mutations in the DNA sequence of K-ras gene, especially in codon 12 or 13. Nowadays, EGFR targeted therapies, such as cetuximab and panitumumab were used for CRC and K-ras mutation analysis has gained importance. In this study, possible relationship between ABO-Rh blood groups factor and K-ras status was investigated.
Material and Methods: In 94 patients with CRC, blood group and Rh factor were examined. The relationship of blood groups with wild type K-ras status was evaluated and compared with the healthy volunteer donors control group of 22,821 people which admitted to Ankara University Medical School Blood Center at 2010.
Results: Information on ABO blood type and Rh factor were available for 94 patients. Of patients 34% (32 patients) was female and 66% (62 patients) was male. The most of patients had (40.4%) blood group A. Overall, the ABO blood group distribution of the 94 patients with CRC was similar to that of the general population. There wasn't statistically significant difference ($p = 0.83$) between groups (see Table 1).
Conclusion: This study is the first study done on this issue. In our study, we didn't find any relationship between K-ras status and ABO blood group and Rh factor. However further studies with larger number of patients are needed to establish the role of blood groups in this population.

Table 1: The blood group distribution of patients and control group

Blood groups	K-ras WT patients		Control group	
	n	%	n	%
A Rh (+)	33	35.1	8795	38,54
A Rh (−)	5	5.3	1130	4,95
B Rh (+)	13	13.8	3185	13,96
B Rh (−)	2	2.1	425	1,86
AB Rh (+)	3	3.2	1581	6,93
AB Rh (−)	1	1.1	205	0,90
0 Rh (+)	32	34.0	6550	28,70
0 Rh (−)	5	5.3	950	4,16
Total	94	100	22821	100

6136 POSTER

Relatively High Discordant Rate of KRas Mutation Between Primary and Metastatic Sites, and a Different Pattern of KRas Mutational Status According to Metastatic Sites in Korean Patients With Colorectal Cancer

M.J. Kim[1], K.W. Lee[1], H.S. Lee[2], J.H. Kim[1], Y.J. Kim[1], J.H. Kwon[3], D.W. Kim[4], S.B. Kang[4], I.S. Choi[5], J.S. Lee[1]. *[1]Seoul National University Bundang Hospital, Internal Medicine, Seongnam, South Korea; [2]Seoul National University Bundang Hospital, Pathology, Seongnam, South Korea; [3]Seoul National University Hospital, Internal Medicine, Seoul, South Korea; [4]Seoul National University Bundang Hospital, Surgery, Seongnam, South Korea; [5]Seoul Municipal Boramae Hospital, Internal Medicine, Seoul, South Korea*

Background: KRAS mutations predict resistance to cetuximab or panitumumab therapy in patients with metastatic or recurred colorectal cancer (MRCRC). The aim of this study was to determine the concordance rate of KRAS mutational status between primary tumours and related metastases, and to find out the association between KRAS mutational status and clinicopathologic features in Korean patients with MRCRC.
Material and Methods: KRAS mutational status in codon 12, 13, and 61 from formalin-fixed sections of primary tumours and related metastases was analyzed. In addition, the association between KRAS mutational status and clinicopathologic features was evaluated.
Results: Of 128 patients whose primary and matched metastatic tissues were analyzed for the KRAS mutation status, 5 patients were excluded because of a failure in the process of KRAS mutation analysis and 123 patients were finally included in this study. Matched metastatic sites for KRAS analysis were liver (n = 46), lung (n = 27), peritoneum (n = 26), distant lymph node (LN; n = 13), ovary (n = 10) and pancreas (n = 1), respectively. KRAS mutation was observed in 52 (42.3%) of primary tumours, and in 54 (43.9%) of related metastatic sites. Discordance of KRAS status between primary and metastatic sites was observed in 18 patients (14.6%; kappa = 0.702) and KRAS mutation rate considering both primary and metastatic sites was 50.4% (62/123). When the association between KRAS status and initial metastatic sites at the time of diagnosis of stage 4 cancer or recurrence (in the cases of initial stages 1−3) was analyzed, a high percentage of wild type KRAS was observed in patients with initial liver or distant LN metastases compared with patients without liver or LN metastases ($P = 0.001$ in liver; $P = 0.037$ in LN). However, patients with initial lung metastases had a higher rate of KRAS mutation than patients without lung metastasis ($P = 0.002$). Other clinicopathologic features including age, gender, histologic grade, primary tumour site, obstruction or perforation of primary site, and microsatellite instability were not related to the KRAS mutational status.
Conclusions: The discordant rate of KRAS mutation status between primary and metastatic sites was relatively high (14.6%) in Korean MRCRC patients compared with the rate (<10%) previously reported in Western patients. The frequency of KRAS mutation was different according to the initial metastatic or recurred sites.

