colorectal cancer treated with CPT-11 were included in our retrospective study. The expression of hMLH1, hMSH2, and Bax was analyzed all tumors by immunohistochemistry. The MSI phenotype could be determined in 44 tumors through the analysis of the mononucleotide tracts located in the coding regions of RII-TGFb, BAX, hMSH3 and hMSH6 genes, and that of BAT26. A partial or minor response to chemotherapy with CPT-11 was observed in 11 patients, disease stabilization in 19 patients and progression in 20 patients. Staining of hMLH1 was undetectable in 3 of the 50 tumors, whereas only 1 tumor lacked hMSH2 expression. We found no association between a defect in hMLH1 staining and the response to CPT-11. Among the five tumors that displayed a MSI+ phenotype, four had frameshift mutations within TGFb-RII, BAX or hMSH3 genes. Among these, 3 tumors displayed mutations in BAX and showed a markedly reduced staining with the anti-Bax antibody. A decrease in Bax expression was associated with a better response to CPT-11 ($p<0.001$). We also found a significant correlation between the MSI+ phenotype and the umor responsiveness to CPT-11 ($p<0.001$). Our preliminary results indicate that MSI screening could help to select patients who would benefit from chemotherapy with CPT-11.

1107 POSTER

Randomized study of postoperative chemotherapy (CT) after preoperative chemoradiation (CTRT) in locally advanced rectal cancer (LARC). Preliminary results

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Purpose: to assess the value of concomitant CTRT as preoperative treatment and of postoperative CT in LARC. Study design: multicentric randomized 9/93-2/01. Random arms: preop. CTRT all cases; Arm A: surgery + observation; Arm B: surgery + postop CT. Endpoints: preop. CTRT: compliance, toxicity and tumor downstaging; postop. CT: freedom from loc. and dist. recurrences, survival.

Matherials and methods: 635 pts. (Arm A 309, Arm B 326). Inclusion criteria: tumor invading the perirectal fat at DRE (fixed or tethered) or at intrarectal US; age below 76; tumor origin lower 2/3; adenocarcinoma. Pts characteristics: males 419, females 216; median age 62 yrs; fixed 109, tethered 425, perirectal fat at US only 95. Preop. CTRT: 45 Gy (180 cGy x 5 weekly); 5-FU 350 mg/mq and Folinic Acid 10 mg/mq days 1 to 5 and 29 to 33, of the RT course. Postop. CT (Arm B): 5-FU 350 mg/mq and Folinic Acid 100 mg/mq days 1 to 5, six cycles, 3 weeks apart.

Results (to be considered preliminary as the data collection is still ongoing): Compliance to preop. CTRT: full treatment 584 (92%); RT only 3; 1 CT course 44; no CT and RT 7. Surgery: inoperable 15; refusal 4; died before surgery for intercurrent death 7; for disease 2; for toxicity 3; missing data 42; undergoing surgery 562 (88.5%%). Type of surgery: APR 188 (33.5%), LAR 340, TEM 24, palliative 10. Perioperative morbidity: anastomotic deiescence 44, perineal abscess 12, intestinal occlusion requiring surgery 12. Clinical downsizing (surgical evaluation): Tumor size reduction > 50% 353 (64.2%). Downstaging (pathological examination): T0 96 (17.4%), T1-T2 201(36.5%), T3 253; N+ 122(22.2%); positive margins 16(2.9%). Compliance to postop. CT: randomised Arm B 326; receiving 6 cycles 149, < 6 cycles 37, refusal 54, missing data 66. Follow-up: data available 536; median length 24.8 months; local recurrence only 28 (5.2%); local and distant 19 (3.5%); distant only 114 (21.3%). OS 5 yrs 67.3%. Prognostic factors: initial T extent $p<0.02$; APR $p< 0.05$; downstaging $p<0.05$; pN+ $p<0.05$. Treatment arms: distribution of prognostic factors well balanced; no difference neither in recurrence rate (n° of events: Arm A 71, Arm B 71) nor in survival (Arm A 63.5%, Arm B 67.5%).

