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Clinical Trial Results

Prolonged Pemetrexed Infusion Plus Gemcitabine in Refractory Metastatic Colorectal Cancer: Preclinical Rationale and Phase II Study Results

Alessandro Passardi,[a] Francesca Fanini,[a] Livia Turci,[a] Flavia Foca,[a] Paola Rosetti,[a] Silvia Ruscelli,[a] Andrea Casadei Gardini,[a] Martina Valgiusti,[a] Claudio Dazzi,[b] Maurizio Marangolo[c]

[a]Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, Meldola, Italy; [b]Santa Maria delle Croci Hospital, Ravenna, Italy; [c]Cooperative Outcome Research (CORE)-Cineca, Bologna, Italy

Trial Information

- **ClinicalTrials.gov Identifier**: NCT01909830
- **Sponsor**: none
- **Principal Investigator**: Alessandro Passardi
- **IRB Approved**: Yes

Lessons Learned

- Difficulties in translating in vitro results into clinical practice are inevitable.
- Further efforts to verify the efficacy of alternative schedules of pemetrexed in solid tumors are encouraged.

Abstract

Background. We investigated the cytotoxic activity of pemetrexed in combination with several drugs (gemcitabine, carboplatin, vinorelbine, and mitomycin C) using different exposure schedules in three colon cancer cell lines. The best results were obtained with the following schedule: a prolonged pemetrexed exposure followed by a 48-hour wash-out and then gemcitabine. This combination was then advanced to a phase II clinical trial.

Methods. Patients with metastatic colorectal cancer in progression after standard treatment were included in the study. Adequate bone marrow reserve, normal hepatic and renal function, and an Eastern Cooperative Oncology Group (ECOG) performance status of 0–2 were required. Treatment consisted of an 8-hour intravenous infusion of pemetrexed 150 mg/m^2 on day 1 and a 30-minute intravenous infusion of gemcitabine 1,000 mg/m^2 on day 3 of each cycle, repeated every 14 days.

Results. Fourteen patients were enrolled onto the study (first step). No objective responses were seen, and evidence of stable disease was observed in only one of the 12 evaluable patients. The most important grade 3–4 side effects were hematological toxicity (neutropenia 64.2%, thrombocytopenia 71.4%, anemia 28.7%), fatigue (50.0%), and stomatitis (21.5%). Median overall survival and time to progression were 5.8 months (95% confidence interval [CI]: 3.9–7.1) and 2.1 months (95% CI: 1.7–2.8), respectively.

Conclusion. The experimental pemetrexed-gemcitabine combination proved to be inactive and moderately toxic. ***The Oncologist*** 2017;22:1–7

Discussion

Although gemcitabine and pemetrexed have shown preclinical and clinical activity in patients with metastatic colorectal cancer, clinical data remain inconclusive [1–8]. A critical review of the available literature shows an evident incoherence between preclinical data and clinical trial design. From in vitro experiments, it seems clear that the administration of pemetrexed should precede all other drugs, with the possible exception of irinotecan, to increase cell kill and induce a synergistic effect [9, 10]. Clinical studies of pemetrexed-containing regimens have ignored this important finding in that the cytotoxics are generally infused concomitantly. Moreover, pemetrexed is commonly administered intravenously as a 10-minute infusion, but there is a strong body of evidence that anti-metabolites such as 5-fluorouracil and gemcitabine, when given in continuous intravenous infusion, show a different efficacy and toxicity pattern compared with the same dose given as an intravenous bolus [11–13]. In particular, our preclinical experience suggests that a prolonged exposure (6 or 12 hours) to pemetrexed leads to higher antitumor activity than a short exposure (<6 hours) and that a wash-out time between each drug administration ranging from 48 to 72 hours is essential for the induction of both cell cycle perturbation and apoptosis [14]. The reasons for this are not clear, and more in-depth pharmacokinetic studies are warranted.

