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## FEASIBILITY AND ACTIVITY OF A 3-DAY PELF-LIKE REGIMEN IN ADVANCED GASTRIC CANCER. PRELIMINARY DATA

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## DETECTION OF COLORECTAL CARCINOMA METASTASES IN BLOOD

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Metastasis is an important factor that determines the prognosis of patients with cancer. A non-invasive method for the early detection of disseminating disease would provide an important predictive tool with respect to recurrence and to result in a more appropriate selection of patients for adjuvant therapy.

We have used a combination of immunomagnetic separation to isolate epithelial cells from whole blood and detection by nested RT-PCR. Monoclonal antibody BerEP4, which recognizes an epitope expressed by epithelial cells, was covalently linked to magnetic beads. The RT-PCR was performed with oligonucleotides derived from the sequence of the keratin 19 gene. Serial dilutions of SW620 tumor cells were performed in normal blood. We were able to detect 1 colorectal carcinoma cell in 1 ml of whole blood.

The clinical applicability of this technique was documented by evaluating patients with a colorectal carcinoma. Two patients presenting a tumor no deeper than the submucosa (stage A) were tested. None of them had epithelial cells in their blood. For 2 out of the 8 patients who had no detected node involvement (stage B) we were able to early identify a disseminating disease. These patients may thus be at high risk of relapse. Eight patients with histologic evidence of locoregional lymph node involvement were analyzed. Six of them had detectable epithelial tumor cells in their blood. This shows that, in addition to lymph node metastases, the majority of these patients have potential distant metastases in their blood. Finally, we have analyzed 5 stage D patients. Four of them had circulating epithelial tumor cells in their blood.

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## MOLECULAR ANALYSIS OF APC MUTATIONS IN FAMILIAL ADENOMATOUS POLYPOSIS

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Recently we identified three mutations in the APC gene, the insertion of one base, the deletion of 4 bp and the deletion of 2 bp in exons 5, 14 and 15, respectively, in FAP patients from the Neapolitan area.

In this study we report two novel germ-line mutations, identified by means of PCR-SSCP analysis, protein truncation test (PTT) and direct sequencing: the deletion of an A residue at nucleotide 2638, and the insertion of an A residue after nucleotide 2803, both in exon 15. These mutations can be analysed by restriction enzyme digestion. Our data are in agreement and enlarge the notion that the majority of APC mutations occur in exon 15 and result in an early stop codon, thus giving rise to a truncated protein. Furthermore, in the course of screening of the APC gene, we have identified a new polymorphism in exon 15, fragment L, that can be used in cosegregation studies for the presymptomatic diagnosis of FAP and for family studies.

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## INDUCTION OF A HEAT SHOCK RESPONSE PROTECTS TUMOUR CELLS FROM MONOCYTE MEDIATED LYSIS.

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**Hypothesis:** The proliferation of tumour cells despite tumouricidal mediators being present could be due induction of a heat shock response, a universal cellular defense mechanism in host cells and possibly tumour cells. Protection may be mediated either by increasing intracellular levels or surface expression of heat shock proteins (HSP)

**Aim:** To assess the effect of heat shock induction on tumour cell protection against host effector cells. **Methods:** The heat shock response was induced in SW707 colorectal cells by either sodium arsenite (SA, 0-320 μM for 6hrs) or by hyperthermia (42°C for 20min). Monocyte mediated cytotoxicity, flow cytometry to assess surface expression of HSP60 and HSP70 or western blots of whole cell lysates to assess total HSP60 and HSP70 were performed. **Results:** Cytotoxicity showed a significant decrease in all treated groups (p<0.002) when compared to the control value of 40.45±0.812%. There was also a significant decrease in all groups (p<0.001) when compared to the 42°C value of 11.50±0.799%. No significant alteration in surface expression of either HSP60 or HSP70 was seen. In whole cell lysates HSP60 expression was unaltered in all groups but HSP70 expression was increased with SA 40-160 μM and with heat treatment. **Conclusion:** Heat shocking tumour cells significantly protects them from Mφ-mediated tumour cell lysis. Since the flow cytometric data indicate that there is no concomitant increase in surface expression of HSP60 and HSP70 on the tumour cell when the cells are heat shocked, it can be inferred from the whole cell lysate data that induction of intracellular HSP70 levels are in part responsible for the protective effect on the tumour cells.