6137 POSTER

Biological Analysis of Phase II Study Evaluating the Activity of Cetuximab Combined to Oxaliplatin and Fluoropirimidine (TEGAFOX-E) as First Line Treatment in Metastatic Colorectal Cancer (mCRC) Pts by the Italian Trials in Medical Oncology (I.T.M.O.) Group

M. Di Bartolomeo[1], K.F. Dotti[1], F. Pietrantonio[1], F. Perrone[2], A. Martinetti[1], S. Pilotti[2], A. Lampis[2], C. Bertan[2], R. Buzzoni[1], E. Bajetta[1]. *[1]Fondazione IRCCS Istituto Nazionale Tumori, Medical Oncology, Milano, Italy; [2]Fondazione IRCCS Istituto Nazionale Tumori, Experimental Molecular Pathology, Milano, Italy*

Background: There is some evidence that *p53* inactivating mutant (mut) confer oxaliplatin resistance, but improve cetuximab benefit in KRAS wt pts *(Oden-Gangloff, 2009)*. On the other hand, KRAS mut predict cetuximab resistance, but increase oxaliplatin sensitivity in a p53-dependent manner (de Bruijn, 2010). We conducted a prospective Phase II evaluating the activity of the cetuximab plus an oxaliplatin-based regimen as first line treatment in mCRC, and the correlation with predictive biomarkers such as *p53* and *KRAS* status.
Methods: Twenty-eight elderly mCRC pts (age $\geqslant$70 yrs) were enrolled in a multicenter, prospective study and treated with TEGAFOX-E regimen (oxaliplatin 120 mg/m^2 d1, UFT 250 mg/m^2 d1−14, cetuximab 400 mg/m^2 initial dose, then 250 mg/m^2 weekly) up to 8 cycles, followed with one-year maintenance cetuximab, or until progressive disease (PD)/unaccectable toxicity. *KRAS, p53*, *BRAF* and *PI3KCA* mut were successfully analyzed by genomic sequencing in 23 samples. A mutant-enriched PCR was performed for codons 12 and 13 of *K-Ras*.
Result: TEGAFOX-E regimen produced a 44% objective response rate. *KRAS* mut were detected in 12/23 (52%) and *p53* mut in 5/21 (24%) samples. Two samples were not evaluable for *p53* status. Except for one case, *PI3KCA* mut (26%) were always coupled with *KRAS* mut. No statistically significant difference could be found between responders and non responders in terms of *KRAS* or *p53* mut. On the basis of their *KRAS* and *p53* status, the samples (21 cases) were molecularly classified in three groups and associated to response and mPFS. Group 1 (*KRAS* mt/*p53* mt): all 2 pts harbouring double mutation showed PD at 9-weeks, i.e., the first reassessment; Groups 2−3 (*KRAS* mt/*p53* wt or *K-ras* wt/*p53* mt): in this cross-interference groups 7/12 pts (58%) showed disease control [5 PR, 2 SD and 5 PD], with mPFS of 11 weeks; Group 4 (*KRAS* wt/*p53* wt group): in this double drug-sensitivity group 86% (5/7) pts benefited from treatment (5 PR, 1 long-lasting SD and 1 PD), with a mPFS of 44 weeks.
Conclusions: TEGAFOX-E combination displayed promising efficacy in pts with both wt *KRAS* and *p53* tumours and these hypothesis-generating results should be verified in larger, prospective and randomised phase III trials.

