A Phase I Study of Pemetrexed (ALIMTA) and Cyclophosphamide in Patients with Locally Advanced or Metastatic Breast Cancer

Christian Dittrich,1 Lubos Petruzela,3 Pavel Vodvarka,4 Margit Gneist,1 Filip Janku,3 Tamara Kysela,4 Allen Melemed,5 Jane Latz,5 Lorinda Simms,5 and Kurt Krejcy2

Abstract

Purpose: Determine the maximum tolerated dose (MTD) of pemetrexed and cyclophosphamide combination therapy for patients with locally advanced or metastatic breast cancer.

Experimental Design: Patients with locally advanced or metastatic breast cancer and WHO performance status 0 to 2 were eligible. Pemetrexed (range, 400–2,400 mg/m²) was administered on day 1 of a 21-day schedule followed by cyclophosphamide (range, 400–800 mg/m²). Folic acid and vitamin B12 supplementation began 1 to 2 weeks before the first pemetrexed dose.

Results: Fifty-seven pretreated patients were enrolled and received 342 cycles (median, 4 cycles; range, 1–26) through 14 dose levels. The MTD of pemetrexed was 2,400 mg/m² (combined with cyclophosphamide, 600 mg/m²) with dose-limiting toxicities of grade 4 neutropenia with grade 4 infection and grade 3 diarrhea. Other grade 3 or 4 toxicities included (febrile) neutropenia, thrombocytopenia, anemia, elevated alanine aminotransferase/aspartate aminotransferase, and diarrhea. Pharmacokinetic analysis indicated that pemetrexed clearance and central volume of distribution were 40% lower than single-agent reference data, yielding a 68% increase in total systemic exposure and a 56% increase in maximal plasma concentration. Among the 50 patients evaluable for efficacy, 13 (26%) patients had a partial response and 17 (34%) patients had stable disease.

Conclusions: Pemetrexed was generally well tolerated. The observed toxicities were consistent with the known toxicity profiles of pemetrexed and cyclophosphamide. Considering the MTD and the toxicity and efficacy results in this and prior studies, a low (600 mg/m²) and high (1,800 mg/m²) dose of pemetrexed with cyclophosphamide (600 mg/m²) will be evaluated in the consecutive prospective randomized phase II study.

Breast cancer is the most common malignancy to affect women in developed countries. Approximately 36% of women with breast cancer are expected to develop fatal metastatic disease (1). In the metastatic setting, anthracyclines and/or taxanes have become standard therapeutics for first-line treatment if they have not already been used in the adjuvant setting. After these agents fail, non-anthracycline-based and non-taxane-based chemotherapy regimens must be developed as the next logical treatment option for these patients.

Pemetrexed, an inhibitor of thymidylate synthase, dihydrofolate reductase, and glycaminide ribonucleotide formyl transferase (2–4), has shown broad-spectrum activity in multiple tumor types (5). The substance has a manageable toxicity profile that includes dose-limiting neutropenia and thrombocytopenia as well as lower grades of reversible hepatotoxicity and gastrointestinal toxicity. Pemetrexed-associated toxicity is increased with a high level of plasma homocysteine, which in turn has been associated with nutritional folate deficiency (6). Folate and vitamin B12 supplementation have been found to reduce plasma homocysteine and, thus, pemetrexed-associated toxicities, allowing dose escalation without apparently compromising antitumor activity (7, 8).

The antitumor activity of pemetrexed against breast cancer has been shown in various preclinical models (9, 10). In addition, pemetrexed is expected to have antitumor activity against breast cancer because other thymidylate synthase inhibitors, such as 5-fluorouracil (5-FU), and other dihydrofolate reductase inhibitors, such as methotrexate, are active substances and well established in the treatment of breast cancer. In addition, because thymidylate synthase and dihydrofolate reductase are not targeted by anthracyclines or taxanes, cross-resistance of pemetrexed with those agents would not be anticipated, and therefore, pemetrexed may be expected to inhibit growth of breast cancer that relapses following exposure to those two classes of agents.

