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ORIGINAL ARTICLE

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Phase II clinical trial of parenteral hydroxyurea in combination with fluorouracil, interferon and filgrastim in the treatment of advanced pancreatic, gastric and neuroendocrine tumors

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Abstract *Purpose*: Combined inhibition of ribonucleotide reductase (RR) and thymidylate synthase (TS), the enzymes responsible for a balanced supply of nucleotides for DNA synthesis, has been shown to induce synergistic antiproliferative effects in vitro. In the clinic, prolonged infusion of the RR inhibitor, hydroxyurea (HU), may be more effective than bolus or oral administration of drug. The purpose of the current study was to determine whether dose intensification of parenteral hydroxyurea in combination with fluorouracil could enhance the response rates of the combination against refractory upper gastrointestinal malignancies. *Methods*: A clinical trial of parenteral, weekly, high-dose HU in combination with weekly, high-dose infusional fluorouracil (5FU) was initiated in patients with advanced pancreatic and gastric cancer. Patients received 5FU 1.3 g/m^2 by continuous intravenous infusion (CIVI) daily over 48 h weekly in combination with HU 4.3 g/m^2 CIVI per day over 48 h weekly. Patients also received the biologic agent interferon alfa-2a 9 MU subcutaneously (s.c.) three times per week and filgrastim 480 μg s.c. on days 3 (starting after midday), 4, 5, and 6 each week. Each cycle required treatment on days 1 and 8 every 22 days. *Results*: Enrolled in the study were 32 patients, of whom 30 were evaluable. The median age was 56 years. Primary sites included pancreas (18), gastric (13) and islet cell (1). Despite filgrastim, the major toxicities were hematologic with 15 of 30 patients developing grade 3/4 granulocytopenia. Of the 30 patients, 4 developed grade 3/4 diarrhea. Interferon-mediated fatigue was mild. Of 12 evaluable patients with gastric cancer, 1 had a partial response, and there were no responders among patients with pancreatic cancer. *Conclusions*: Combined inhibition of RR and TS using this high-dose, weekly, 48-h infusional regimen is not an improvement over single-agent therapy in these tumor types.

Keywords Hydroxyurea · Fluorouracil · Ribonucleotide reductase · Thymidylate synthase · Pancreatic cancer · Gastric cancer

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Introduction

Systemic therapies for adenocarcinoma of the upper gastrointestinal (GI) tract have had very limited efficacy. Gemcitabine is the only approved agent for the treatment of pancreatic cancer, but the median survival in patients with metastatic disease treated with this drug is less than 6 months [1]. Systemic therapy for stomach cancer offers more options, but outcomes remain poor. Anthracycline-based regimens have demonstrated clinical activity, but without an improvement in median survival (reviewed in reference 2). In a large trial conducted by the European Organization for Research and Treatment of Cancer (EORTC), in which ELF (etoposide, leucovorin, fluorouracil), FUP (infusional fluoro-

uracil and cisplatin) and EAP (etoposide, Adriamycin, cisplatin) were compared, the median survival times were 6.7–7.2 months [3]. Recently, the combination of irinotecan and cisplatin has been shown to have clinical activity [4]; however, median survival was only 9 months. Therefore, better systemic therapies for these refractory malignancies are required.

Both gemcitabine and fluorouracil (5FU), mainstays of treatment for upper GI malignancies, act by inhibiting synthesis of DNA precursors in cancer cells, with the goals of growth inhibition and of induction of DNA damage, leading to apoptosis. One cellular target for gemcitabine is ribonucleotide reductase (RR), an anabolic enzyme necessary for cellular purine synthesis [5], and a comparable target for fluorouracil is thymidylate synthase, necessary for synthesis of DNA thymidine precursors [6]. Thus, it is logical to conclude that combined inhibition of RR and thymidylate synthase may be more effective than strategies targeting either enzyme alone.

