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EGFR downstream signaling pathway in primary colorectal tumours and related metastatic sites: optimizing EGFR targeted treatment options

M. Scartozzi[1], I. Bearzi[2], R. Berardi[1], A. Mandolesi[2], C. Pierantoni[1], S. Cascinu[1]. [1]*Azienda Ospedaliera Ospedali Riuniti-Universita' P, Clinica Di Oncologia Medica, Ancona, Italy;* [2]*Azienda Ospedaliera Ospedali Riuniti-Universita' P, Anatomia Patologica, Ancona, Italy*

Although multiple therapeutic options targeting the EGFR-driven molecular pathway have been recently proposed for the treatment of advanced colorectal tumours, the biologic mechanisms underlying the activity of these drugs "in vivo" are still to be fully investigated. In fact, most of the scientific data available so far were unable to correctly identify molecular markers predicting response (or resistance) to EGFR-targeted agents.
After our previous findings of a lack of EGFR status correlation, we analysed the expression of phosphorylated Akt and MAPK in primary tumours and corresponding metastatic sites with the aim to better define the EGFR-related molecular profile of colorectal cancer, in order to serve as a tool for treatment selection Ninety-nine cases (paired primary tumours and metastases with an already determined EGFR status) were available for our study, to date we completed Akt and MAPK analysis in 52 primary tumours and 55 paired metastases. In primary tumours immunohystochemically determined phosforilated Akt and MAPK were positive in 33 (63%) and 32 (62%) cases respectively, whereas Akt and MAPK were positive in 32 (58%) and 36 (65%) metastatic sites respectively. Interestingly EGFR negative primary tumours (27 cases, 52%) expressed Akt and MAPK in 16 (59%) and 17 (63%) cases. Accordingly with these findings also in EGFR negative metastases Akt was expressed in 19 (59%) and MAPK was expressed in 24 (75%) metastatic samples. Akt expression in primary colorectal tumours changed from positive to negative in 11 (21%) paired metastases and from negative to positive in 6 (11%) related metastatic sites. MAPK status in primary tumours changed from positive to negative in 8 (15%) paired metastases and from negative to positive in 11 (21%) related metastatic sites. Taken together our findings suggest that akt and MAPK status in primary tumours does not correlate with Akt and MAPK status in corresponding metastases. Moreover EGFR downstream signaling pathway can be over-activated even in the absence of EGFR expression in a considerable proportion of patients, thus making the use of anti-EGFR treatment with monoclonal antibodies at least theoretically inappropriate in these tumours. On the contrary, in these cases the use of a treatment strategy including small tyrosine kinase inhibitors that can interfere with the downstream pathway of the EGFR, seem more appealing. Complete analysis of all 99 cases will be presented at the meeting.

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Analysis of the SDF-1/CXCR4 chemokine-chemokine receptor axis and downstream effector pathways in human colorectal carcinomas

V. Valera[1], B. Walter[2,3], Y. Koyama[1], M. Kawahara[1], N. Yokoyama[1], T. Iiai[1], H. Okamoto[1], K. Hatakeyama[1]. [1]*Niigata University, First Department of Surgery, Niigata, Japan;* [2]*Niigata University, Division of Otolaryngology, Niigata, Japan;* [3]*Niigata University, Division of Microscopic Anatomy and Bio-imaging, Niigata, Japan*

