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Circulating tumour cells in colorectal cancer: multi-gene expression analysis during chemotherapy

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Background: We have developed a new preanalytical enrichment method for circulating carcinoma cells (CTC) based on EpCAM and MUC1 specific antibodies coupled to immunomagnetic beads. Molecular detection and tumor cell characterization was performed with a multimarker panel by real-time RT-PCR. Here we present first results of a universal marker panel for local advanced and/or metastatic colorectal cancer.
Methods: Samples from patients were divided in native probes and matched calibrator probes containing 2 and 10 carcinoma tumor cells (ETC). The high affinity antibodies BM7 (MUC-1) and VU1D9 (EpCAM) were used for immunomagnetic tumor cell enrichment from 10 ml peripheral EDTA-blood of patients with documented metastatic and/or local advanced disease. Separated cells were lysed and used for mRNA isolation and c-DNA synthesis. Real-time quantitative RT-PCR approaches with SYBR assays (Eurogentec) and FAM-labeled TaqMan probes selected with the UniversalProbeLibrary system (Roche AG, Basel, CH) were developed for the epithelial markers cytokeratin19 and 20 (CK19/20), EpCAM, CEA, Survivin, CD276, metastasis associated in colon cancer (MACC) transketolase TKTL1 and HIF-1alpha.
Results: Sensitivity of the the multimarker panel was validated in calibration tests with 2 cells and 10 cells (embedded tumor cell calibrators, ETC) and the specificity of the panel was confirmed by examination of blood from healthy donors. Positivity rate of ETC controlled real-time RT-PCR on the basis of the multimarker panel was 76% (13 of 17 patients) with local advanced and/or metastatic disease. 11 patients (65%) showed two or more positive markers. The marker with the highest prevalence was Survivin (53%) followed by EpCAM (47%), CEA (29%), CK20 (29%), MACC (24%) and CK19 (12%).
Conclusion: We have used embedded tumor cells (ETC) as internal calibrators for accurate process control and normalization of the immunobead quantitative RT-PCR technique. The newly introduced surrogate marker panel is linked to cellular characteristics as apoptosis, invasion, angiogenesis and stem cell phenotype. Therefore it may help to give additional information on prognosis and therapy response. It can be used in patient follow-up to detect early metastasis. It also might lead to both tailored therapy regimes and revised indications for adjuvant treatment, independent of lymph node involvement.

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Single nucleotide polymorphisms in the vascular endothelial growth factor A gene predicts response to chemotherapy in patients with metastatic colorectal cancer

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Background: Besides the cytotoxic effect on cancer cells, chemotherapy may exert a similar effect on the rapidly dividing endothelial cells involved in the tumour associated neoangiogenesis. These immature endothelial cells are known to be dependent on vascular endothelial growth factor A (VEGF-A) as a survival factor. Recent evidence suggests a clinical importance of single nucleotide polymorphisms (SNP's) in the *VEGF-A* gene, but a possible association between these SNP's and the efficacy of chemotherapy has not been elucidated. The aim of this study was to investigate the predictive and prognostic role of SNP's in the *VEGF-A* gene in relation to first line treatment with capecitabine and oxaliplatin in patients with metastatic colorectal cancer (mCRC).
Materials and Methods: The study prospectively included 72 patients with mCRC. Genomic DNA was isolated from whole blood, and 4 SNP's in the *VEGF-A* gene were analysed by polymerase chain reaction. Clinical response was assessed by radiologic examination. The response evaluation criteria in solid tumors (RECIST) were used for evaluation, and compared by a chi-square test. Progression free survival (PFS), according to genotypes, was compared using the Kaplan-Meier method and the log rank test, and the Cox regression method was used for multivariate analysis.
Results: Three of the 4 SNP's demonstrated a significant association with response to chemotherapy. Response was observed in approximately 25% of the patients with heterozygous genotypes, compared to 55% in the patients with homozygous genotypes; the +405 G/C SNP, $p = 0.02$; the −460 C/T SNP, $p = 0.008$; and the −2578 C/A SNP, $p = 0.001$. Heterozygosity in two of these 3 SNP's were significantly associated with PFS, but only the +405 GC genotype remained an independent prognostic marker after multivariate analysis, hazard ratio 2.07, 95% confidence interval 1.05−4.10, $p = 0.04$.
Conclusions: The results demonstrated an association between 3 SNP's in the *VEGF-A* gene and response to first line treatment with capecitabine and oxaliplatin in patients with mCRC, which translated to a significant difference in PFS. The results call for validation in a bigger prospective trial.

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Genotype subset selection of multi-UGT1As polymorphisms can predict severe neutropenia and tumour responses of metastatic CRC patients received FOLFIRI regimen

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Introduction: The pharmacogenetics of irinotecan indicate that a common polymorphism in the uridine diphosphate glucuronosyltransferase 1As (UGT1As) gene predict severe toxicity. However the tumor response to irinotecan is variable and unpredictable.
Methods: Two multi-center phase II studies of FOLFIRI (FLIGHT-1 and FLIGHT-2 study) were conducted for patients with metastatic colorectal cancer (CRC) in Japan. FLIGHT-1 study was first-line chemotherapy, and FLIGHT-2 study was FOLFOX-refractory second-line chemotherapy. 103 patients have been enrolled in these studies (53 patients in FLIGHT-1 and 50 patients in FLIGHT-2) from 20 institutions by April 2007 from November 2005. Seventy one patients were analyzed UGT1As polymorphisms, UGT1A1*28 (TA6>TA7), UGT1A1*6 (G>A), UGT1A1*60 (T>C), UGT1A7 (N129K; T>G), UGT1A7 (−57 T>G), UGT1A9*22 (T10>T9).
Results: Out of 71 patients, 34 had G3/4 neutropenia or leukopenia, and 23 had tumor responses (CR + PR). G3/4 neutropenia was more frequent in patients with *6, N129K(G), −57(G), *22 allele than patients without these allele ($p < 0.05$). Other polymorphism was not the predictive factor for toxicity and tumor response, independently. On the other hand, genotype subset selection of multi-UGT1As polymorphisms was useful to predict severe toxicities and tumor responses.
Twelve out of 16 patients with G3/4 neutropenia had either, UGT1A1*28 (TA6/6) & UGT1A9*22 (T9/9) or UGT1A1*28 (TA6/7) & UGT1A7 (−57; T/G). While, 22 out of 29 patients with G0/1/2 neutropenia had either UGT1A1*28 (TA6/6) & UGT1A9*22 (T10/10), UGT1A1*28 (TA6/7) & UGT1A7 (−57; T/T) or UGT1A1*6 (G/G) & UGT1A1*60 (T/T).
Thirteen patients out of 15 non-response patients had either UGT1A1*60 (G/G), UGT1A1*6 (G/G) & UGT1A7 (−57; T/G), UGT1A1*28 (TA6/7) & UGT1A1*6 (G/G), UGT1A1*28 (TA6/7) & UGT1A9*22 (T9/10). While, 8 out of 12 response patients had either UGT1A1*6 (G/A) & UGT1A9*22 (T9/9), UGT1A9*22 (T10/10) & UGT1A1*60 (T/G), UGT1A1*28 (TA6/6) & UGT1A1*60 (T/G) & UGT1A7 (−57; T/G), UGT1A1*28 (TA6/6) & UGT1A7 (−57; G/G).
Conclusions: Genotype subset selection of multi-UGT1As polymorphisms were the excellent predictor for severe toxicities and tumor responses of metastatic CRC patients received FOLFIRI regimen.