Single-agent pemetrexed has shown activity in patients with advanced breast cancer; most of whom were previously treated in the metastatic and/or adjuvant settings, yielding response...
rates of 28% (11), 26% (12), or 20% (13, 14). In a study of heavily pretreated patients with metastatic breast cancer who had received three or more regimens, including prior anthracyclines, taxanes, and capecitabine, pemetrexed showed only modest antitumor activity (8% response rate) but was well tolerated and efficacious in stabilizing disease and providing symptom palliation (15). In a single study testing pemetrexed in previously untreated breast cancer, a response rate of 35% was reported (16). Given these single-agent results, the pemetrexed and cyclophosphamide combination was expected to offer additional efficacy benefits.

Among the many cytotoxic agents registered for breast cancer, cyclophosphamide is one of the most widely used alkylating agents with antineoplastic activity (17). Single-agent cyclophosphamide is associated with objective response rates ranging from 10% to 62% in patients with breast cancer (18, 19) and with an average response rate of ~34% (20). The cyclophosphamide-based combination regimen cyclophosphamide, methotrexate, and 5-FU is well established in breast cancer both as adjuvant therapy (21, 22) and as therapy for advanced disease with an overall response rate of ~50% (23).

To determine whether pemetrexed-containing combination therapy might provide an effective alternative to cyclophosphamide, methotrexate, and 5-FU, we proposed replacing the thymidylate synthase inhibitor 5-FU and the dihydrofolate reductase inhibitor methotrexate with the multitargeted antifolate pemetrexed. Thus, we designed a phase I/II study to evaluate the combination of pemetrexed and cyclophosphamide in patients with locally advanced or metastatic breast cancer. The phase I study results are reported here.

The primary objective of this multicenter phase I study was to determine the maximum tolerated dose (MTD) of pemetrexed and cyclophosphamide combination therapy. Secondary objectives included the evaluation of quantitative and qualitative toxicities, the assessment of the effect of a patient’s baseline nutritional status on safety as measured by homocysteine levels, a pharmacokinetic assessment of systemic exposure to the chemotherapy based on a limited blood sampling scheme during dose escalation, and a pharmacodynamic assessment of the relationship between systemic drug exposure and toxicities.

**Materials and Methods**

**Patient selection.** Major eligibility criteria included histologic or cytologic diagnosis of locally advanced or metastatic breast cancer, performance status 0 to 2 on the WHO scale, and an estimated life expectancy of at least 12 weeks. Patients were not allowed to receive other forms of treatment for at least 4 weeks before study enrollment with the exception of radiotherapy (to <25% of the bone marrow) and endocrine therapy (except luteinizing hormone-releasing hormone analogues). Patients were required to have adequate bone marrow function (absolute neutrophil count ≥1.5 × 10^9/L, platelet count ≥100 × 10^9/L, and hemoglobin ≥9 g/dL), adequate liver function (bilirubin ≤1.5 times the upper limit of normal, alkaline phosphatase, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) ≤3.0 times the upper limit of normal), and normal renal function (calculated creatinine clearance ≥50 mL/min).

Patients were excluded from the study for the following reasons: chemotherapy within 4 weeks before study enrollment, treatment with any drug within the last 30 days that had not received regulatory approval at the time of study entry; previous participation in a clinical study involving pemetrexed; serious concomitant systemic disorders, including active infection, second primary malignancy, and symptoms of central nervous system metastases; inability to take folic acid or vitamin B12 supplementation or dexamethasone (or an equivalent corticosteroid); and inability to stop treatment with nonsteroidal anti-inflammatory drugs. In addition, patients were excluded from the study for pregnancy, breast-feeding, and/or childbearing potential without adequate contraception.

All patients were required to provide written informed consent before study entry. This study was conducted according to the guidelines of each participating institution and was in compliance with Good Clinical Practice and the tenets of the Declaration of Helsinki.