6138 POSTER

Prognostic Role of KRas and BRAF Mutation in Colorectal Cancer

M. Baek[1], T. Ahn[1], D. Jung[2], S. Park[2], H. Kim[2], W. Lee[2], D. Park[2], S. Lee[3], D. Park[4]. *[1]Soonchunhyang University Cheonan, Surgery, Cheonan, Korea; [2]Soonchunhyang University College of Medicine, Pathology, Cheonan, Korea; [3]Chungbuk National University Hospital, Surgery, Cheongju, Korea; [4]Dankook University Hospital, Surgery, Cheonan, Korea*

Background: In numerous studies on the development of cancer induced by K-Ras mutation, raf kinase has been shown to be an important signal transducer in the activation of Ras oncogene. To see whether the mutation of K-Ras gene and BRAF gene is associated with colorectal cancer, in colorectal cancer patients, we compared the presence or absence of the mutation of K-Ras gene and BRAF gene with the clinicopathological characteristics of colorectal cancer patients, and examined the effect of the mutations on survival rate.
Material and Methods: DNA was extracted from 162 cases of colorectal cancer tissues of patients performed surgery for colorectal cancer from 2002 to 2007 and the presence or absence of the mutation of K-Ras and BRAF was assessed by the use of the K-Ras and BRAF detection test kits applying PNA PCR claming method developed recently. The presence or absence of the mutation of K-Ras as well as BRAF was compared with various clinicopathological factors. 5-year survival rate was analyzed by the Kaplan–Meier survival analysis.

Results: Among 162 colon cancer subjects, K-Ras 12 codon mutation was detected in 55 cases (34.0%) and K-Ras 13 codon mutation was detected in 16 cases (9.9%). BRAF mutation was detected in 26 cases (16.0%). It was observed that K-Ras 12 or 13 mutations was not associated with the age, gender, TNM stage, the lymph node metastasis, differentiation. In the Kaplan–Meier survival analysis, K-Ras mutation was not significantly associated with 5-year survival rate ($p = 0.73$, $p = 0.52$). In cases expressing BRAF mutation, it was shown to be not associated with the age, gender, TNM stage, lymph node metastasis, differentiation. Nevertheless, in the Kaplan–Meier survival analysis, 5-year survival rate of cases with BRAF mutation was significantly decreased ($p = 0.02$).
Conclusion: Taken together, it appears that K-Ras mutation is not associated with various clinicopathological factors of colorectal cancer patients, and it does not correlate to survival rate. Nonetheless, it was confirmed that survival rate was reduced in colorectal cancer patients expressing BRAF mutation, and it is considered that BRAF mutation could be a prognostic factor in colorectal cancer patients.

6139 POSTER

Incidence of VEGFR-2, PDGFRα and PDGFRβ Mutations in Colorectal Cancer and Potential Value as Prognostic Markers

P. Estevez Garcia[1], A. Castaño[2], A.C. Martin[3], F. Lopez-Rios[4], J. Iglesias[5], I. Lopez-Calderero[1], S. Molina-Pinelo[1], M.D. Pastor[1], L. Paz-Ares[1], R. Garcia-Carbonero[1]. *[1]Hospital Virgen del Rocio/Instituto de Biomedicina de Sevilla (IBIS), Medical Oncology, Sevilla, Spain; [2]Hospital de Fuenlabrada, Pathology Department, Madrid, Spain; [3]Bionostra, Aplicaciones Biotecnológicas S.L.U., Madrid, Spain; [4]Centro Integral Oncológico Clara Campal, Pathology Department, Madrid, Spain; [5]Bionostra, Aplicaciones Biotecnológicas S.L.U, Madrid, Spain*

