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High prevalence of DNA mismatch-repair-deficiency in Peruvian colorectal tumours

P. Montenegro[1], C. Alenda[2], A. Paya[3], M.I. Castillejo[2], A. Martinez-Canto[2], C. Barrionuevo[4], L. Casanova[5], J. Leon[5], A. Carrato[6], J.L. Soto[2]. *[1]Instituto Nacional De Enfermedades Neoplásicas, Medical Oncology, Lima, Peru; [2]Hospital General Universitario Elche, Molecular Oncology Lab, Elche, Spain; [3]Hospital General Universitario Alicante, Pathology Department, Alicante, Spain; [4]Instituto Nacional De Enfermedades Neoplásicas, Pathology Department, Lima, Peru; [5]Instituto Nacional De Enfermedades Neoplásicas, Medical Oncology, Lima, Peru; [6]Hospital General Universitario Elche, Medical Oncology, Elche, Spain*

Background: Colorectal cancer (CRC) is the fifth and the fourth cause of cancer in Peruvian males and females, respectively. DNA mismatch repair (MMR) deficiency in CRC has been reported in a minority of cases ranging from 8% to 18% in all series of unselected tumours. The MMR status has treatment selection and prognostic value. Alteration of the MMR system is the molecular hallmark of the Lynch syndrome tumours.
Materials and Methods: Subjects: a series of 90 unselected CRC patients from Peru with a median age of 59.5 years old (ranged from 22 to 89). Fifty one percent were females and 49% males. CR tumours where analyzed for *MLH1*, *MSH2*, *MSH6* and *PMS2* protein expression by immunohistochemistry and for microsatellite instability (MSI) using the *BAT26* marker. Family history of cancer, clinical and pathological features of all cases where also tested in a univariate analysis.
Results: The association between MSI and MMR protein expression was highly significant ($p < 0.0001$). We found a total of 35 cases with loss of expression of, at least, one of the MMR protein and/or MSI (35/90 = 38.8%). To our knowledge, this is the highest level of MMR deficiency reported to date.
MMR deficient tumours were associated to right colon location, high grade, intratumoral lymphocytosis and special histological type (medullar, signet ring, mucinous, undifferenciated and mixed types) as previously described ($p < 0.0001$). Up to 72% of the analyzed proximal colon tumours showed MSI (23/32). There was also, significant association with female ($p = 0.044$) and, unexpected association to older patients. Median age of patients at diagnosis was 64 and 55 years old for MMR-deficient and proficient tumours respectively ($p = 0.003$). No association was detected between the MMR status with tumour stage and with the Bethesda criteria (guidelines for MSI detection in the screening for Lynch syndrome).
Conclusions: Peruvian CRC show the highest prevalence of MSI reported to date (38%). The expected hereditary component is also high. The age of onset of these MSI tumours is higher than non-MSI, suggesting the ineffectiveness of the Bethesda criteria for Lynch syndrome screening in Peru.

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Safety and pharmacokinetic (PK) profile in high risk group based on UGT1A1*6 and *28 polymorphisms: detailed analysis of UGT0601 genotype-directed dose finding study

Y. Sakata[1], T. Satoh[2], T. Tsujinaka[3], T. Ura[4], Y. Sasaki[5], K. Yamazaki[6], Y. Yamada[7], M. Munakata[8], N. Ishizuka[9], I. Hyodo[10]. *[1]Misawa City Hospital, Medical Oncology, Misawa, Japan; [2]Kinki University School of Medicine, Department of Medical Oncology, Osakasayama, Japan; [3]NHO Osaka National Hospital, Department of Surgery, Osaka, Japan; [4]Aichi Cancer Center, Department of Clinical Oncology, Nagoya, Japan; [5]Saitama Medical Univ. International Medical Center, Department of Medical Oncology, Hidaka, Japan; [6]Shizuoka Cancer Center, Division of Gastrointestinal Oncology and Endoscopy, Sunto-gun, Japan; [7]National Cancer Center Hospital, Medical Oncology Division, Tokyo, Japan; [8]Misawa City Hospital, Department of Internal Medicine, Misawa, Japan; [9]International Medial Center of Japan, Division of Preventive Medicine, Tokyo, Japan; [10]Tsukuba University Institute of Clinical Medicine, Division of Gastroenterology, Tsukuba, Japan*

