6097 POSTER

The recovery of circulating dendritic cells during anti-VEGF treatment is related to clinical outcome in advanced colorectal cancer patients

M. Manzoni[1], B. Rovati[1], S. Delfanti[1], S. Chatzileontiadou[1], K. Bencardino[2], M. Ronzoni[2], S. Brugnatelli[1], F. Luopakis[3], A. Falcone[3], M. Danova[1]. [1]*IRCCS Foundation Policlinico S. Matteo, Medical Oncology, Pavia, Italy;* [2]*S. Raffaele Scientific Institute, Medical Oncology, Milan, Italy;* [3]*Azienda USL-6 Livorno and University of Pisa, Medical Oncology, Livorno, Italy*

Background: The proved clinical efficacy of Bevacizumab (BEV) in metastatic colorectal cancer (mCRC) could be related not only to its well-established effect on tumor neoangiogenesis but also to a counteraction of VEGF-mediated dendritic cell (DC) abnormalities. BEV addiction to chemotherapy (CT) may improve the number and function of blood DCs in cancer pts. We have focused on the correlation between this immunological favourable effect and the clinical efficacy of a multicyclic BEV-based, 1st-line treatment for mCRC.
Material and Methods: Starting from January 2007 we performed a flow cytometric analysis of PB lymphocytes and DC subsets (DC1 and DC2) in 53 mCRC pts who had not received prior CT for metastatic disease or for whom 6 months had relapsed since adjuvant CT (M/F: 31/22, median age: 59 yrs; range 32−75; ECOG PS <2), before and every 3 courses of a BEV+CT (5-FU± CPT11± Oxaliplatin) program. Biological data of the 42 evaluable pts that received all the planned treatment were correlated to both tumor response (OR) and progression free survival (PFS).
Results: During treatment, DCs and their subsets showed a progressive, significant increase in absolute number, with respect to baseline, both in responder (CR, PR, SD) (67%) and in non responder pts; only responder pts keep this immunological effect at the moment of clinico-radiological revaluation, performed at 3 weeks since the last course administration. The DC and DC1 absolute number of pts with PFS > 15 months (58%) increased more evidently during antiangiogenetic-therapy and was significantly higher after therapy completion with respect to DC of pts with shorter PFS ($p < 0.02$).
Conclusions: First-line BEV-based therapy in mCRC pts improves the number of blood DCs, pointing out a potential additional anticancer mechanism of this drug. The recovery of DC does not influence OR but correlates with longer PFS. This suggests that BEV can influence tumor regrowth by contributing to overcome the impairment of the host immune surveillance induced by VEGF.

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Clinicopathologic significance of nuclear factor kappa B, hypoxia-inducible factor 1 alfa, and vascular endothelial growth factor expression in stage III colorectal cancer

H. Kwon[1], S.H. Kim[1], S.Y. Oh[1], S. Lee[1], K.A. Kwon[1], J.H. Lee[1], H.J. Choi[2], K.J. Park[2], M.S. Roh[3], H.J. Kim[3]. [1]*Dong-A University Medical Center, Internal Medicine, Busan, Korea;* [2]*Dong-A University Medical Center, Surgery, Busan, Korea;* [3]*Dong-A University Medical Center, Pathology, Busan, Korea*

Background: Activation of transcription factor nuclear factor-κB (NF-κB) has a role in cell proliferation, invasion and angiogenesis. Hypoxia-inducible factor 1α (HIF-1α) and vascular endothelial growth factor (VEGF) are involved in tumor growth and metastases. The aim of this study was to evaluate the prognostic significances of the expressions of NF-κB, HIF-1α, and VEGF in stage III colorectal cancer.
Materials and Methods: The tumor tissues of 148 patients with stage III colorectal carcinoma that underwent potentially curative resection followed by postoperative adjuvant therapy were investigated immunohistochemically using monoclonal antibodies against NF-κB, HIF-1α, and VEGF. Clinical information, including tumor size, grades, vessel invasion, number of involved lymph nodes, carcinoembryonic antigen (CEA) levels, and disease-free survival and overall survival were evaluated with respect to the expressions of NF-κB, HIF-1α, and VEGF.
Results: Median follow-up duration was 53.2 months, and median patient age was 60±11 years (range 22−82). Positivity rates of NF-κB, HIF-1α, and VEGF were 52.7%, 47.3%, and 61.5%, respectively. NF-κB expression in tumor tissue was found to be significantly correlated with the expression of HIF-1α ($P < 0.001$), VEGF ($P = 0.044$), and presence of vessel invasion ($P = 0.013$). No relationships were found between the expressions of HIF-1α or VEGF and clinicopathologic variables. Univariate analysis showed that NF-κB expression was associated with poor 5-year overall survival (55.8 months vs. 76.9 months, $P = 0.012$). Multivariate analysis confirmed that NF-κB was independently associated with an adverse outcome (Relative risk: 1.59, $P = 0.049$). However, HIF-1α and VEGF were not found to be related to clinical outcome.
Conclusion: NF-κB expression in tumor tissue is associated with angiogenesis and a poor 5-year overall survival in stage III colorectal cancer patients.