Hydroxyurea (HU) is an effective inhibitor of the small subunit of RR (reviewed in reference 7). Preclinical studies of the combination of HU and 5FU have demonstrated that combining these agents results in enhanced depletion of nucleotide pools and synergistic antiproliferative effects against human colon carcinoma cell lines [8]. Of interest, treatment with HU is associated with a two- to fourfold induction of both subunits of RR, suggesting that inhibition of the enzyme causes a compensatory increase in synthesis of RR mRNA resulting in synthesis of new, uninhibited enzyme within 24 h of drug exposure [9]. Therefore, our initial trial design incorporated a prolonged 24-h infusion of HU with infusional 5FU administered on a weekly basis [10] in order to sustain inhibition of target enzyme.

The biologic agent, interferon (IFN), has also been shown to enhance the antiproliferative effects of both 5FU and HU in vitro; therefore, this agent was incorporated into the trial design. IFN at nontoxic concentrations decreases the IC_{50} of HU from 368 μM to 215 μM ($P < 0.01$) in human colon cancer cell lines in vitro. The mechanism of action is unclear: detailed studies have revealed that IFN at a clinically achievable concentration (500 U/ml) fails to augment the effects of HU on RR protein levels, RR mRNA levels or RR enzyme activity in either wild-type or HU-resistant cells, suggesting that the mechanism by which IFN augments the effects of HU in the wild-type cells is independent of the effects of HU on M2, the small subunit of RR. In contrast IFN has been shown to enhance the cytotoxic effects of 5FU both in vitro and in vivo by inducing expression of thymidine phosphorylase, thereby increasing levels of FdUMP, the active anabolite of 5FU.

Initial studies with the combination of 5FU and HU administered as a weekly, high-dose 24-h infusion in patients with advanced gastric cancer were encouraging, with 44% response rates and a median survival of 10 months [10]. Nevertheless, resistance to HU remains a central problem. The mechanism of resistance is complex. Preclinical studies have demonstrated overexpression of M2, the small subunit of RR, in HU-resistant cell lines. In cells resistant to gemcitabine, another inhibitor of RR, enhanced expression of RR results from both gene amplification [11] and upregulation of M2 transactivation, likely related to transcriptional regulation of CCAAT motifs in the M2 promoter [12, 13, 14]. Preclinical studies in human colon cancer cells in vitro have demonstrated that longer exposures to HU result in greater accumulation of cells in S phase at the G_2 boundary and greater perturbation of both purine and pyrimidine pools, an effect associated with greater cytotoxicity in this system [8]. We reasoned that prolonged inhibition of RR might overcome RR resistance, and postulated that prolonging the infusion of HU from 24 to 48 h might result in an enhanced therapeutic effect. A phase I trial demonstrated the tolerability of such an intensified regimen when administered with filgrastim on days 3–6 [10].

Therefore, a phase II trial of 48-h 5FU and HU administered as a weekly, high-dose prolonged infusion in combination with IFN was initiated in patients with advanced gastric and pancreatic cancer. As the major toxicity of parenteral HU is myelosuppression, we also administered filgrastim in an attempt to maintain the dose intensity of this regimen.

Patients and methods

Administrative

This was a single institution, prospective phase II trial, which was approved by the Protocol Review Committee of the Albert Einstein Cancer Center and the Institutional Review Board of Montefiore Medical Center. The primary aim of the study was to determine the objective response rates to this regimen in patients with refractory tumors of the upper GI tract. While we did not anticipate that we would be able to accrue sufficient numbers of patients with neuroendocrine tumors in a single institution study to determine the response rate to this regimen with a high degree of confidence, we nevertheless included this patient population because IFN is active in this group of patients as a single agent and because we had seen clinical activity for the combination against neuroendocrine tumors in previous 5FU/HU/IFN combination studies, and therefore we thought we could generate sufficient pilot data for a future multi-institutional study.

