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

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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/m² on day 1 and a 30-minute intravenous infusion of gemcitabine 1,000 mg/m² 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;22:886–e79

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...
continuous intravenous infusion followed by a 48-hour wash-out 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/m² 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.

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.

## 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                |
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 $\alpha$ and $\beta$ 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 (m²) |
| Route                | Continuous intravenous infusion (cIV) |

**Schedule of Administration**

Eight-hour intravenous infusion of pemetrexed 150 mg/m² on day 1 and a 30-minute intravenous infusion of gemcitabine 1,000 mg/m² 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 (m²) |
| Route                | IV          |

**Schedule of Administration**

Eight-hour intravenous infusion of pemetrexed 150 mg/m² on day 1 and a 30-minute intravenous infusion of gemcitabine 1000 mg/m² 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       | $n = 0$ |
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, 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 glycineamide 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 xenographs. Phase I clinical trials established pemetrexed 600 mg/m² 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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### Adverse Events: Phase II Pemetrexed/Gemcitabine

| Name                        | 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
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/m² 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 71.1% 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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**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 cell 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.

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

| 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.