6099 POSTER

IVS10+12A>G polymorphism in hMSH2 gene associated with prognosis for patients with colorectal cancer

J.G. Kim[1], Y.S. Chae[1], B.W. Kang[1], S.N. Kim[1], S.J. Lee[1], K.H. Lim[2], H.Y. Chung[2], G.S. Choi[2], S.H. Jun[2]. [1]*Kyungpook National University Hospital, Oncology/Hematology, Daegu, South Korea;* [2]*Kyungpook National University Hospital, Surgery, Daegu, South Korea*

Background: The polymorphisms in DNA repair genes may contribute to a variation in the DNA repair capacity, thereby affecting the risk of carcinogenesis and prognosis of colorectal cancer. Accordingly, the present study analyzed 14 polymorphisms in DNA repair genes and their impact on the prognosis for patients with colorectal cancer.
Materials and Methods: Three hundred and ninety-seven consecutive patients with curatively resected colorectal adenocarcinoma were enrolled in the present study. The genomic DNA was extracted from fresh colorectal tissue and 14 polymorphisms of DNA repair genes (XRCC1, hMSH2, ERCC2, ERCC4, VARS2[rs2074511, rs2249459], XPA, XPC, POLR2A, POLR2B, RFC1, RFC4, XAB2, DNMT3B) determined using a real-time PCR genotyping assay.
Results: The median age of the patients was 63 years (range, 21−85), and 218 (54.9%) patients had colon cancer, while 179 (45.1%) patients had rectal cancer. The pathologic stages after surgery were as follows: stage 0/I (n = 86, 21.7%), stage II (n = 146, 36.8%), stage III (n = 145, 36.5%), and stage IV (n = 20, 5.0%). A multivariate survival analysis, including age, differentiation, CEA level, and stage, revealed a better survival for the patients with the combined IVS10+12AG and GG genotype than for the patients with the IVS10+12AA genotype (disease-free survival [DFS]: hazard ratio [HR] = 0.47, 95% confidence interval [CI], 0.30−0.75, P = 0.002; overall survival [OS]: HR = 0.50, 95% CI, 0.26−0.98, P = 0.042). None of the other polymorphisms was associated with survival.
Conclusions: The IVS10+12A>G polymorphism in the *hMSH2* gene was found to be an independent prognostic marker for patients with colorectal cancer. Accordingly, in addition to the pathologic stage, an analysis of the IVS10+12A>G polymorphism in the *hMSH2* gene could help identify patient subgroups with a high risk of a poor disease outcome.

6100 POSTER

S-1 enhances radiosensitivity in a mouse xenograft model of human colon cancer

C. Murayama[1], A. Kamijo[2], M. Isozaki[1], Y. Maeda[2], H. Kobayashi[1], S. Sadahiro[2]. [1]*Tokai University School of Medicine, Clinical Pharmacology, Isehara, Japan;* [2]*Tokai University School of Medicine, Surgery, Isehara, Japan*

Background: Preoperative radiation therapy (XRT) with concurrent protracted venous infusion (PVI) 5-fluorouracil (5-FU) has become the standard of care for patients with locally advanced rectal cancer (LARC). Oral fluoropyrimidines have been developed as a therapeutic alternative to PVI 5-FU. S-1, a novel oral fluoropyrimidine, has demonstrated comparable therapeutic efficacy with UFT plus leucovorin (LV) in advanced colorectal cancer. In the present study, we compared the radiosensitization by S-1 with UFT or UFT/LV in a mouse xenograft model of human colon cancer.
Material and Methods: KM20C, human colon cancer cells, were implanted in the right hind leg of female BALC/cA-nu mice (N = 10 each group). Fractionated daily dose of 2 Gy was given to tumors on days 1−4 by 6MV X-rays locally. S-1 (5.7 mg/kg/day), UFT (13.9 mg/kg/day) and UFT (13.9 mg/kg/day)/LV (10 mg/kg/day) were administered orally 1 hr before irradiation for 2 consecutive weeks. Equitoxic doses of S-1 and UFT were selected. Tumor response to the treatment was assessed by calculating the tumor growth time (TGT), defined as the time required for the initial tumor volume to grow 5-fold after treatment. The growth delay (GD) was calculated by the formula: GD of each tumor = TGT (treated) of each tumor − average TGT (untreated). Synergy ratio was calculated by the following formula: (GD induced by combination treatment)/[(GD by chemotherapy) + (GD by XRT)]. A result greater than 1 indicates a greater than additive response. The GD produced by each treatment was analyzed by Mann-Whitney's U test. $P < 0.05$ was considered to denote statistical significance.
Results: With regard to GD by chemotherapy alone, there was no significant difference between S-1 and UFT/LV, although UFT/LV was demonstrated significantly greater GD than UFT. The GD by S-1+XRT was significantly greater than that by UFT/LV+XRT, but there was no significant difference between the GD by UFT/LV+XRT and that by UFT+XRT. The combination of S-1 plus XRT demonstrated a greater than additive

