DPD levels. TS and DPD quantitation may be helpful to evaluate prognosis of patients receiving adjuvant 5-FU therapy.

1110 POSTER

Identification of mutation in the dihydropirimidine dehydrogenase gene - clinical implications in 5-FU treatment

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Purpose: 5-Fluourouracil (5-FU) remains one of the most frequently prescribed chemotherapeutic drugs for the treatment of gastrointestinal tract, breast and head and neck cancers.

5-FU is a pyrimidine analogue and greater than 80% of a dose is degraded in a three-step pathway, initially catalysed by the enzyme dihydropyrimidine dehydrogenase (DPD). Deficiency in DPD enzyme activity is associated with a considerable delay in clearance of 5-FU from plasma, leading to severe, life-threatening diarrhoea, neutropenia and in some cases neurotoxicity.

Recently, it has been show that patients suffering from severe or even life-threatening toxicity after the administration of 5-FU are often genotypically heterozygous for a mutant allele of the gene encoding DPD. Our aim was to screen mutations in DPD gene in colorectal (CRC) cancer patients submited to treatment with 5-FU.

Methods: We studied 40 cases of sporadic CRC, treated with surgery followed by adjuvant chemotherapy with 5-FU. In each case the DNA was amplified by PCR using specific primers for the exon 14 from the DPD gene, and analysed by automated sequencing. The grade of treatment associated toxicity was evaluated following the National Cancer Institute toxicity guidelines.

Results: Analysis of DPD gene of 40 patients revealed the presence of a novel mutation 1845G®T (E615D) in only one patient. With respect to toxicity we found that this patient developed a grade IV hematological toxicity, a grade III mucocutaneous toxicity and some others bacterial associated infections.

Conclusion: Finding this mutation in a patient with severe toxicity to 5-FU is in keeping with a role of DPD gene mutations in the development of 5-FU associated toxicity.

1111 POSTER

Systematic identification of genes with coding microsatellites mutated in DNA mismatch repair-deficient cancer cells

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Microsatellite instability (MSI) caused by defective DNA mismatch repair (MMR) is a hallmark of tumors of the hereditary non-polyposis colorectal cancers (HNPCC) syndrome but also occurs in about 15% of sporadic tumors. If instability affects microsatellites in coding regions, frameshift mutations inevitably lead to functional inactivation of affected genes. Recently, coding microsatellites in several genes were shown to be mutated in MSI cancers thereby providing a selective growth advantage to MMR deficient cells thus contributing to MSI tumorigenesis. We initiated a systematic database search in 33595 annotated human genes and identified about 17000 coding mononucleotide repeats (cMNR) and about 2000 coding dinucleotide repeats (cDNR) consisting of $n > 6$ and $n > 4$ repeat units, respectively. Mutation analysis of 25 novel cMNR's with the longest repeats revealed instability frequencies of 5-96% in 30 MSI colorectal cancer tumors and cell lines, whereas microsatellite stable cancers lacked such alterations. All four cDNR's did not show MSI at all. RT-PCR analysis showed that most of the analyzed genes (19/25; 76%) were highly expressed in tumor cells. No correlation between mutation frequency and expression pattern was observed. Some of the cMNR's displayed significant differences in frameshift mutation frequencies among MSI colorectal and endometrial cancers. The approach outlined here enables us to identify comprehensively coding microsatellite genes as frameshift mutation targets in MSI tumor cells. The knowledge of these mutated genes ultimately points to key pathways altered during MSI tumorigenesis. This will lead to the development of novel and highly specific diagnostic and therapeutic strategies for MSI cancers.

1112 POSTER

An ongoing phase II study of tomudex (raltitrexed) plus irinotecan in advanced colorectal cancer

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Aims: To assess the efficacy and tolerability of a Tomudex (TOM) and Irinotecan (CPT) combination in patients with previously untreated Advanced Colorectal Cancer (ACC).

Patients and Methods: Inclusion criteria: Advanced Colorectal Adenocarcinoma confirmed by biopsy, aged > 18 years, WHO performance status score < or = 2, satisfactory haematological, renal and hepatic functions, and with at least one assessable or measurable lesion. Exclusion criteria: presence of any cerebral metastasis, concomitant use of any anticancer treatment either previous or adjuvant in the 6 months before.

CPT 350 mg/m2 was administered as c.i. over 90 min. followed, 1 hour later, by TOM 3 mg/m2 over 15 min. iv infusion, once every 21 days. All patients who received at least one cycle were evaluated for toxicity and those who received more than three cycles for efficacy.

Results: From March to October 2000, 72 patients in 14 Spanish centres (OncoPaz/Associated Hospitals Group) were included. In this preliminary analysis, toxicity data from 72 patients and efficacy data from 50 patients were available. Mean age was 60,3 (range: 35-77), median: 63 years. ECOG at inclusion was: 0 in 50%; 1 in 40% and 2 in 10%. Primary tumor was located in colon in 45 (62,5%). The most common metastases locations were liver 51 (47,6%) and lung 21 (19,5%). A total of 18 patients shown 1 metastases (25%), other 27 shown 2-3 mestastases (37,5%) and the remaining 27 shown more than 3 metastases (37,5%). A total of 367 cycles were administered with a mean of 5,1 cycles per patient (range:1-16), median 4,5. Moderate/severe or grade III-IV toxicity was assessed. The most frequent toxicity's were: early diarrhoea 8 (11,1%), nausea and vomiting 6 (8,3%), late diarrhoea 3 (4,1%), liver 3 (4,1%), anaemia 2 (2,7%), and mucositis 2 (2,7%).

Of 50 patients to value for efficacy, 2 (4,0%)had a complete response and 18 (36,0%)a partial response. OR 40,0% (I.C.:53,7%-26,3%), 18 patients (36,0%) showed stable disease and 12 (24,0%), progressive disease. The study is ongoing.

Preliminary Conclusions: A combination of Irinotecan and Tomudex is well tolerated, being diarrhoea the most frequent toxicity. Although there are very preliminary data, it can be stated that it is an effective treatment for ACC, obtaining a good objective response percentage: 40,6%. It has a convenient dosing schedule, every 21 days, which confers additional advantages for such population.

1113 POSTER

Informativity and results of LOH analysis of five APC gene polymorphic markers in sporadic colon cancer

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We examined 46 cases of human sporadic colon cancer and corresponding normal tissue samples to evaluate the loss of heterozygosity (LOH) at the APC gene loci.

DNAs were used for PCR, RFLP, VNTR and LOH analysis. To analyze LOH at the APC gene loci we used five polymorfic markers: three RFLP intragenic markers (exon 11 RsaI, exon 15 MspI, and exon 15 AspHI) and two VNTR flanking markers (D5S409 and D5S433).

The informativity for all three intragenic RFLP markers was 50.0% (23 of 46 assayed), and 21.7% of tumors (5 of 23 informative) demonstrated LOH. The informativity for VNTR flanking markers D5S409 and D5S433 was 60.9% (28 of 46 asseyed) and 87.0% (40 of 46 asseyed) respectively. Eight of 28 informative tumors (28.6%) demonstrated LOH for marker D5S409, and 14 of 40 informative tumors (35.0%) demonstrated LOH for marker D5S433. The informativity for all five APC loci was 100% and 14 of 46 tumors (30.4%) demonstrated LOH.

Our study showed that it was necessary to use VNTR flanking markers D5S409 and D5S433 to increase the number of informative cases (from 50% with intragenic markers) to 100%. The highest informativity was observed for VNTR marker D5S433, 87%.