**6107** POSTER

**A pitfall of KRAS sequencing using a small amount of DNA extracted from formalin-fixed paraffin-embedded (FFPE) colorectal cancer tissues**

H. Bando[1], K. Tsuchihara[2], T. Yoshino[1], A. Ochiai[2], M. Kojima[2], N. Fuse[1], M. Tahara[1], T. Doi[1], H. Esumi[2], A. Ohtsu[1]. *[1]National Cancer Center East, Division of Gastrointestinal Oncology, Kashiwa, Japan; [2]National Cancer Center East, Research Center for Innovative Oncology, Kashiwa, Japan*

**Background:** The direct sequencing of PCR-amplified *KRAS* gene fragments is the most widely accepted method for *KRAS* testing and has long been the gold standard for mutation detection. Genomic DNA samples are routinely obtained from formalin-fixed paraffin-embedded (FFPE) tissues. However, when insufficient amounts of template DNA from FFPE samples are used, artificial mutations caused by the cross-linking of cytosine nucleotides and the terminal transferase activity of *Taq* DNA polymerase are frequently observed. Therefore, a minimum of 30 ng of template DNA is recommended to avoid overestimating mutations (Jimeno et al., J Clin Oncol 27:1130–1136, 2009). Since *KRAS* testing is pivotal in colorectal cancer patients prior to the administration of cetuximab, we attempted to optimize the *KRAS* testing method using clinical samples.
**Materials and Methods:** Surgically resected FFPE tissues were used for the analysis. Alternatively, biopsy tissues were used when resected tissues were not available. The tumor cells were macroscopically dissected, and the genomic DNA was extracted using the QIAamp® DNA FFPE Tissue Kit (QIAGEN). The extracted DNA was spectrophotometrically quantified using Nano Drop 1000® (Thermo Fisher Scientific). The *KRAS* gene fragments including exon 2 were amplified using 40 cycles of PCR, and both strand sequences were determined using Big Dye® Terminator v3.1 and an ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems). Seven major types of mutations in codon 12 or 13 were identified.
**Results:** Between September 2008 and March 2009, 110 specimens from 104 patients (95 surgically resected specimens, 15 biopsy specimens) were analyzed. The median amount of DNA used for the PCR analysis was 100.1 ng (range: 10.9–1068.3 ng), and the success rate of the PCR analysis and subsequent sequencing was 100%. We detected 32 *KRAS* mutants in the 110 specimens (29.1%). When the samples were stratified according to the amount of DNA used for the PCR analysis, one of 15 (6.7%) specimens containing less than 250 ng of DNA was found to contain a mutation. In contrast, the mutant ratio among samples with more than 250 ng of DNA was 32.6% (31 out of 95 specimens), which was consistent with previous reports. When 8 of the 15 specimens in which a relatively small amount of DNA was initially examined were re-examined using a larger amount of DNA, 4 of the 8 specimens were found to carry a mutation.
**Conclusions:** A sufficient amount of DNA from FFPE samples is required to avoid underestimating the incidence of *KRAS* mutations.

**6108** POSTER

**Association of FcGammaRIIa-FcGammaRIIIa polymorphisms and KRAS mutations with clinical outcome in advanced colorectal cancer patients treated with antiEGFR based treatment**

D. Paez[1], I. Espinosa[2], A. Altés[3], E. del Rio[4], J. Salazar[4], A. Barnadas[1], E. Marcuello[1], M. Baiget[4]. *[1]Hospital de la Santa Creu i Sant Pau, Medical Oncology, Barcelona, Spain; [2]Hospital de la Santa Creu i Sant Pau, Pathology, Barcelona, Spain; [3]Fundacion Althaia. Manresa, Hematology, Barcelona, Spain; [4]Hospital de la Santa Creu i Sant Pau, Genetic, Barcelona, Spain*

