7) and never smokers (68.6), p < 0.0001. 20% (n = 49) of current smokers with CRC were under 50 years old, vs 10.2% (n = 104) of never smokers (p < 0.0001). Less women were current or ex-smokers (29%, n = 236) than men (67%, n = 550) (p < 0.0001).
Current smokers had significantly less screen-detected CRC: 4.5% (n = 11), compared with never smokers, 8.8% (n = 90, p = 0.02). Smokers were more likely to be diagnosed with rectal cancer (38%, n = 93) than ex- or never smokers (31%, n = 487) (p = 0.04). Smokers presented with fewer stage III cancers than never smokers (20.8%vs29.2%, p = 0.009). Median body mass index (BMI) was lower for current smokers (25.4kg/m^2) than ex- or never smokers (26.75kg/m^2, p = 0.0009). There were no significant differences for diabetes status or lymph node yield.
Despite being younger, significantly more post-surgical medical and surgical complications occurred in current smokers. Return to theatre occurred in 8.2% of current smokers (n = 20) vs 2.2% of never smokers (n = 22), p < 0.0001. Post-operative inpatient death occurred in 3.7% (n = 9) of current smokers vs 1.8% (n = 18) of never smokers (p = 0.08).
Conclusions: Smokers present with CRC at a younger age; are less likely to be screen-detected; have lower BMI; have more rectal rather than colon cancer; and have more post-surgical complications despite being younger. An independent association for CRC and BMI will be presented in multivariate analysis. Given that 20% of smokers were diagnosed under 50 years of age, screening for smokers from a younger age than current guidelines, may be appropriate.

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Calcium/magnesium (CaMg) reduces grade 2+ oxaliplatin-induced neurotoxicity in patients with glutathione S-transferase pi 1 (GSTP1) I105V polymorphism

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Background: FOLFOX has emerged as a standard therapy in colorectal cancer. Oxaliplatin's dose-limiting toxicity is a cumulative sensory neurotoxicity (sNT) which commonly requires stop of therapy before tumor progression. Polymorphisms in GSTP1 have been implicated in susceptibility for early onset of sNT on FOLFOX. We analyzed GSTP1 in patients (pts) enrolled in N04C7, a phase III trial to evaluate IV CaMg vs placebo as neuroprotectant for adjuvant FOLFOX in colorectal cancer.
Materials and Methods: Of 102 pts enrolled, 98 evaluable pts provided blood for DNA extraction. TaqMan was used for GSTP1 I105V genotyping. Primary endpoint of N04C7 was to compare grade 2+ chronic sNT during or after therapy between CaMg and placebo. Secondary endpoints were to compare among GSTP1 subgroups (A/A wildtype, A/G heterozygous, G/G I105V polymorphism): overall grade 2+ sNT, time to grade 2+ sNT, and time to grade 2+ sNT before reaching cumulative oxaliplatin dose of 800 mg/m^2.
Results: No difference was found (Chi-square, p = 0.8) in grade 2+ NT among the GSTP1 subgroups: A/A (14/44), A/G (11/38), G/G (6/16). Interestingly, in GSTP1 I105V pts (i.e., G/G, n = 16), 0/6 pts treated with CaMg experienced grade 2+ NT compared to 6/10 pts treated with placebo (Fisher's Exact Test, p = 0.03). In contrast, in GSTP1 A/A or A/G pts (n = 82), 11/42 pts treated with CaMg experienced grade 2+ NT compared to 14/40 pts treated with placebo (Chi-square, p = 0.4). Finally, we observed no association between GSTP1 subgroups and time to grade 2+ sNT or time to grade 2+ sNT before reaching cumulative oxaliplatin dose of 800 mg/m^2 (Kaplan-Meier, all p > 0.5).
Conclusions: This study provides preliminary evidence that pts with the GSTP1 I105V polymorphism could benefit from CaMg as neuroprotectant against oxaliplatin-induced sNT. Further prospective validation of GSTP1 I105V and its association with the effectiveness of CaMg is warranted.

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Heterogeneous expression of cyclooxygenase-2 and inducible nitric oxide synthase within colorectal tumors: correlation with tumor angiogenesis

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Background: Recent studies have shown that the cyclooxygenase (COX) and the inducible nitric oxide synthase (iNOS) pathways are involved in the development of tumor angiogenesis in human cancers. We aimed at investigating whether a different pattern of COX-2 and iNOS expression/activity exists within different areas of colorectal tumors and to analyze the relationship between these two enzymes and tumor angiogenesis.
Methods: Microvessel density (MVD) and COX-2, iNOS, vascular endothelial growth factor (VEGF) and VEGF receptor-2 (VEGFR-2) protein expression were evaluated at both the invasive front (IF) and the tumor center (TC) in 46 human colorectal cancer specimens. We also investigated the concentration of PGE_2 and NO at the same sites.
Results: COX-2 and iNOS protein expression and activity were significantly higher within the IF than the TC of the tumor specimens. Similarly, MVD and VEGF/VEGFR-2 expression significantly increased from the TC to the IF. Only COX-2 expression was significantly correlated with MVD and VEGF/VEGFR-2 expression at both the TC and IF.
Conclusion: Our study shows a heterogeneous expression of COX-2 and iNOS in colorectal cancer. The up-regulation of COX-2 at the IF parallels an increase in vessel density and VEGF/VEGFR-2 expression, thus supporting the hypothesis that the tumor periphery is the most aggressive portion of a colorectal tumor.

