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Short Communication

Phase II Trial of *N*-(phosphonacetyl)-L-aspartate (PALA), 5-Fluorouracil and Recombinant Interferon-α-2b in Patients with Advanced Gastric Carcinoma

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The aspartate transcarbamoylase inhibitor, *N*-(phosphonacetyl)-L-aspartate (PALA), synergistically enhanced the cytotoxicity of a combination of 5-fluorouracil (5-FU) and interferon-α (IFN) against human colon cancer cell lines *in vitro*. To test the efficacy of this combination in the clinical setting, patients with locally advanced or advanced gastric carcinoma were treated with the combination of PALA, 5-FU and IFN (PFI). Patients were required to have biopsy-proven disease beyond the scope of surgical resection, measurable disease, no prior chemotherapy, adequate bone marrow, renal and hepatic function, to be fully ambulatory and to have given informed consent. Drug was administered as follows: PALA, 250 mg/m^2, 15 min i.v. infusion, days 1, 15, 22, 29, and then weekly; 5-FU, 750 mg/m^2 daily × 5 as a continuous i.v. infusion beginning day 2, then at 750 mg/m^2 days 16, 23 and 30, then weekly; IFN, 9 MU subcutaneously three times per week beginning day 2. There were 22 patients enrolled. The major toxicities were fatigue and associated neurotoxicity, with acceptable gastrointestinal and haematological toxicities. There was one complete responder (5%) and 3 partial responders (14%); two of these responses were durable (> 3 years). Despite this modest clinical activity, other regimens for advanced gastric cancer such as FAMTX and ELF appear to have greater activity with comparable toxicity.

Key words: gastric cancer, 5-fluorouracil, *N*-(phosphonacetyl)-L-aspartate (PALA), interferon-a-2b, biochemical modulation

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INTRODUCTION

THERAPY FOR metastatic or recurrent gastric carcinomas is currently inadequate. While combination chemotherapy is superior to single agent therapy, overall survival remains poor (reviewed in [1]). More recent trials, employing intensified cisplatin-based therapies, have appeared promising, but with median survivals in the 6–8 month range [2, 3]. In contrast, regimens designed to exploit the principles of biochemical modulation may have equivalent or greater activity to combination chemotherapy regimens. For example, the ELF regimen, which combines 5-fluorouracil (5-FU) with the modulating agent, leucovorin, was designed for the treatment of patients who were not candidates for EAP and may yield comparable results to cisplatin-based therapies [4]. Furthermore, the FAMTX regimen, which exploits the sequence-dependent modulation of 5-FU by methotrexate was therapeutically equivalent to EAP with less toxicity [5]. Thus, it is reasonable to continue to investigate biochemical modulation as a treatment strategy in gastric cancer.

PALA, an inhibitor of cytidine synthesis, has been shown to enhance the cytotoxic effects of 5-FU *in vitro* by at least five potentially important mechanisms (reviewed in [6]). The combination of 5-FU + PALA has already demonstrated clinical activity in patients with advanced gastric carcinoma [7]. Interferon has been shown to enhance synergistically the cytotoxicity of 5-FU *in vitro* and may enhance the clinical activity of 5-FU in patients with carcinomas of the colon and

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oesophagus (reviewed in [8]). To determine whether double modulation of 5-FU with the combination of PALA and IFN was feasible, and to determine the activity of this triple combination (PFI), a clinical trial was initiated in patients with advanced gastric carcinoma.

PATIENTS AND METHODS

Eligibility

Patients were required to have biopsy-proven adenocarcinoma of the stomach which was either recurrent following definitive resection, or metastatic and beyond the scope of surgical resection. All patients had measurable disease and were previously untreated with chemotherapy or radiotherapy. All patients were fully ambulatory and had adequate bone marrow, renal and hepatic function defined as leucocyte count ≥ 4000 cells/mm^3, platelets ≥ 100000 cells/mm^3, bilirubin ≤ 1.5 mg/dl, SGOT $< 3\times$ upper limit of normal, alkaline phosphatase $\leq 3\times$ the upper limit of normal, and creatinine ≤ 2.0 mg/dl. Patients had to have adequately recovered from surgery and could not have co-morbid conditions which precluded administration of this regimen, such as uncontrolled infections, heart disease or diabetes, and were required to have adequate nutritional intake. All patients gave informed consent.

Study design

The objectives of this study were to determine the overall response rates and toxicity of the combination of PALA–5-FU–IFN in a phase II clinical trial in patients with advanced gastric cancer. The study was conducted jointly by the Albert Einstein College of Medicine (Bronx, New York, U.S.A.) and the University Hospital Benjamin Franklin (Freie University, Berlin, Germany). Accrual goals and statistical analysis met standard National Cancer Institute (NCI) criteria for phase II studies [9]. PALA was supplied by U.S. Bioscience, West Conshohocken, Pennsylvania, U.S.A., or by U.S. Bioscience, Europe, Watford, U.K. Interferon-α-2b was supplied by Schering-Plough Pharmaceuticals, Kenilworth, New Jersey, U.S.A., or Essex Pharmaceuticals (Munich, Germany). 5-FU was purchased commercially.

PALA, 250 mg/m^2, was administered on days 1, 15, 22, 29 and then weekly, exactly 24 h prior to administration of 5-FU, in a 15 min i.v. infusion. 5-FU was administered at 750 mg/m^2 daily $\times 5$ as a continuous i.v. infusion beginning day 2, then at 750 mg/m^2 on days 16, 23, 30 and thereafter weekly by rapid i.v. infusion exactly 24 h after administration of PALA. Patients were instructed to suck on ice chips from 15 min before to 1 h after administration of 5-FU. IFN was administered at 9 MU subcutaneously three times per week starting day 2 with at least 24 h between each administration, and subsequently on each day that 5-FU was received.