The experimental regimen was based on these assumptions, in particular the administration of pemetrexed as a

Correspondence: Alessandro Passardi, Ph.D., Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, via P. Maroncelli 40, Meldola (FC) 47014, Italy. Telephone: 0039543739100; e-mail: alessandro.passardi@irst.emr.it Received March 14, 2017; accepted for publication May 5, 2017. http://dx.doi.org/10.1634/theoncologist.2017-0206

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Table 1. Patient characteristics

Characteristic	No. of patients, $n = 14$, n (%)
Median age, years (range)	57 (48–77)
Gender	
Male	8 (57.1)
Female	6 (42.9)
PS (ECOG)	
0	9 (64.3)
1	4 (28.6)
2	1 (7.1)
Ethnic origin	
White	14 (100.0)
Stage IV colorectal cancer	14 (100.0)
Prior chemotherapy for metastatic disease	14 (100.0)
Primary tumor localization	
Rectum	3 (21.4)
Colon	11 (78.6)
Site of metastasis	
Lung and liver	8 (57.1)
Liver and other sites	3 (21.4)
Liver	2 (14.3)
Lymph nodes and bone	1 (7.2)
Prior radiotherapy	
Performed	4 (28.6)
Not performed	10 (71.4)
Prior surgery	
Left hemicolectomy	8 (57.1)
Right hemicolectomy	3 (21.4)
Metastases resection	2 (14.3)
Other	1 (28.6)

Abbreviations: PS, performance score; ECOG, eastern cooperative oncology group.

continuous intravenous infusion followed by a 48-hour washout and then gemcitabine infusion. The chosen dose of pemetrexed derived from a phase Ib trial performed at our institute, which showed the feasibility of a 12-hour continuous infusion of pemetrexed 200 mg/m^2 every 2 weeks in patients with cancer who were previously treated (data not published). However, clinical results of the present study were not in line with the preclinical rationale, and the study was closed at the first step because of important toxicity and the absence of proven significant activity.

Trial Information	
Disease	Colorectal cancer
Stage of Disease/Treatment	Metastatic/advanced
Prior Therapy	More than two prior regimens
Type of Study - 1	Phase II
Type of Study - 2	Single arm
Primary Endpoint	Overall response rate
Secondary Endpoint	Toxicity
Secondary Endpoint	Time to progression
Secondary Endpoint	Overall survival

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Additional Details of Endpoints or Study Design

A minimax two-stage Simon design was employed. A 10% response would preclude further study, whereas a 30% response rate would indicate that further study would be warranted. Using α and β errors of 0.10 and 0.10, respectively, 12 patients were required in the first stage, and if 1 or 0 responses were observed, the trial had to be terminated. Otherwise, an additional 23 patients were to be enrolled. If 5 or fewer responses were observed in 35 patients, the combination would not have been considered worthy of further study; however, if 6 or more responses were observed, the combination would have been considered sufficiently active to warrant further testing.

Investigator's Analysis	Level of activity did not meet planned endpoint

Drug Information for Phase II Pemetrexed/Gemcitabine

Drug 1	
Generic/Working name	Pemetrexed
Trade name	Alimta
Company name	Lilly
Drug type	
Drug class	Antimetabolite
Dose	150 milligrams (mg) per squared meter (m^2)
Route	Continuous intravenous infusion (cIV)
Schedule of Administration	Eight-hour intravenous infusion of pemetrexed 150 mg/m^2 on day 1 and a 30-minute intravenous infusion of gemcitabine 1,000 mg/m^2 on day 3 of each cycle, repeated every 14 days
Drug 2	
Generic/Working name	Gemcitabine
Drug type	
Drug class	Antimetabolite
Dose	1,000 milligrams (mg) per squared meter (m^2)
Route	IV
Schedule of Administration	Eight-hour intravenous infusion of pemetrexed 150 mg/m^2 on day 1 and a 30-minute intravenous infusion of gemcitabine 1000 mg/m^2 on day 3 of each cycle, repeated every 14 days.