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## COMBINED RADIOCHEMOTHERAPY IN PATIENTS WITH LOCALLY ADVANCED PANCREATIC CANCER (PC)

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20 patients (12 males, 8 females) with unresectable locally advanced PC were treated with combined modality therapy. Median age was 57 years (range, 31-70 yrs.) and median WHO performance status was 1 (range, 0-2). 8 pts. had stage II and 12 pts. had stage III, histology was ascertained during explorative laparotomy or by CT-guided aspiration cytology. Treatment consisted of 4-6 intravenous courses of 5-fluorouracil 400 mg/m<sup>2</sup>, leucovorin 200 mg/m<sup>2</sup>, and cisplatin 20 mg/m<sup>2</sup>. All drugs were given on days 1-4; courses were repeated every 28 days. During the 2nd and 3rd course (that was reduced in dose by 25 %) radiation therapy (cobalt 60 or linear accelerated 6MV, 3-field wedged technique 2 Gy/day, 5 days/week) was administered concomitantly for a total dose of 50-60 Gy. 15 pts. are currently evaluable for response and toxicity, 5 pts. are too early. The overall response rate was 53 % (8/15 pts.), including 1 pathologically documented complete response and 7 partial responses; 3 pts. (20 %) had stable disease and 4 pts. (27 %) had progressive disease. Median survival has not been reached, 65 % are currently alive. Severe (WHO grade 3 or 4) toxicity was uncommon and included neutropenia in 3 (20 %), thrombocytopenia in 2 (13 %), diarrhea in 2 (13 %) and nausea in 3 pts. (20 %). All other side effects were mild to moderate, renal or neurological toxicity were not recorded. Preliminary results obtained with this combined modality regimen are encouraging, and the study remains in progress.

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## LOCAL RECURRENCE AFTER TOTAL RECTAL RESECTION, MESORECTUM EXCISION AND COLOENDOANAL ANASTOMOSIS FOR TREATMENT OF LOW RECTAL CANCER.

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Total rectal resection (TRR) with coloendoanal anastomosis (CEAA), mesorectum "en bloc" removal, radical abdomino-pelvic lymphadenectomy (RAPL) and J colic reservoir represents a valid alternative to the traditional surgery for the management of rectal cancer. Globally at the NCI of Milano from March 1990 to November 1995, 141 CEAs associated with a colic reservoir were done. The present abstract concerns 115 CEAs performed in 111 consecutive patients affected with primary rectal carcinoma (24 pts Dukes A; 26 pts Dukes B; 52 pts Dukes C and 9 pts Dukes D). All lesions were located in the lower third of the rectum with a distance from the anal verge ranging from 4 to 7 cm. The follow up period ranged from 3 to 53 months (median 24). The distance of the distal tumor margin from the resection edge of the rectum ranged from 1 to 6 cm. All Dukes A patients did not show local relapse while only 15 Dukes C/B patients (2 pts Dukes B and 13 pts Dukes C) presented pelvic relapses after TRR and CEAA from 7 to 40 months. Only one case showed this recurrence at the para-anastomotic site. Post-operative morbidity due to procedure was low. A perfect continence was documented in 66% of cases after colostomy closure and many patients (63%) referred one or two bowel movements a day. Presently 78 patients of this series are alive, 73 of whom without actual evidence of disease. At present it is unanimously accepted that minimum distance edge from the neoplasm must not be more than 2 cm. However some literature data and our personal experience show that free distal margin from neoplasm is less important than thought in the past, what regard to local relapse and survival time, but a careful total mesorectum excision seems to be the most important factor in reducing incidence of local and pelvic recurrence. We conclude that a conservative surgical approach such TRR and CEAA can be considered a feasible option to the traditional abdomino perineal resection for primary cancers in the low rectum.