**Background:** The anti-epidermal growth factor receptor antibodies show activity in metastatic colorectal cancer (mCRC). Besides the inhibition of the EGFR pathway, Cetuximab may exert anti-tumor effects through antibody-dependent cell-mediated cytotoxicity (ADCC), an anticancer mechanism in which antibody Fc portion interacts with Fc receptors (FcγRs) expressed by immune effector cells.
**Objective:** The aim of this analysis was to investigate the association of FcγR polymorphisms and KRAS mutation with the clinical outcome of mCRC patients treated with Cetuximab in combination with chemotherapy (mainly irinotecan).
**Patients and Methods:** Genomic DNA was obtained from peripheral blood samples of 93 mCRC patients. FcγRIIa-H131R and FcγRIIIa-V158F polymorphisms were analysed by means of Real-Time PCR on an ABI PRISM 7000 Sequence Detection System. Data were analyzed using Allelic Discrimination Program (Applied Biosystems). Tumour tissues were screened for KRAS mutations.
Patient clinical determinants were classified according to European Organization for the Research and Treatment of Cancer (EORTC model). The results were correlated with response rate (RR) according to RECIST criteria and progression-free survival (PFS).
**Results:**
- 20 patients (21.3%) were responders.
- KRAS mutation, detected in 26.3% of patients was associated with lower RR (4.8% vs 27.3% in nonmutated patients; p = 0.03) and shorter PFS (4 vs 8 months; p = 0.3) (table)
- In the logistic regression analysis: (i) KRAS mutation (p = 0.03), (ii) Performance Status (p = 0.04) and (iii) H131R polymorphism in the FcγRIIa gene (p = 0.06) were correlated with response. In the multivariate Cox model, only the clinical risk classification (EORTC model) was found to be independent risk factor for disease progression (p = 0.005).

| Polymorphism | n (%) | Response (%) | PFS (months) |
|---|---|---|---|
| FcγRIIa | | | |
| H/H | 23 (24.7) | 14.3 | 4 |
| H/R | 50 (53.8) | 17 | 6 |
| R/R | 20 (21.5) | 40 (p = 0.05) | 7 |
| FcγRIIIa | | | |
| V/V | 14 (15) | 21 | 4 |
| V/F | 38 (41) | 20 | 5 |
| F/F | 41 (44) | 23 | 7 |

**Conclusions:** The present study confirms that KRAS mutation in tumors is highly predictive of a non-response to antiEGFR-based therapy. In addition patients with R/R genotype (FcγRIIa) showed better response rate than H/H genotype patients. FcγRIIa and/or FcγRIIIa genotypes were not associated with clinical outcome. The independent prognostic marker in our group of patients was the clinical risk classification.

**6109** POSTER

**Shift from cytoplasmic to nuclear maspin expression correlates with shorter overall survival in nodal negative colorectal cancer**

H. Arnholdt[1], G. Schenkirsch[2], R. Herrmann[1], K.H. Haude[3], H. Spatz[4], M. Anthuber[4], G. Schlimok[5], D. Oruzio[5], B. Märkl[1]. *[1]Klinikum Augsburg, Pathology, Augsburg, Germany; [2]Klinikum Augsburg, Clinical and Population Based Cancer Registry Augsburg, Augsburg, Germany; [3]Klinikum Augsburg, Department of Clinical Communication and Information Technology, Augsburg, Germany; [4]Klinikum Augsburg, Department of Visceral Surgery, Augsburg, Germany; [5]Klinikum Augsburg, II. Medical Clinic, Augsburg, Germany*

**Background:** Maspin is a serine protease inhibitor which is related to the serpin family and has been characterised as a potent tumour suppressor. In contrast, in stage III colon cancer an association with shorter overall survival (OS) as well as sensitivity to chemotherapy was found for cases with nuclear maspin expression. Since a certain proportion of nodal negative colorectal cancer cases show a fatal clinical course, we hypothesised that immunohistochemical maspin expression could be of help to identify higher risk cases.
**Methods:** We analysed survival in a study employing 156 cases of stage I/II colorectal cases out of the years 2000–2004.
**Results:** Immunohistochemical cytoplasmic and/or nuclear maspin expression was found in 72% and 48% of the cases, respectively. Significant correlations between cytoplasmic expression and high tumour grade ($p < 0.01$) and between nuclear expression and tumour budding ($p < 0.001$) were shown. No differences concerning OS and immunohistochemical maspin expression were found when the complete collective was analysed. However, evaluation of the pT3 cases revealed a highly significant worse mean OS of cases with a combination of nuclear expression and cytoplasmic loss of maspin compared to cases with the opposite expression pattern nuclear loss and cytoplasmic expression (mean OS 40 vs. 63 months, respectively; $p < 0.001$). The other possible combinations (complete positive and complete negative) showed intermediate mean OS times with 54 and 49 months, respectively.
**Conclusions:** Our findings suggest a compartment dependent function of maspin in colorectal cancer which can be useful in identifying stage II cases with a higher risk for fatal outcome. In addition these patients could benefit from adjuvant chemotherapy. Further prospective studies restricted to stage II cases should confirm that.