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High-dose Folinic Acid, 5-Fluorouracil Bolus and Infusion in Advanced Pancreatic Adenocarcinoma: a Pilot Study

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MOST PATIENTS with pancreatic adenocarcinoma present with unresectable advanced disease. 5-Fluorouracil (5FU), streptozotocin and mitomycin-C have demonstrated limited antitumoral activity [1] and combined regimens did not improve results [2]. Folinic acid has been reported to enhance the activity of 5FU [3]. 5FU was given as a continuous infusion with an added bolus in a 2-day/2-week schedule to maximise 5FU doses and to avoid the cumulative toxicity of 5-day consecutive regimens. This combination has been used with low toxicity in advanced colorectal [4] and gastric cancers [5].

20 previously untreated patients (13 males/7 females, mean age: 53 years, range 30–76) with advanced non-resectable measurable histologically confirmed pancreatic adenocarcinoma entered the study. They received a 2-h infusion of folinic acid 200 mg/m^2, followed by 5FU 400 mg/m^2 bolus and a further 600 mg/m^2 in a 22-h infusion on days 1 and 2, every 2 weeks (LV5FU2). Performance status was 0 in 4 patients, 1 in 9, 2 in 5 and 3 in 2. 16 patients had liver metastases, 6 had peritoneal carcinomatosis, 5 had lymph node involvement and 3 had pleural or lung metastases. Extension and tumour size were evaluated by computed tomography scan prior to therapy. The first evaluation was made after 3 months and subsequently at 3-month intervals. Responders and stable patients continued on the same treatment until progression. Response was evaluated according to WHO criteria. Duration of response and survival were calculated from the beginning of treatment until progressive disease or death, respectively. Radiotherapy was given in patients with pain, as evaluated on distant metastases. The median follow-up time in October 1992 was 25 months.

Two partial responses (10.5%; 95% confidence interval: 0.1–24.6%) and six stabilisations (one minor response) (31.6%) were observed in 19 evaluable patients. Partial responses lasted 10+ and 12 months and the minor response lasted 17+ months. Median survival was 6 months, 4 patients were alive at 1 year. Treatment was well tolerated, grade 2 and 3 WHO toxicity was as follows: diarrhoea 2 patients, nausea and vomiting 3, mucocitis 2 and anaemia 2 patients. Of the stable patients and responders, performance status and symptoms improved in 3 patients, remained stable in 4 and worsened in 1.

The regimen is well tolerated but only achieved a 10% response rate and 6 months median survival. This agrees with two previous studies of 5FU/leucovorin which also reported poor results in advanced pancreatic cancer [2, 6]. Furthermore, no response was obtained in 6 patients with advanced pancreatic carcinoma treated with the same regimen [7]. The poor median survival led to a discontinuation of the pilot study. This combination is not recommended in the treatment of advanced pancreatic adenocarcinoma.

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Low Frequency of NF1 Gene Mutations in Malignant Gliomas

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DIFFERENT MUTATIONS of the NF1 gene have been described in patients with type 1 neurofibromatosis, an autosomal dominant disorder associated with cutaneous and subcutaneous tumours,

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optic nerve gliomas and neurofibromas, and increased risk of brain tumours [1, 2].

The NF1 gene product contains a GTPase activating protein (GAP) domain which is capable of downregulating the p21*ras* signal [3–5]: one exon encoding part of this GAP domain is characterised by the presence of a highly conserved phenylalanine–leucine arginine (FLR) motif [6]. Therefore, mutations in this region could affect the *ras* signalling pathway and contribute to tumorigenesis.

Interestingly, an altered lysine residue in the FLR exon of NF1 has been detected in three tumour types: colon adenocarcinoma, myelodysplastic syndrome and grade II astrocytoma (1 case out of 10 studied) and expression analysis demonstrated that GAP activities of these NF1 mutant proteins can be severely reduced [7].

It is well known that mutations of tumour suppressor genes, such as the p53 gene, can be involved in the development of tumours in the human nervous system [8]. However, the mutation reported by Li *et al.* [7] is the only one involving the NF1 gene in astrocytomas, the most frequent among brain tumours.

If NF1 mutations have a role in the neoplastic progression of astrocytomas one should expect to find them in glioblastomas (grade III astrocytomas), which represent the most malignant stage in such evolution.

In order to verify this hypothesis we have amplified the FLR exon in 18 cases of glioblastoma. Tumour DNA was prepared by phenol extraction [9] and two intronic oligonucleotide primers were used to amplify the FLR exon. The sense primer was NF1 (5′ CAAACCTTATACTCAATTCTCAACTC 3′) and the antisense primer NF2 (5′ AAGGGGAATTTAAGATAGCTAGATTATC 3′) [7]. Each polymerase chain reaction (PCR) mixture (100 μl) contained 2 μg of genomic DNA, 50 pmol of each primer, 200 μmol/l for each deoxynucleotide, 2 mmol/l $MgCl_2$, 50 mmol/l KCl, 10 mmol/l Tris–Cl (pH 8.3) and gelatine 0.1% (w/v). Thirty-five cycles of amplification were carried out at 94°C for 1 min, 58°C for 1 min, and 72°C for 1 min, with 5 min of initial denaturation, on a Trio Thermoblock incubator (Biometra). Direct sequencing was done by the authomatic method using "Taq Dydeoxy Terminator Cycle Sequencing Kit" and the Sequencer model 373A (Applied Biosystems). Primers used to amplify DNA fragments from the NF1 gene were also used for sequencing reactions.

Direct sequencing of both strands failed to identify any mutation. Therefore, if we consider our data together with those of Li *et al.* [7] only one out of the 28 astrocytomas examined is carrying a mutation of the FLR exon of NF1.

The number of patients examined can certainly be extended and the assessment of the role of NF1 alterations in astrocytomas requires a further study of the other domains in this large gene. Nevertheless, our data suggest that mutations of tumour suppressor genes others than NF1 may be crucial for the development of these highly fatal malignancies.

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Neurotoxicity Following Weekly Therapy with Folinic Acid and High-dose 5-Fluorouracil 24-h Infusion in Patients with Gastrointestinal Malignancies

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IN RECENT months we have observed clinically relevant neurotoxicity in 4 out of 64 patients with various gastrointestinal malignancies treated weekly with folinic acid (FA) (500 mg/m^2) as a 1-h infusion and high-dose fluorouracil (5-FU) (2.600 mg/m^2) as a 24-h infusion [1].

The first patient, a 64-year-old male, had had several seizures of unknown aetiology in 1977, but since that time he has been seizure-free without medication. In May 1992 therapy with FA and 5-FU was started for an inoperable local relapse of a colon cancer. Six days after the fifth infusion the patient had a generalised seizure of grand mal type. Shortly thereafter, neurological examination and computed tomography of the brain were unremarkable. As the patient responded to the treatment, prophylactic anticonvulsant therapy with phenytoin was started and so far three additional 5-FU infusions have been given without any neurotoxicity.

The second patient, a previously healthy 60-year-old woman, had a left sided hemicolectomy in February 1990 for colon carcinoma. In June 1992 amyotrophic lateral sclerosis was highly suspected for muscular weakness with muscular atrophy and hyper-reflexia, sensations of discomfort in several muscles and visible fascicular twitchings of muscle fibres. Electroencephalogram (EEG) revealed signs of general increased activity. Cranial

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