Introduction: We have previously reported (ECCO12) that the CXC chemokine receptor 4 (CXCR4) is mostly downregulated in colorectal carcinomas (CRC) and that this is correlated with tumor progression. In order to further elucidate the factors involved in this phenomenon, we aimed to: a) evaluate the status of the ligand (SDF-1); b) dissect receptor-ligand downstream signalling pathways and c) evaluate the SDF-1/CXCR4 relationship with standard clinicopathologic variables including patients' survival.
Methods: CXCR4 and SDF-1 mRNA and protein expression was examined by real time RT-PCR, immunohistochemistry and western blot analysis in 134 tissue samples including 56 perfectly matched tumour and normal tissues, normal and metastatic lymph nodes (n = 16) and normal and metastatic liver tissues (n = 6). Protein expression was also analysed in 60 independent samples (tissue microarray) including primary and metastatic tumours and in 30 different human CRC cell lines. Gene expression differences between up, down and unregulated CXCR4 groups were evaluated by a low density oligonucleotide microarray approach using unsupervised clustering analysis and relevance gene networks. Analysis of the phosphorylation status of the MAPK family members ERK 1/2, JNK and P38 at the CXCR4 downstream pathway was performed by western blot.
Results: CXCR4 mRNA and protein were found to be 1.8 and 3.3 fold downregulated as compared with the corresponding normal tissue in 37 (66.1%) of the cases ($P < 0.0001$ and $P < 0.05$ respectively) with no significant changes in SDF-1 levels. Amongst the 90 genes analysed by microarrays, 20 (22%) were found to be differentially expressed between the CXCR4 up and downregulated cases. Genes involved in the MAPK pathway were the most differentially regulated, with differences ranging from 2 to 10 fold in change. These changes were confirmed at the protein level with significant low phoshorylation levels for the ERK 1/2 MAP kinase and higher phosphorylation status for the JNK and P38 MAP kinases resulting in different proliferation-apoptosis patterns. Finally, patients with tumors expressing low CXCR4 levels showed poorer survival probabilities when compared to the upregulated cases.
Conclusion: Our results show that CXCR4 is mostly downregulated in CRC without change in SDF-1 levels. The alterations in downstream signaling pathways following CXCR4 downregulation are likely to be responsible for the different tumor progression profiles observed.

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Indomethacin and Wnt signalling in HT-29 colon cancer cells

S. Kapitanovic[1], T. Cacev[1], M. Antica[1], M. Kralj[1], G. Cavrić[1], K. Pavelic[1], R. Spaventi[2]. [1]*Rudjer Boskovic Institute, Division of Molecular Medicine, Zagreb, Croatia;* [2]*PLIVA D.D., PLIVA Research Institute D.O.O., Zagreb, Croatia*

Nonsteroidal anti-inflammatory drugs (NSAIDs) lower the incidence of and mortality from colon cancer. Although there is much evidence from epidemiological and laboratory studies that NSAIDs have antitumor activity and reduce the incidence of colon cancer, the mechanism of action remains unknown. In this paper, we present the effect of indomethacin on growth inhibition, induction of apoptosis, and alterations in the expression of several genes involved in Wnt signalling in HT-29 colon cancer cells.
We have shown that indomethacin reduces the proliferation rate of HT-29 colon cancer cells and induces apoptosis. Concentrations of indomethacin from 10^{-4} to 10^{-3} M strongly inhibited the growth of HT-29 cells. The inhibition of growth, as well as induction of apoptosis was dose and time dependent. The treatment of cells with 4×10^{-4}M indomethacin caused strong inhibition of cell growth (about 70%), enhanced expression of APC, decreased expression of beta-catenin and induced expression of E-cadherin proteins. Expression of beta-catenin was not markedly reduced instead, beta-catenin was translocated from the nucleus and cytoplasm to the plasma membrane. These results were confirmed by real-time RT-PCR analysis on mRNA level. At a concentration of 4×10^{-4} M indomethacin there was increased expression of *APC* gene (10.9-fold induction; delta delta Ct = 3.43) and *E-cadherin* gene (3.5-fold induction; delta delta Ct = 1.79).
These results suggest the antiproliferative effect of indomethacin may contribute to enhanced cell adhesion through increased expression of E-cadherin and translocation of beta-catenin from the nucleus to the cell membrane.