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BRAF V600E mutation and Amphiregulin (AR) immunohistochemical expression in the prediction of benefit from cetuximab plus irinotecan in KRAS wild-type metastatic colorectal cancer (mCRC) patients

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Background: *BRAF* V600E mutation is suggested to predict resistance to anti-EGFR monoclonal antibodies, in *KRAS* wild-type (wt) mCRC patients. Also the expression of the endogenous EGFR ligand AR might play a predictive/prognostic role.
Materials and Methods: We retrospectively assessed *KRAS* codon 12−13, *BRAF* V600E mutations and AR expression at immunohistochemistry (IHC) in 86 mCRC patients treated with cetuximab plus irinotecan. *KRAS* and *BRAF* mutations were detected by PCR and sequencing and AR-IHC was performed on tissue sections from paraffin-embedded tumors.
The correlation among *BRAF* mutations, AR expression (as a continuous variable) and clinical outcome was investigated in the subgroup of *KRAS* wt patients.

Results: 86 patients were included. M/F = 44/42, median age = 67 (41−78), median number of previous lines of chemotherapy = 2 (1−5). In the subgroup of 52 (60%) *KRAS* wt patients, *BRAF* mutation was associated with a trend toward lower response rate (RR 1/10, 10% vs 12/42, 29%; OR: 3.86 [95%CI: 0.44−33.88], p = 0.224) and with significantly shorter PFS (HR: 2.33 [95%CI: 1.12−4.84], p = 0.023) and OS (HR: 3.51 [95%CI:1.55−7.98], p = 0.003). *KRAS* wt patients with higher AR expression showed a trend toward better RR (OR: 0.94 [95%CI: 0.88−1.02], p = 0.119) and PFS (HR: 0.971 [95%CI:0.938−1.005], p = 0.095) that translated into significantly longer OS (HR: 0.950 [95%CI: 0.907−0.995], p = 0.030. A strong association between *BRAF* mutations and lower AR levels was found both in the overall population (t-test; p = 0.0005) and in *KRAS* wt subgroup (t-test; p = 0.0023). In the subgroup of 40 (47%) *KRAS* and *BRAF* wt patients AR expression did not predict RR (OR: 0.969 [95%CI: 0.898−1.046], p = 0.422) nor PFS (HR: 0.983 [95%CI: 0.948−1.019], p = 0.345) nor OS (HR: 0.968 [95%CI: 0.924−1.014], p = 0.175).
In *KRAS* wt subgroup, at the multivariate analysis *BRAF* mutation retained its predictive value in terms of both PFS (HR: 2.577 [95%CI: 1.103−6.022], p = 0.029) and OS (HR: 3.472 [95%CI: 1.417−8.506], p = 0.007), while AR expression did not predict PFS (HR: 0.982 [95%CI: 0.947−1.018], p = 0.320) nor OS (HR: 0.968 [95%CI: 0.924−1.014], p = 0.17).
Conclusions: *KRAS* and *BRAF* mutations are confirmed as predictors of resistance to cetuximab plus irinotecan. The significant association between *BRAF* mutations and lower AR expression suggests that decreasing levels of AR expression may be an epiphenomenon of *BRAF* mutations. Future studies of potential predictors of benefit should take into account their possible overlap.

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K-ras and B-raf mutation analysis has clinical value in stage III colon carcinoma