Patient Characteristics for Phase II Pemetrexed/Gemcitabine

Number of patients, male	8
Number of patients, female	6
Stage	Stage IV colorectal cancer
Age	Median (range): 57 (48–77)
Number of prior systemic therapies	Median (range): $\geq$2 in all patients
Performance Status: ECOG	0 — 9
	1 — 4
	2 — 1
	3 — 0
	Unknown — 0
Other	Not Collected
Cancer Types or Histologic Subtypes	

Primary Assessment Method for Phase II Pemetrexed/Gemcitabine

Number of patients screened	14
Number of patients enrolled	14
Number of patients evaluable for toxicity	14
Number of patients evaluated for efficacy	12
Evaluation method	RECIST 1.0
Response assessment CR	$n = 0$

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Response assessment PR	$n = 0$
Response assessment SD	$n = 1$
Response assessment PD	$n = 11$
Response assessment OTHER	$n = 0$
(Median) duration assessments PFS	2.1 months, CI: 1.7–2.8
(Median) duration assessments OS	5.8 months, CI: 3.9–7.1

Adverse Events: Phase II Pemetrexed/Gemcitabine

All Dose Levels, Cycle 1							
Name	**NC/NA**	**1**	**2**	**3**	**4**	**5**	**All Grades**
Fatigue	14%	7%	29%	50%	0%	0%	86%
Mucositis oral	21%	29%	29%	14%	7%	0%	79%
Anemia	42%	0%	29%	29%	0%	0%	58%
Febrile neutropenia	71%	0%	0%	29%	0%	0%	29%
Platelet count decreased	21%	0%	7%	36%	36%	0%	79%
Neutrophil count decreased	29%	7%	0%	7%	57%	0%	71%

Adverse events among patients undergoing at least one treatment cycle
Abbreviation: NC/NA, no change from baseline/no adverse event.

Assessment, Analysis, and Discussion

Completion	Study terminated before completion
Terminated reason	Toxicity
Pharmacokinetics/Pharmacodynamics	Not collected
Investigator's Assessment	Level of activity did not meet planned endpoint

Metastatic colorectal cancer (mCRC) patients are usually treated with 5-fluorouracil, oxaliplatin, irinotecan, bevacizumab-based regimens, and, in selected cases, cetuximab. Regorafenib and TAS-102 have recently been introduced for the treatment of refractory disease. There are no further treatment options for fit patients who are resistant to these drugs. Although gemcitabine and pemetrexed have shown preclinical and clinical activity in patients with mCRC, clinical data remain inconclusive [1–8].

Gemcitabine has recognized broad-spectrum activity and is recommended for treatment in an increasing number of tumors. However, there is still little clinical proof of its efficacy in mCRC. A review by Merl et al. evaluating the efficacy and safety of fluoropyrimidine plus gemcitabine in patients with mCRC reported objective response rates (ORR) of 30%–38.3%, median time to progression (TTP) of 4–8.3 months, and median overall survival (OS) of 9.8–18 months [1].

Pemetrexed is a pyrrolopyrimidine-based antifolate with proven in vitro activity against folate-requiring enzymes, including thymidylate synthase, dihydrofolate reductase, and glycinamide ribonucleotide formyltransferase [2]. In vitro studies have shown that pemetrexed is active against a wide range of human cell lines, including colon cells and also VRC5 and HXGC3 human colon xenografts. Phase I clinical trials established pemetrexed 600 mg/m^2 once every 21 days as a suitable schedule for phase II trials [3]. Phase II studies of single-agent pemetrexed in mCRC reported an overall response rate of about 15% in the first-line setting and no observable responses in refractory patients [4, 5]. In preclinical models, a synergism between pemetrexed, oxaliplatin, and irinotecan was observed, attesting to the potential for using these combination regimens in clinical trials [6]. Pemetrexed has been studied in combination with oxaliplatin as first-line in mCRC, showing ORR of 29.6%, TTP of 5.3 months, and OS of 12.3 months [7]. The drug has also produced interesting ORRs when used in combination with irinotecan as first- and second-line treatment [8, 9].