Conclusion: concomitant CTRT was proved feasible as preop. treatment in LARC and resulted in a high downsizing/downstaging and in a low local recurrence rate. Postop. CT had a low compliance and did not result at the moment in any advantage on relapse rate or OS.

1108 POSTER

Evaluation of the clinical impact of serum tumor markers in colorectal cancer. A prospective longitudinal study

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Purpose: A controlled prospective study was designed to establish the efficacy of CEA, CA 19-9, and CA 72-4 serum markers as "decision making" clinical parameters when used in combination with the accepted diagnostic procedures in colorectal cancer, and to determine whether or not early treatment will have an impact on patient survival.

Methods: 315 consecutive patients with pathologically confirmed adenocarcinoma of the colorectal tract were assigned to a Study (n=220) or Control Arm (n=95), and followed for at least 3 years after surgery, or until the time of diagnosis of recurrence. Blood was taken for tumor marker estimation at each follow-up. An increase in serum marker levels was considered significant either when negative serum levels became positive or when an increase of greater than 50% of the mean of two previous positive levels was detected.

Results: The three serum markers paralleled the status of the disease in approximately 70% of the cases. No false positive were observed. The time interval between tumor marker increase and clinical diagnosis of recurrence was significantly shortened in Study compared to Control Arm, allowing an earlier diagnosis and treatment of recurrence ($p<0.0001$). For all markers the majority of the patients in the control arm had times to restaging greater than 150 days. As a result, radical surgery for recurrence increased from 28.1% (Control Arm) to 40.3% (Study Arm). Moreover, when the two arms were compared, an increased survival time for patients undergoing radical surgery for recurrent disease (Log Rank= 11.3, $p< 0.001$) was observed in the Study Arm. Patients in the Study Arm who received chemotherapy had a median survival time longer than patients in the Control Arm (Log Rank= 8.53, $p= 0.0035$). A significant improvement of the overall survival rate of colorectal cancer patients was observed in the Study Arm (log Rank= 16.7, $p< 0.0001$).

Conclusions: We can conclude that serum tumor markers can be used in combination to "guide" the timing for diagnostic imaging procedures during post-surgical follow-up of colorectal cancer patients. The adjunct of serum tumor markers in the post-surgical follow-up of colorectal cancer patients will allow an early diagnosis of recurrent disease, which may still be treatable by radical surgery, thus significantly increasing the overall survival.

1109 POSTER

Thymidylate synthase and dihydropyrimidine dehydroge-nase expression in stage II and III colorectal cancer patients receiving adjuvant 5-fluorouracil

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Purpose: To investigate the importance of thymidylate synthase (TS) and dihydropyrimidine dehydrogenase (DPD) expression for the disease specific survival of patients with colorectal cancer (CRC) receiving adjuvant 5-FU chemotherapy.

Methods: In paraffin-embedded primary tumor sections of 309 patients which participated in our adjuvant studies of colon (FOGT-1) or rectum (FOGT-2) cancer TS and DPD gene expression analysis could be successfully performed. mRNA quantitation was performed using a reverse transcription polymerase chain reaction technique with b-actin as internal standard.

Results: The median TS level was 0.75 (range: 0.21 - 7.21) and the median DPD levels was 0.28 (range: 0.01 - 1.62). The effect of TS and DPD on survival was analyzed in 295 patients. Univariate analysis revealed that only nodal stage, UICC stage, and TS were associated with disease specific survival, while DPD showed a slight tendency. Thus, patients with lower nodal stage, lower tumor stage, or higher TS (> 0.6) survived longer and patients with lower DPD levels (£ 0.4) tended to survive longer. Multiple Cox regression analysis showed that besides tumor stage only the combination of TS and DPD expression turned out to be a prognostic factor for disease specific survival.

Conclusions: Among patients receiving 5-FU therapy these with high TS and low DPD levels have longer survival than those with low TS and high

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%.