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Background: Mutations in the k-ras pathway have been widely studied in metastatic colon carcinoma due to their value as predictive markers of response to anti-epithelial growth factor receptor therapy. However the value of disruption of this pathway in other stages of colon carcinoma remains unknown.
Our aim is to study the clinical value of mutations in the *k-ras* and *b-raf* genes in a well defined and clinically homogeneous group of stage III colon carcinoma patients.
Patients and Methods: 213 patients with stage III disease treated with surgery followed by 5-FU based adjuvant therapy were selected. DNA was isolated from selected areas of paraffin material, after determination of percentage of tumoral cells. K-ras mutations in codons 12 and 13 were determined by sequencing. The V600E mutation in the B-raf gene was studied by real time PCR with specific probes for the mutated and the wild type allele. MSI status was determined by typing the BAT 26 marker which is positive in 99% of MSI positive Caucasian patients.
Results: Median age of the group was 64 years (30−83), median follow up was 47 months (4–133). 56.8% of the patients was male and 52.6% had a right sided tumor. 76.4% of the patients had less than 4 positive lymph nodes at diagnosis and 73.7% had a T_3 tumor. 14% was MSI positive, 19.5% had a mutation in the *b-raf* gene and 35% had a mutation in the *k-ras* gene. Mutations in the *b-raf* and *k-ras* genes were mutually exclusive. There was a significant relationship between B-raf mutation and MSI positive tumors (p < 0.0001) and between B-raf mutation and right sided disease (p < 0.0001). In our group the presence of a mutation in the *k-ras* gene significantly correlated with developing a distant metastasis or local recidive during follow-up (p = 0.009).
In a multivariate survival analysis adjusting for known prognostic factors like lymph node status, T status, age, gender, tumor location, MSI, B-raf and K-ras mutations; the V600E mutation in B-raf was an independent factor significantly predicting a worse overall survival (p = 0.006 95% CI (0.21−0.78)). K-ras mutations was an independent factor predicting shorter disease free survival (p = 0.028 95% CI (0.34−0.94)).
Conclusion: We conclude that mutation analysis of the K-ras pathway is a useful clinical tool to predict overall survival and disease free survival in stage III colon carcinoma patients.

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VEGF gene polymorphisms in the prediction of benefit from first-line FOLFIRI plus bevacizumab (BV) in metastatic colorectal cancer (mCRC) patients (pts)

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Background: Addition of BV to first-line irinotecan plus 5FU improves PFS and OS of mCRC pts. Meanwhile, the anti-VEGF causes specific toxicities and increases costs of treatment. At the same time, not all pts derive an equal benefit from the VEGF inhibitor. So far, molecular predictors of BV efficacy have not yet been identified. Specific *VEGF* polymorphisms may affect gene transcription, thus indirectly influencing efficacy of BV.
Materials and Methods: Peripheral blood samples for genomic DNA extraction were collected from consecutive mCRC pts receiving FOLFIRI plus BV as first-line treatment (BV-group). *VEGF* −2578A/C, −460C/T, +405C/G, +936C/T polymorphisms were analysed by means of PCR-RFLP. One-hundred-seven pts, treated with FOLFIRI alone, served as historical control group.
Results: One-hundred-eleven pts were included in the BV-group. M/F = 57/54, median age = 63 (34−82), Köhne score (low/intermediate/high/data missing) = 57/39/12/3. Sixty-nine out of 111 pts achieved response (RR = 62%). Median PFS (mPFS) and median OS (mOS) were 10.2 and 22.2 months, respectively. *VEGF* −460C/C, C/T and T/T allelic variants were found in 20%, 54% and 26% of pts, respectively. −460 T allele demonstrated shorter PFS and OS with an additive effect of each T allele (PFS: HR = 2.65, [1.49−6.62], p = 0.003; OS: 2.47, [0.91−7.66], p = 0.074). −460C/C pts achieved significantly longer PFS and OS in comparison to pts carrying at least one T allele (mPFS: 12.8 vs 9.8 months; HR = 0.48 [0.28−0.85], p = 0.012; mOS: 27.3 vs 20.5 months; HR = 0.38 [0.19−0.94], p = 0.034). In the control group mPFS and mOS were 8.2 and 20.6 months; −460C/C, C/T and T/T variants were found in 23%, 52% and 25% of pts, respectively; there was no significant association with PFS or OS. Other investigated polymorphisms did not affect outcome neither in BV-group nor in the control group.
Conclusions: At our knowledge this is the first report of a pharmacogenetic determinant of improved PFS and OS for mCRC pts treated with first-line BV-containing therapy. The observation that *VEGF* −460C/T variants did not influence the outcome in the control group led to hypothesize a predictive other than a prognostic role for such genetic signature. These preliminary data deserve investigation in prospective, randomized, validating trials.

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Epidermal growth factor receptor (EGFR) expression in stage II-III colon carcinoma (CC) – nine years of follow-up

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Background: Epidermal growth factor receptor (EGFR) is a protoncogen that is found overexpressed in colorectal carcinomas and it correlates with a worse prognosis. The aim was to describe EGFR overexpression patterns in non-metastatic CC and to correlate these data with follow-up.
Methods: We analyzed a series of 194 CC. Inclusion criteria were: a) resected primary adenocarcinoma; b) curative surgery; c) pT3 N0−2 M0 without progression during the first 6 months post surgery; d) minimum follow-up over 8 years. EGFR overexpression was analyzed by immunohistochemistry (IHC) using the Dako PharmaDx kit (Glostrup, Denmark). As positive control the Dako slides and a bloc cell of A431-AAM cells were used. Presence of cytoplasmic and membrane patterns (intensity 1(+), 2(+) and 3(+)) were evaluated as well as the percentage of positive cells. Statistical analysis: association between qualitative variables was analyzed by Fisher's exact test. Disease-free and overall survival distributions were estimated by the Kaplan-Meier method and were analyzed with the log rank test. All *P* values are from two-sided statistical tests.