Background: Angiogenesis plays an essential role in tumour growth and metastasis, being a major target in cancer research. Vascular endothelial growth factor receptor (VEGFR) and platelet-derived growth factor receptor (PDGFR) families are largely involved in this process, but incidence of mutations in these receptors and their potential value as prognostic or predictive markers in colorectal cancer (CRC) has not been fully assessed.
Methods: VEGFR2, PDGFRα and PDGFRβ mutations were evaluated by sequencing their tyrosine kinase domains in 8 CRC cell lines (T84, LOVO, LS174T, HT 29, LS180, SW48, SW480, COLO205) and in 92 samples of patients with colorectal adenocarcinoma. Potential correlations with clinicopathological features and survival of these patients were analyzed.
Results: Three genetic variations were identified in PDGFRα and one in PDGFRβ, all of them corresponding to single nucleotide polymorphisms (SNP 11A, 12A, 16A and 19B), whereas no VEGFR-2 mutations were detected. SNP 11A and 16A were present in 100% of cell lines and tumour samples in homozygosis. SNP 12A was detected in 2 colorectal cell lines (LS174T, LS180) and SNP19B in 4 (LS174T, LS180, SW48, COLO205). Patient characeristics were representative of a standard CRC population: median age 68 years, 63% males, 75% colon and 25% rectal cancer, and typical stage distribution (I:9%,II:24%,III:26%,IV:40%). SNP 19B was found in 45 patients (49%) and SNP 12A in 13 (14%). Five-year overall survival was significantly higher for patients with PDGFR-B19 wild type (WT) tumours than for those harbouring SNP B19 (54% vs 22%; $p = 0.047$), but no survival difference was observed according to PDGFR-A12 status. Multivariate analysis revealed PDGFR SNP B19 ($p = 0.037$), age ($p = 0.002$), TNM stage ($p < 0.001$) and CEA ($p = 0.012$) as independent prognostic factors for decreased survival.
Conclusions: PDGFR SNP B19 is associated with poorer survival and could be a promising new prognostic marker in colorectal cancer patients. Further studies to validate our data are warranted.

6140 POSTER

Cetuximab-mediated Immune-enhancing Effects in Vitro and in Metastatic Colorectal Cancer (mCRC) Patients

P. Correale[1], C. Botta[1], M.G. Cusi[2], E. Bestoso[1], S. Apollinari[1], M. Caraglia[3], M.T. Del Vecchio[4], A. Abbruzzese[3], A. Aquino[5], P. Tagliaferri[6]. *[1]"S. Maria alle Scotte" Siena University Hospital, Department of Oncology, Siena, Italy; [2]"S. Maria alle Scotte" Siena University Hospital, Department of Molecular Biology Microbiology Section, Siena, Italy; [3]II University of Naples, Department of Biochemistry and Biophysics, Naples, Italy; [4]"S. Maria alle Scotte" University Hospital of Siena, Section of Pathological Anatomy Department of Human Pathology and Oncology, Siena, Italy; [5]"Tor Vergata" University, Department of Neurosciences Pharmacology Section, Rome, Italy; [6]Magna Græcia University and Tommaso Campanella Cancer Center Catanzaro, Medical Oncology, Catanzaro, Italy*