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Intraperitoneal application of the trifunctional antibody catumaxomab (anti-EpcAM x anti-CD3) for the treatment of peritoneal carcinomatosis due to GI cancer: Results of a phase I trial

M. Stroehlein[1], F. Lordick[2], D. Ruettinger[3], U. Gruetzner[3], H. Menzel[2], K. Bartelheim[4], M. Jaeger[5], H. Lindhofer[5], K. Jauch[3], M.M. Heiss[1]. [1]*University Witten-Herdecke, Surgery, Cologne, Germany;* [2]*Technical University of Munich, Internal Medicine, Munich, Germany;* [3]*University of Munich, Surgery, Munich, Germany;* [4]*Fresenius Biotech, Munich, Germany;* [5]*TRION Research, Munich, Germany*

Background: Peritoneal carcinomatosis (PC) due to GI cancer is associated with a survival of 3–6 months. Due to the lack of effective therapies no standard treatment is currently recommended. The trifunctional antibody (trAb) catumaxomab (anti-EpCAM x anti-CD3) belongs to a new class of intact antibodies. While binding simultaneously to EpCAM+ tumor cells, T cells, and via the intact Fc region to accessory cells, it is able to induce effective tumor cell killing. In a phase I trial the intraperitoneal application of catumaxomab is being investigated in patients with PC due to GI cancer.
Patients and methods: 17 patients after diagnosis of EpCAM+ PC (7 gastric-ca, 9 colon-ca, 1 CUP) have been evaluated up to now. Treatment consisted of 4 escalating doses of catumaxomab administered intraperitoneally within 10 days. The MTD was defined for each of the 1st, 2nd, 3rd and 4th infusion according to a dynamic escalation schedule. Anti-tumor efficacy was evaluated by immunocytochemical analysis of peritoneal lavages before and after treatment. Patients were free to get any further chemo- or radiotherapy after trAb treatment and were frequently followed up. Patient enrollment into additional subgroups is ongoing. Currently 23 patients have been treated. In these patients, shorter duration of infusion with first pharmacokinetic (pk) data and further dose escalation with dexamethasone premedication is investigated.

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.

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

J.L. Soto[1,2,3], N. Garrigos[1,3], J. Gallego-Plazas[1,2], P. Guaraz[1], M. Garcia-Bautista[1,3], A. Castillejo[1,3], A. Gomez[3], C. Casado-Llavona[1,2], A. Rodriguez-Lescure[1,2], A. Carrato[1,2,3]. *[1]Elche University General Hospital, Molecular Oncology Research Unit, Elche, Spain; [2]Eche University General Hospital, Medical Oncology Department, Elche, Spain; [3]Miguel Hernandez University, Institute of Molecular and Cellular Biology, Eclhe, Spain*

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.

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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

J. Kralovánszky[1], B. Budai[1], E. Pandi[1], E. Hitre[1], V. Adleff[1], C. Katona[2], A. Réti[1], V. Komlósi[1], Z. Orosz[1], I. Láng[1]. *[1]National Institute of Oncology, Clinical Research, Budapest, Hungary; [2]Uzsoki Hospital, Oncoradiology Center, Budapest, Hungary*

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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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)

P. Innominato[1,2], G.A. Bjarnason[3], C. Focan[4], C. Garufi[5], S. Iacobelli[6], M.C. Mormont[1,2], J. Waterhouse[7], T. Gorlia[8], F. Levi[1,2]. *or The EORTC Chronotherapy Group*

1.INSERM & Paris XI University, INSERM E0354, Villejuif Cedex, France [2]Paul Brousse Hospital, Oncology Department, Villejuif Cedex, France [3]Toronto-Sunnybrook Regional Cancer Centre, Toronto, Canada [4]Les Cliniques Saint-Joseph, Liège, Belgium [5]Regina Elena Cancer Institute, Roma, Italy [6]"G. D'Annunzio" University, Chieti, Italy [7]University of Manchester, Manchester, United Kingdom [8]EORTC Data Center, Brussels, Belgium

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