A critical review of the available literature shows an evident incoherence between preclinical data and clinical trial design. From in vitro experiments, it seems clear that the administration of pemetrexed should precede all other drugs, with the possible exception of irinotecan, to increase cell kill and induce a synergistic effect [10, 11]. Clinical studies of pemetrexed-containing regimens have ignored this important indication in that the cytotoxics are generally infused concomitantly. Moreover, pemetrexed is commonly administered intravenously as a 10-minute infusion, but there is a strong body of evidence that anti-metabolites such as 5-fluorouracil and gemcitabine, when given in continuous intravenous infusion, show a different efficacy and toxicity pattern compared to the same dose given as an intravenous bolus [12–14]. In particular, our preclinical experience suggests that a prolonged exposure (6 or 12 hours) to pemetrexed leads to higher antitumor activity than a short exposure (<6 hours) and that a wash-out time between each

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drug administration ranging from 48 to 72 hours is essential for the induction of both cell cycle perturbation and apoptosis [15]. The reasons for this are not clear; it can be argued that a longer exposure of tumor cells to lower doses of the drug may optimize the carrier system much more than higher doses administered over a shorter time. More sophisticated pharmacokinetic studies (intracellular drug concentration) are warranted.

The experimental regimen was based on these assumptions, in particular the administration of pemetrexed as a continuous intravenous infusion followed by a 48-hour wash-out and then gemcitabine infusion (Fig. 1). The chosen dose of pemetrexed was derived from a phase Ib trial performed at our institute that showed the feasibility of a 12-hour continuous infusion of pemetrexed 200 mg/m^2 every 2 weeks in pretreated cancer patients (data not published). However, clinical results were not in line with the preclinical rationale and the study was closed at the first step because of important toxicity and the absence of proven significant activity. In particular, no objective responses or even minor tumor shrinkage (enough to satisfy partial response (PR) criteria) were seen. The only patient with stable disease after 8 weeks' treatment showed clinical progression immediately after the radiological evaluation. One reason for such a negative result might be the low activity of gemcitabine and pemetrexed in mCRC. Enrolled patients had also received several treatments and were presumed to have chemoresistant disease (Table 1). In addition, the toxicity profile (Table 2) was not favorable. Grade 4 neutropenia and thrombocytopenia were observed in 57.1% and 35.7% of patients, respectively, and 4 (28.7%) patients experienced febrile neutropenia. The most important grade 3–4 nonhematological adverse events were fatigue (50%) and stomatitis (21.5%).

Difficulties in translating in vitro results into clinical practice are inevitable. In our study the duration of pemetrexed continuous infusion and the length of the washout period between treatments were defined on an empirical basis. This is an important aspect to consider when conducting translational research, as it may affect clinical results. Taking into account the low efficacy and high toxicity associated with the experimental regimen, clinical development is not recommended. However, further efforts are warranted to verify the efficacy of alternative schedules of pemetrexed in solid tumors.

Disclosures

The authors indicated no financial relationships.