Eligibility

Patients were required to have histologically confirmed adenocarcinoma of the stomach or pancreas beyond the scope of surgical resection. Neuroendocrine tumors of the pancreas were also allowed. Patients with hepatobiliary tumors were included in this trial; results with these patients will be reported separately. All patients had measurable disease. Patients must have received no prior systemic chemotherapy for advanced disease, but were allowed prior 5FU or gemcitabine as a radiation sensitizer for locally advanced disease. For patients who had received prior radiation therapy, a 1-month recovery time was required. All patients had an Eastern Cooperative Oncology Group (ECOG) performance status < 2, an adequate leukocyte count ($> 4.0/mm^3$), adequate platelet count ($> 100{,}000/mm^3$), adequate serum creatinine (< 2.0 mg/dl), and adequate hepatic function (AST and serum bilirubin less than three times normal). All patients gave informed consent.

Table 1 Schema (*F* 48-h infusion of 5FU beginning day 1 of each treatment week of treatment; *I* IFN treatment days 1, 3 and 5 of each treatment week; *H* 48-h infusion of HU beginning day 1 of each treatment week; *G* G-CSF (filgrastim) days 3, 4, 5 and 6 of each treatment week; *O* no treatment)

	Week			
	1	2	3	4
5FU 1.3 g/m^2 CIVI per day over 48 h weekly	F	F	O	F
IFNα 9 MU s.c. three times per week	III	III	OOO	III
HU 4.3 g/m^2 CIVI per day over 48 h weekly	H	H	O	H
Filgrastim 480 μg s.c. days 3 (starting after midday), 4, 5 and each week	GGGG	GGGG	OOOO	GGGG

Study design

The schema, which includes the doses of drug employed, for the study is shown in Table 1. Patients were treated for 2 weeks, followed by 1-week rest. Treatment resumed on day 1 of week 4. One cycle was defined as 6 weeks of treatment. Both 5FU and HU were administered by CADD pump or other comparable portable pump device in the outpatient setting. Patients had double-lumen Port-a-caths placed prior to initiating therapy. Acetaminophen 650 mg orally was administered 0.5 h prior to administration of IFN then every 4–6 h as needed for fevers, and was the only routine supportive care required. Filgrastim was administered 4 days per week in order to maintain the dose intensity of the regimen. Doses were modified for diarrhea, stomatitis, IFN-mediated constitutional symptoms including fatigue, neurotoxicity or decreased performance status, and myelosuppression. Criteria for response followed ECOG guidelines [15].

Statistical analysis

The aim of this study was to determine whether this regimen had a response rate $>40\%$ or $<20\%$ in patients with gastric tumors and a response rate $>20\%$ or $<5\%$ in patients with pancreatic cancer. Simon's optimal two-stage design for phase II clinical trials was used. The trial had an 80% power to distinguish between a response rate of $<20\%$ and $>40\%$ with a significance level of 0.05. For patients with pancreatic cancer, the trial had an 80% power to distinguish between a response rate of $<5\%$ and $>20\%$ with a significance level of 0.05.

Results and discussion

As shown in Table 2, 32 patients were enrolled in this trial. After the first treatment, two patients refused further therapy, and were therefore considered inevaluable. There were 18 patients with pancreatic cancer, 13 with gastric cancer and 1 with islet cell tumor. The majority of the patients had liver metastases, as expected. As shown in Table 3, the major toxicities among all cycles of therapy were hematologic, despite aggressive prophylactic therapy with filgrastim. Nevertheless, only 4 of 30 evaluable patients had serious or life-threatening infections (sepsis 1, cellulitis 1, port infection 2). Four of 30 evaluable patients also developed serious diarrhea. IFN-related symptoms, including fever and fatigue, were tolerable.