response, while UFT/LV+XRT and UFT+XRT showed almost additive effects.

Group	Treatment	GD (days, mean±SD)		Synergy ratio
G1	UFT	2.00±0.25		–
G2	UFT/LV	4.75±0.83	P < 0.05 vs G1	–
G3	S-1	6.00±0.96	n.s. vs G2	–
G4	XRT	5.08±0.91		–
G5	UFT+XRT	7.75±0.92		1.09
G6	UFT/LV+XRT	9.25±1.01	n.s. vs G5	0.94
G7	S-1+XRT	15.25±3.10	P < 0.05 vs G6	1.38

Conclusion: S-1 showed a stronger radiosensitizing activity than UFT or UFT/LV in a mouse xenograft model of human colon cancer. These results suggest that S-1 is a promising new candidate in combination with preoperative XRT in LARC.

6101 POSTER

"Determina KRAS" Project: what have we learned after nine months?

J. Garcia-Foncillas[1], B. Honorato[2], T. Caldes[3], M. de la Hoya[3], J. Hernandez[4], S. Landolfi[4], M. Mata[5], S. Gallach[5], M.D. Bautista[6], M. Benavides[7]. *[1]Clinica Universitaria Navarra, Department of Oncology and Radiotherapy, Pamplona, Spain; [2]Clinica Universitaria Navarra, Unit of Clinical Genetics, Pamplona, Spain; [3]Hospital Clinico Universitario "San Carlos", Oncologia, Madrid, Spain; [4]Hospital Universitario Valle de Hebron, Anatomia Patologica, Barcelona, Spain; [5]Hospital General, Oncologia, Valencia, Spain; [6]Hospital Universitario "Carlos Haya", Anatomia patologica, Malaga, Spain; [7]Hospital Universitario "Carlos Haya", Oncologia, Malaga, Spain*

Background: ASCO addresses the utility of KRAS gene mutation testing in patients with metastatic colorectal cancer (mCRC) to predict response to anti-EGFR MoAb therapy. Recent results from phase II and III studies demonstrate that patients who have mutations in codons 12 or 13 of KRAS gene do not benefit from this therapy and patients with wild-type KRAS had better clinical response in terms of prolonged median progression-free survival and overall response rates when compared to mutant KRAS.
Screening for colorectal tumors lacking KRAS mutations may facilitate identification of patients most likely to benefit from anti-EGFR therapies. CRC in Spain is an important issue. As of May 2008, it was not feasible to determine KRAS mutational status in much of the country. The Spanish project "Determina KRAS" was developed in order to answer this need allowing access to the test throughout the country. Results of the first nine months of development of this project are shown.
Material and Methods: Five well-known Spanish centres were adequately trained and provided with the equipment necessary to analyse KRAS status over a period of five working days. Testing for the mutation began at the first centre in July 2008, and the last centre in October 2008.
KRAS mutations were detected using a validated KRAS mutation kit (DxS Ltd, Manchester, United Kingdom) which identifies seven different somatic mutations located in codons 12 and 13 using allele-specific real-time polymerase chain reaction.
Results: In total, 4169 samples from mCRC patients were analyzed (39% in first line). 89% of samples analyzed were paraffin-embedded. There were no technical problems in 97% of cases. 1901 samples (53.8%) demonstrated wild-type KRAS status and the G12D mutation was the most frequently observed mutation.
Conclusions: Recent clinical data have provided substantial evidence that KRAS mutational status should be used as a predictive marker of response to anti-EGFR therapies in colorectal cancer.
The "Determina Kras" Project makes the analysis available to every patient. A slight difference was observed between our data and those reported in the literature with regards to the percentage of patients harbouring the KRAS mutation. No significant differences were observed in the type of mutations. Because KRAS status is essential for treatment decision-making in mCRC, access to this test must be guaranteed.