Background: We conducted the prospective study to determine the initial dose of irinotecan based on UGT1A1*28 and UGT1A1*6 polymorphisms in patients (pts) with gastrointestinal cancer in the UGT0601 study. The maximum tolerated dose (MTD) and the recommended dose (RD) in Hetero, and the MTD in Homo was determined 150 mg/m^2. Pts with homozygote showed the different trend of pharmacokinetic (PK) and pharmacodynamics compared with those of other groups. We analyzed the association with homozygote and the clinical outcomes.
Material and Methods: Pts received prior chemotherapies except for CPT-11 for metastatic gastrointestinal cancer were enrolled. UGT1A1 polymorphisms were categorized into no mutation (Wild: *1/*1), heterozygous (Hetero: *1/*28, *1/*6), and homozygous (Homo: *28/*28, *6/*6, *28/*6). CPT-11 was administered biweekly. Starting doses were 150 mg/m^2 in Wild, 100 mg/m^2 in Hetero, and 75 mg/m^2 in Homo. Dose-limiting toxicity (DLT) was defined as grade 4 hematological, or grade 3 non-hematological toxicity. MTD was defined as the closest-dose 30% to DLT appearance. DLT was guided by the continual reassessment method in the cohort of Hetero and Homo. DLT and PK sampling was evaluated during the 1st cycle. This study is registered with UMIN Clinical Trial Registry, number UMIN000000618 and supported by Yakult Honsha Co., Ltd.
Results: From Nov 2006 to Oct 2008, 361 pts were screened for UGT1A1 test and then 82 pts enrolled (Wild, Hetero, Homo: 41, 20, and 21, respectively). At 150 mg/m^2, DLT was observed in 6 of 21 pts in Homo (grade 4 neutropenia, grade 3 diarrhea: 6 and 1, respectively). The probability of DLTs were 37.4% at 150 mg/m^2 and the MTD was determined 150 mg/m^2. However, the grade 3 to 4 neutropenia during the 1st cycle occurred in 10 of 16 pts (62.5%; $P < 0.001$). And treatment delay of the 2nd administration occurred in 10 of 16 (62.5%) pts and 4 of 10 pts required the dose reduction. Four of 16 (25%) pts completed entire courses of therapy without treatment delay and dose reduction. One treatment-related death due to septic shock occurred in 1 pt of Homo for *28/*28 during the 2nd cycle. In PK analysis, pts with Homo showed significantly higher SN-38 AUC0−24 h (mean ± SD: 509.8±261.8 ng*hr/mL; $P = 0.002$) and lower AUCSN-38G/AUCSN-38 ratio (1.85±1.13; $P < 0.001$).
Conclusions: The 150 mg/m^2 dose q2w is difficult to recommend in Homo because of the large interpatient variavility of PK. Other predictive factors are needed to determine RD in this group.

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Frequency of KRAS/BRAF mutations as predictive markers for Cetuximab in Japanese colorectal cancer patients

T. Yokota[1], N. Shibata[2], T. Ura[1], D. Takahari[1], K. Shitara[1], K. Muro[1], Y. Yatabe[2]. *[1]Aichi Cancer Center Hospital, Clinical Oncology, Nagoya, Japan; [2]Aichi Cancer Center Hospital, Pathology and Molecular Diagnostics, Nagoya, Japan*

Background: Recent studies from Western countries have suggested that *KRAS* and *BRAF* mutations were present in approximately 40% and 10% of colorectal cancer (CRC) patients, respectively, and either *KRAS* or *BRAF* mutation affects the response to EGFR-targeted therapies against CRC. However, the valid assays using pathological specimens have not been yet established. In this study, we analyzed the frequency of *KRAS*/*BRAF* mutations in Japanese CRC patients, by using a rapid, sensitive assay for *KRAS* genotyping.
Material and Methods: Cycleave PCR was used to detect the *KRAS*/*BRAF* mutations in CRC with a chimeric DNA-RNA-DNA probe labeled with fluorescent dye and quencher, with results obtained within 4 hours. Template DNA was extracted from formalin-fixed, paraffin-embedded specimens, which were surgically resected or biopsied in 245 CRC patients in our institution from 2001 to 2009. The data from Cycleave PCR was confirmed by reverse transcriptase-PCR-coupled direct sequencing (RT-PCR-DS) to evaluate Cycleave PCR accuracy.
Results: *KRAS* mutations were present in 90 (37%) of 245 patients, including 65 (27%) and 25 (10%) in codon 12 and 13 mutations, respectively. However, there was only 4 (1.8%) of 227 patients with *BRAF* mutation. None of the CRC patients carried both *KRAS* and *BRAF* mutations, as expected. Concordant results between Cycleave PCR and RT-PCR-DS in *KRAS* codon 12/13 and *BRAF* were found in 35/36 (97%), 23/23 (100%), and 41/41 (100%), respectively. Although some of the surgical specimens could not be evaluated with Cycleave PCR, corresponding biopsy specimens could be used alternatively. Because biopsy specimens were fixed by formalin for a shorter period, thus such fixation of surgical specimens may have contributed to PCR failure. Indeed, over-fixation by formalin impaired PCR amplification of *KRAS* in time-dependently. Further, results of 4 randomly selected biopsy specimens subjected to Cycleave PCR were consistent with those of surgical specimens.
Conclusion: Cycleave PCR of biopsy specimens in paraffin is accurate, rapid, and useful to detect *KRAS*/*BRAF* mutations in CRC patients. The frequency of CRC patients with *BRAF* mutation was lower than expected by literatures from Western countries. Therefore, *BRAF* genotyping in clinical practice might be less significant than *KRAS* for Japanese CRC patients.