Table 2 Demographics

Entered (*n*)	32
Eligible (*n*)	32
Evaluable (*n*)	30
Age (years)	
Median	56
Range	28–76
Gender (*n*)	
Male	16
Female	16
Race	
White	16
Hispanic	9
Black	7
Prior therapy (*n*)	
Chemotherapy	3
Radiation	3
Primary site (*n*)	
Pancreas	18
Stomach	13
Neuroendocrine	1
Sites of metastases (*n*)	
Liver	19
Lymph nodes	8
Local	5
Peritoneal cavity	5
Lung	2
Pelvis	1
Bone	1

Table 3 Toxicities ($n=31$) (*ND* not done)

	NCI Common Toxicity Criteria Grade					ND
	0	1	2	3	4	
Neutropenia	6	3	11	10	1	0
Granulocytopenia	9	0	6	13	2	1
Thrombocytopenia	10	15	2	3	1	0
Anemia	1	8	12	9	1	0
Infection	23	3	1	3	1	0
Fever	3	16	12	0	0	0
Diarrhea	11	11	5	1	3	1
Fatigue	13	12	2	1	0	3

The median duration on study was 2.5 months (range 1–8 months). Only 1 of 12 evaluable patients with gastric cancer showed a partial response. There were no responders among the 18 patients with pancreatic cancer. The one patient with the islet cell tumor was inevaluable. Only 3 of 30 evaluable patients remained alive at the time of this report, with three additional patients lost to follow-up.

Despite the initial encouraging results of our initial phase II trial in gastric cancer, the 24-h regimen failed to show comparable results in patients with gastric cancer in a trial conducted by the ECOG [16]. The confounding factor in that trial was that patients had a threefold higher incidence of metastases to the peritoneum, omentum and ovary and a threefold higher incidence of ascites ($P=0.01$) than the patients in the original phase II study. The 48-h regimen, despite the 2/3 week trial design, had a 78%

increase in the dose intensity of the HU. Furthermore, the prolonged exposure to HU, was postulated to be more effective as an inhibitor of RR. Therefore, the poor response rates were discouraging.

The results of this trial do not provide a ready explanation for the lack of activity in gastric cancer. Patients had a similar performance status, age range and sites of metastases as patients in our prior, single-institution phase II trial employing the 24-h infusional regimen. Furthermore, despite the greater dose intensity of the HU in this 48-h trial, there was no suggestion of toxicities worsening to the extent of impairing the delivery of drug. The drug concentration of 5FU was likely somewhat lower because the 2.6 g/m^2 dose was administered over 48 h rather than 24 h, as had been administered previously; however, it is not clear why this would compromise the efficacy of the regimen. It may be that patient selection resulted in a worse clinical outcome, or alternatively, that intensifying the HU diminished the synergy with 5FU observed in our previous study by altering the pyrimidine:purine balances in a disadvantageous fashion.

Parenteral HU was employed because of the more predictable pharmacokinetics versus oral HU. Oral administration of HU results in unpredictable serum levels. In detailed pharmacokinetic studies [17], oral administration of HU 800 mg/m^2 every 4 h for 18 doses resulted in peak levels of 800 μM (700–900 μM), 1080 μM (770–1250 μM), and 2480 μM (1550–3200 μM) after doses 1, 7 and 13 respectively; however, trough levels were only 410 μM (290–530 μM), 730 μM (680–770 μM) and 550 μM (450–650 μM) after the same doses. In contrast, studies of 72-h parenteral HU administration by the same investigators revealed essentially steady-state plasma levels for 72 h at 488, 460, 537, 760, and 1090 μM for doses of HU in the range 2–3 mg/m^2 per min (2.9–4.3 g/m^2 per day). Thus, the failure of earlier regimens employing oral HU administration in patients with solid tumors may have resulted at least in part from the lack of consistent plasma levels of drug allowing recovery of RR kinetic function at the cellular level.

RR remains an attractive target for the development of new agents. Parenteral HU, while still of interest, will probably not undergo further development. In addition to gemcitabine and HU, new agents in clinical trials which target RR include triapine (3-amino-pyridine-2-carboxaldehyde thiosemicarbazone, 3-AP) and the antisense compound, GTI-2040, which is directed against the R1 component. Further studies should include identification of more effective inhibitors of the enzyme and strategies to determine whether combined inhibition of RR and other synthetic enzymes is more efficacious than targeting a single enzyme.

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