6102 POSTER

Neutrophil/lymphocyte ratio as a predictor of response and survival in metastatic colorectal cancer

W. Chua[1], P. Beale[1], L. Horvath[1], S.J. Clarke[1]. *[1]Sydney Cancer Centre, Department of Medical Oncology, Sydney NSW, Australia*

Background: Accurate, reliable and easily accessible predictors of chemotherapy response, toxicity and survival are needed in metastatic colorectal cancer (mCRC) to allow improved tailoring of chemotherapy and to avoid potential life-threatening toxicities. Neutrophil/lymphocyte ratio (NLR) has been used as an accurate predictor of survival after resection of colorectal and non-small cell lung cancers. The aim of this study was to identify NLR as a predictive factor in addition to other clinical and laboratory predictors in the treatment of mCRC.
Methods and Methods: Clinical and laboratory variables were analysed in patients enrolled in 10 first-line chemotherapy trials for mCRC from 1999–2007 at our centre. Prospectively collected data regarding response, toxicity and survival were analysed according to clinical and laboratory variables prior to chemotherapy commencement. Chi square tests were used for correlation between variables and response or toxicity. Cox regression analysis was used to examine the effects of variables on PFS and OS.
Results: A total of 171 patients were enrolled with a median age of 61 (range 33−84) and were predominantly male (64%) with ECOG performance status (PS) 0 or 1 (97%). Younger age ($\leqslant$65) ($p = 0.032$), PS 0 ($p = 0.04$), combination chemotherapy ($p < 0.001$) and neutrophil/lymphocyte ratio (NLR) $\leqslant 5$ ($p = 0.017$) predicted for clinical response (CR and PR). Only combination treatment ($p = 0.025$) predicted for worse grade 3 or 4 toxicity during chemotherapy. ECOG PS 0 ($p = 0.001$), combination chemotherapy ($p < 0.001$) and NLR $\leqslant 5$ ($p = 0.008$) predicted for improved PFS. Predictors of improved OS were PS 0 ($p = 0.01$), combination chemotherapy ($p = 0.039$), normal alkaline phosphatase ($p = 0.023$) and NLR $\leqslant 5$ ($p = 0.014$).
Conclusion: These results have identified NLR as a potential laboratory variable for predicting response, PFS and OS. Further studies are required to confirm the utility of NLR and the role of other inflammatory markers in this setting.

6103 POSTER

Expression and role of placenta growth factor (PlGF) on colorectal carcinomas

M. Baek[1], T. Ahn[1], D. Jeong[2], S. Cho[3], J. Kim[3], E. Shin[4], O. Song[4]. *[1]Soonchunhyang University Cheonan, Surgery, Cheonan, Korea; [2]Soonchunhyang University Cheonan, Pathology, Cheonan, Korea; [3]Soonchunhyang University Hospital, Surgery, Seoul, Korea; [4]Soonchunhyang University Bucheon, Surgery, Bucheon, Korea*

Background: Placenta growth factor (PlGF) is a member of the vascular endothelial growth factor (VEGF) family. PlGF is implicated in several pathologic processes, including the growth, spread of cancer and tumor angiogenesis. The aim of this study was to evaluate expression and clinical implications of PlGF in colorectal cancer.
Methods: In order to ascertain the clinical significance of PlGF expression in colorectal cancer, we analysed the expression pattern of PlGF using both immunohistochemical method and real time quantitative PCR and attempted to establish if a relationship existed between PlGF and microvessel density (MVD), and subsequently between PlGF and the predicted prognosis. We investigated 72 tumor/non-tumor pairs of fresh colorectal tissues for real time PCR and a total of 83 patients with colorectal cancer were included for immunohistochemical staining. Clinicopathological characteristics were defined according to the TNM criteria of the UICC. Clinico-pathologic factors, such as age, sex, histological types of tumors, tumor cell grade, TNM stage, lymphovascular invasion, lymph node metastasis, were reviewed.
Results: The mRNA expression levels for PlGF was significantly higher in tumor than in non-tumor tissues ($p = 0.001$). The ratio of PlGF expression in tumor to non-tumor in the advanced staged group was significantly higher than for the early staged group. PlGF mRNA was significantly higher in III-IV stage patients than in stage I-II patients ($p = 0.011$). The relationship between PlGF mRNA expression and sex, histological type, lymph node status was otherwise not statistically significant. PlGF protein expression level was significantly correlated with MVD, patient survival, and clinicopathological factors such as lymph node metastasis, TNM staging, lymphatic invasion and vascular invasion in this study.
Conclusions: PlGF may be an important angiogenic factor in human colorectal cancer, and PlGF expression level was significantly correlated with positive lymph node metastases, tumor stages, and patient survival. These findings suggest that PlGF expression correlates with disease progression and may be used as a tumor marker for colorectal cancer.