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Figures and Tables

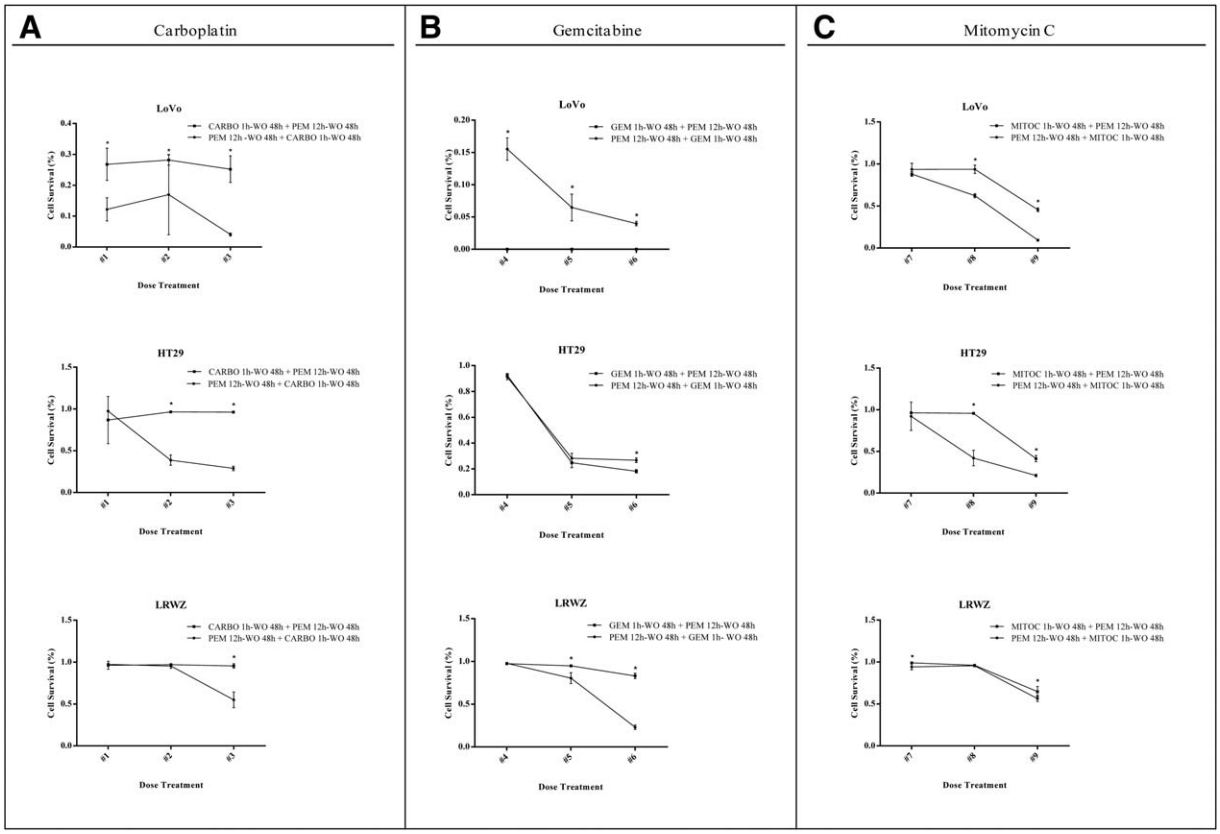

Figure 1. Effect of different schedules of combination of pemetrexed (PEM) with carboplatin (CARBO) **(A)**, gemcitabine (GEM) **(B)**, or mitomycin C (MITOC) **(C)** on three colon cancer cells lines. Cell viability was determined by SRB assay, and the results were expressed as the mean of an octuplicate from three independent experiments. Bars represent standard deviation (*$p < .05$). Statistical significance was determined using the GraphPad Prism Software Holm-Sidak method. Treatment dose: #1 (PEM 1 mM - CARBO 0.8 mM), #2 (PEM 10 mM - CARBO 8 mM), #3 (PEM 100 mM - CARBO 80 mM), #4 (PEM 1 mM - GEM 0.4 mM), #5 (PEM 10 mM - GEM 4 mM), #6 (PEM 100 mM - GEM 40 mM), #7 (PEM 1 mM - MITOC 0.03 mM), #8 (PEM 10 mM - MITOC 0.3 mM), and #9 (PEM 100 mM - MITOC 3 mM).

Abbreviations: CARBO, carboplatin; GEM, gemcitabine, MITOC, mitomycin C; SRB, sulforhodamine B; WO, wash-out.

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Table 2. Adverse events among patients undergoing at least one treatment cycle

	No. patients, $n = 14$, n (%)			
Adverse Events	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**
Neutropenia	1 (7.1)	—	1 (7.1)	8 (57.1)
Febrile neutropenia	—	—	4 (28.7)	—
Leukopenia	—	—	—	1 (7.1)
Thrombocytopenia	—	1 (7.1)	5 (35.7)	5 (35.7)
Anemia	—	4 (28.7)	4 (28.7)	—
Fatigue	1 (7.1)	4 (28.7)	7 (50.0)	—
Rash/desquamation	1 (7.1)	1 (7.1)	—	—
Pruritus	—	1 (7.1)	—	—
Nausea	3 (21.4)	2 (14.4)	—	—
Anorexia	—	—	1 (7.1)	—
Stomatitis/pharyngitis	4 (28.7)	4 (28.7)	2 (14.4)	1 (7.1)
Diarrhea	1 (7.1)	1 (7.1)	—	—
Constipation	—	1 (7.1)	—	—
Fever	7 (50.0)	—	—	—
Pain	1 (7.1)	4 (28.7)	1 (7.1)	—
Hepatotoxicity	—	1 (7.1)	1 (7.1)	—
Other	1 (7.1)	8 (57.1)	—	—

Abbreviation: —, no data.