Dose modifications were based on NCI Common Toxicity Criteria [10]. The dose of IFN was reduced by 50% without interruption of therapy for severe neurotoxicity or decrease in performance status by two levels persisting for > 7 days. 5-FU was withheld for grade 2 diarrhoea or any watery diarrhoea, reduced by 25% for three episodes of watery diarrhoea in 1 day or grade 3 diarrhoea and restarted at 75% of the dose, and similarly for grade 2–3 stomatitis developing during bolus therapy, or for $\geq$ grade 2 leucopenia or granulocytopenia. Patients were removed from study for grade 4 non-haematological toxicity or grade 4 haematological toxicity associated with haemorrhage or severe infection, or for progressive disease.

Table 1. Demographic characteristics

	n
Patients enrolled	22
Performance status 0:1	14:5*
Age (years)	
Median	55
Range	24–76
Disease status	
Locally advanced	13
Distance metastases	16
Liver	9
Lymph nodes	12
Lung	2
Peritoneum	5
Ovary	1

*3 patients not recorded.

Patients were required to have measurable disease. All patients were evaluated with an abdominal computed tomographic scan prior to study entry, then every 6 weeks, as well as by measurement of serum carcinoembyonic antigen, CA-19-9 and CA-125. Standard ECOG response criteria were employed [11].

RESULTS

Demographic characteristics

A total of 22 patients with metastatic or recurrent gastric cancer were enrolled, including 7 from Albert Einstein and 15 from the Freie University. Over half the patients had locally advanced disease and over half had distant metastatic disease, with 7 patients having evidence of both. Patient characteristics are shown in Table 1.

Toxicity

The toxicity is shown in Table 2. All patients experienced IFN-induced constitutional symptoms. The major toxicity of this regimen was fatigue with associated somnolence and decreased performance status. One patient with pre-existing Parkinson's disease had worsening of his symptoms. One patient developed a focal sclerosing glomerulonephritis, most likely secondary to IFN treatment, which required dialysis.

Table 2. Toxicities (n = 22)

	ECOG grade			
	1	2	3	4
Thrombocytopenia	1	2	0	0
Leucopenia	1	3	5	0
Anaemia	4	2	0	0
Alopecia	2	2	0	0
'Flu'-like syndrome	1	5	7	0
Nausea	3	3	2	0
Diarrhoea	1	4	2	0
Stomatitis	0	4	2	0
Fatigue	0	2	4	0
Mood swings	1	2	0	0
Infection	1	0	1	0
Neurotoxicity	2	0	0	1*

*Worsening of symptoms of Parkinson's disease.

One patient developed severe enterocolitis and withdrew from the trial after one cycle. One patient withdrew after one cycle because of IFN-associated symptoms. Diarrhoea, nausea and vomiting, and myelosuppression were all within acceptable limits.

Response

There were four responders [one complete response (5%) and three partial responses (14%)] among the 22 patients. The overall response rate was 18% (95% CI, 8–30%). All responders were among the 7 patients treated at Albert Einstein ($P = 0.0023$).

DISCUSSION

PALA is a potent inhibitor of aspartate transcarbamoylase (ATCase), a critical enzyme for an early step in the synthesis of pyrimidines [6]. Treatment of patients with PALA results in inhibition of ATCase activity and depletion of pyrimidine nucleotide pools [12]. PALA enhances the cytotoxic effects of 5-FU *in vitro* by depletion of pools of UTP, thus increasing the FUTP:UTP ratio and incorporation of FUTP into RNA [13], or alternatively by increasing the ratio of FdUMP:dUMP with augmented inhibition of thymidylate synthase [14].

It is of interest that the triple combination of PALA + 5-FU + IFN (PFI) demonstrated synergistic cytotoxicity *in vitro* against two human colon cancer cell lines, which was greater than that achieved with the doublets, 5-FU + IFN or PALA + 5-FU [15]. Treatment with this combination resulted in depletion of pools of both pyrimidine nucleotides, TTP and dCTP, but the mechanism of interaction most likely involved increased incorporation of 5-FU anabolites into RNA. Thus, double modulation of 5-FU with both PALA and IFN appeared to be a promising approach in the treatment of solid tumours and formed, in part, the rationale for a clinical trial in patients with gastric carcinoma.

The results of our trial, while provocative, do not support the routine use of this regimen in the treatment of gastric cancer. Although the overall response rate, 4 of 22 (18%), was not substantially different from that of 5-FU alone, it was of interest that while 0 of 15 patients at the Berlin campus responded to treatment, 4 of 7 (57%) patients at the Albert Einstein campus demonstrated important clinical responses. These included 2 of 4 with durable responses, a response in a patient with massive retroperitoneal tumours, and a long-term response in a patient with linitis plastica histology, a histology which almost always confers a dismal prognosis. The difference in outcome between the two campuses most likely reflects either a biological difference between the two patient populations, a subtle difference in patient selection factors, or simply a difference by chance.

The positive clinical results with regimens such as ELF or FAMTX suggest that biochemical modulation is a viable strategy for future therapeutic development against gastric carcinoma [4, 5, 7]. A combination of PALA, 5-FU and IFNα, which appears to target RNA, rather than DNA or DNA precursors, remains of interest, and further studies may be worthwhile, although the development of interferon-induced renal failure in a single patient mitigates caution in developing this regimen. Finally, further U.S.–European collaborative efforts may allow the investigation of a broader spectrum of patients with results that are more generalisable than single institution studies, and should be encouraged.

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