Introduction: Cetuximab is a chimeric human-murine monoclonal IgG_1 to the Epidermal-growth-factor-Receptor, approved for colorectal cancer treatment with chemotherapy. Its human constant fragment (Fc), throughout receptor binding, trigger additional immune-mediated effects, which may offer significant contribute to the final therapeutic effect. We investigated cetuximab ability *in vitro* to promote colon cancer cell phagocytosis and antigen-cross-priming by dendritic cells (DCs) to tumour-specific cytotoxic-T-cell (CTL) precursors. We also carried-out an immunological study in 26 mCRC patients enrolled in an on-going phase 2 trial, receiving an experimental biochemotherapy (GILFICet) regimen combining gemcitabine, irinotecan, fluoruracil, levofolinate, cetuximab, and metronomic sc. aldesleukine.
Material and Methods: Transmission-electron-microscopy (TEM) and Flow cytometry were used to evaluate the susceptibility of multiple colon cancer cell lines to DC-mediated phagocytosis. Human DCs loaded with cetuximab-coated colon cancer cells, exposed or not to a combination of anti-cancer drugs, were used to *in vitro* sensitize human PBMCs from normal donors and cancer patients collected at baseline and after 3 GILFICet courses. T-cell cultures were characterized for immune-phenotype and tumour-antigen specific CTL activity by Flow cytometry, LDH release and IFNγ-ELISPOT.
Results: ILF (irinotecan+ folinate+5-flurouracil) and GILF (gemcitabine+ILF) poly-chemotherapies confirmed their ability to induce antigen remodelling and danger signals in colon cancer cells. After exposure to poly-chemotherapy and cetuximab these cells became highly susceptible to Fc-receptor-mediated phagocytosis/trogocytosis by human DCs, promoted their activation, and increased their ability *to* elicit a highly efficient CTL response on human PBMCs *in vitro*. Our study on the PBMCs of colon cancer patients enrolled in the GILFICet trial, revealed a significant treatment-related increase in naïve and central-memory-T-cells, activated-CTLs, NK/NKTs, mature-activated DCs and IFNγ-releasing cells. Substantial differences were observed in T-cell lines generated from patients' PBMCs taken before and after biochemotherapy. In the latter group there was in fact, a remarkable increase in proliferating $CD8^+Ki67^+$CTLs and tumour-antigen specific CTLs' precursors.
Discussion: These results suggest that cetuximab may exert immune-enhancing effects with potential antitumour activity.

6141 POSTER

Frequent Molecular Alterations in Brain Metastases From Colorectal Tumours

M. Denis[1], D. Loussouarn[2], S. Theoleyre[1], D. Vansteene[3], J. Bennouna[3]. *[1]CHU Nantes- Hotel Dieu, Laboratoire de Biochimie, Nantes, France; [2]CHU Nantes- Hopital Laennec, Anatomie Cytologie Pathologiques, Nantes, France; [3]Institut de Cancérologie de l'Ouest, Oncologie Médicale, Saint Herblain, France*

Background: Brain metastases associated with colorectal cancer are relatively rare, but a frequent cause of death. The aim of our study was to analyze brain metastases from colorectal tumour, and identify cancer characteristics which were associated with their development.
Material and Methods: We performed a retrospective study of 19 patients (11 males and 8 females, 50−88 years, mean 67 years) who underwent brain surgery. DNA was extracted from FFPE sections following macrodissection using an automated iPrep system (InVitrogen). RNA was extracted from serial sections using the RNeasy FFPE kit (Qiagen), and cDNA was synthesized using the Quantitect reverse transcription kit (Qiagen). *KRAS* and *BRAF* mutations were tested using allele-specific PCR. PTEN expression was determined by immunohistochemistry. Amphiregulin expression was measured by real-time PCR.
Results: *KRAS* mutations were found in 13 (68.4%) brain metastases: c.35G >T, c.35G >A, c.35G >C and c.38G >A mutations were found in 5, 3, 3 and 2 samples, respectively. One tumour (5.3%, KRAS wt) presented the *BRAF* p.V600E mutation. PTEN was detected by immunohistochemistry in 12 tumours (63.2%), but 7 cases (36.8%) were negative for PTEN expression. Of these 7 tumours, 5 presented a *KRAS* mutation, and 1 was *BRAF* V600E. Amphiregulin expression was found to be very low in 10 tumours, intermediate in 6 samples, and high in the remaining 3 tumours. Two of these 3 tumours presented a mutated KRAS and the third one was PTEN negative. *KRAS*, *BRAF* and PTEN status were fully concordant between primary cancer and brain metastasis from the same individual. However, we did not find any correlation in the expression level of amphiregulin between the primary tumour and the metastatic site.
Conclusions: *KRAS* mutation prevalence was high in this series of patients presenting with cerebral metastases. Loss of PTEN was strongly associated with *KRAS*/*BRAF* alterations. Altogether, the overwhelming majority of the tumours tested (16/19, 84.2%) presented at least one molecular alteration. Further characterization of these tumours might yield better insight into their development, and potentially the treatment of these patients with poor prognosis.
Acknowledgements: samples were kindly provided by Drs S. Martin, M.F. Heymann, G. Aillet, N. Rioux-Leclercq, C. Magois, and J.-J. Auger.