**Treatment and dose escalation scheme.** Beginning at the low dose of 400 mg/m² and increasing to a maximum of 2,400 mg/m², pemetrexed was administered i.v. over 10 minutes (and up to 30 minutes for doses ≥1,800 mg/m² to avoid hot flashes observed in some patients) on day 1 of a 21-day schedule. Cyclophosphamide was administered i.v. over 30 minutes starting at 400 mg/m² and increased to a maximum of 800 mg/m² on day 1 of a 21-day cycle beginning ~10 minutes after the completion of the pemetrexed infusion. To reduce pemetrexed-associated toxicities, patients were required to take oral folic acid and to get i.m. injections of vitamin B12. Patients were to take oral folic acid (350-1,000 µg) daily beginning approximately 1 to 2 weeks before the start of cycle 1 and continuing daily until 3 weeks after the last dose of study therapy. Patients were also required to receive vitamin B12 injections (1,000 µg) approximately 1 to 2 weeks before the start of cycle 1, and injections were repeated approximately every 9 weeks until 3 weeks after the last dose of study therapy. Prophylactic dexamethasone (4 mg twice daily) was administered on the day before, the day of, and the day after each dose of pemetrexed to prevent rash. Patients continued treatment until disease progression; however, a patient could have discontinued earlier in case of the following: unacceptable toxicity; pregnancy or failure to use adequate birth control; failure to stop using nonsteroidal anti-inflammatory drugs 2 to 5 days before, during, and 2 days after therapy administration; in case of patient request; or at the discretion of the investigator, the patient’s physician, or Eli Lilly and Company.

The decision to escalate the doses of pemetrexed and cyclophosphamide from the respective starting doses of 400 mg/m² to the next dose levels was based on toxicities observed during cycle 1. If a patient did not have a dose-limiting toxicity (DLT) at cycle 1 at a given dose level, then two additional patients were treated at that dose level before patients were allocated to the next higher dose level. If a DLT was observed in one of three patients at cycle 1, then a maximum of three additional patients were entered at that dose level. The same pattern of enrollment was followed for all dose levels. The MTD was reached if the same DLT occurred in two or more of six patients at a given dose level; the dose level recommended for phase II studies was defined as the next lower dose level than the MTD.

A DLT was defined as follows: (a) Common Toxicity Criteria grade 4 neutropenia lasting ≥5 days, (b) febrile neutropenia, (c) grade 4 thrombocytopenia or grade 3 thrombocytopenia with bleeding requiring platelet transfusion, and (d) grade ≥3 nonhematologic toxicity, excluding alopecia, nausea, and vomiting manageable with antiemetic therapy and excluding isolated Common Toxicity Criteria grade 3 ALT or AST that returned to grade 1 or pretreatment values within 3 weeks.

**Baseline and treatment assessments.** Assessments at baseline included a complete medical history and physical examination, evaluation of performance status (WHO), tumor measurement of palpable or visible lesions, hematologic and blood chemistry assessment, and vitamin metabolite panel. Hematology and blood chemistry were monitored weekly throughout the study and within 4 days before each cycle. Hematologic and nonhematologic data were assessed according to the National Cancer Institute Common Toxicity Criteria rating scale version 2.0 (24). Patients were considered evaluable for MTD and safety if they received at least one dose of either study drug.
For determination of plasma pemetrexed concentration, two blood samples were collected from each patient. Samples were taken at the end of the 10-minute pemetrexed infusion (30-minute infusion for dosages from 1,800 to 2,400 mg/m²) and 6 to 8 hours later. Plasma samples obtained during this study were analyzed for pemetrexed at Taylor Technology, Inc. (Princeton, NJ). Heparinized human plasma samples were analyzed for pemetrexed using a high-performance liquid chromatography electrospray ionization tandem mass spectrometry method over the concentration range of 10.0 to 200,000.0 ng/mL (25). Efficacy was not a primary or secondary end point of the phase I portion of this study; however, lesions were followed with appropriate radiological imaging every other cycle to exclude progressive disease. Anitumor activity was documented using Southwest Oncology Group criteria (26). A complete response was defined as complete absence of measurable, evaluable, or nonmeasurable disease with no new lesions and no disease-related symptoms. A partial response was defined as ≥50% reduction from baseline of the sum of the products of perpendicular diameters of all measurable lesions, with no progression of evaluable disease and no new lesions. Any complete or partial response required confirmation 4 weeks later. Tumor progression was defined as a 50% increase or an increase of 10 cm² (whichever was smaller) in the sum of products of all measurable lesions over the smallest sum observed or a clear worsening of any evaluable disease or the reappearance of any lesion that had disappeared or the appearance of any new lesion/site. Stable disease was that which did not qualify for complete, partial, or nonmeasurable disease. Patients were evaluated for tumor response (during treatment and poststudy follow-up) if they had measurable disease and had received at least one complete cycle of both study drugs.

**Statistical and pharmacokinetic analyses.** Plasma pemetrexed concentration versus time data from the current study (pemetrexed administered in combination with cyclophosphamide) was compared with reference data of 1,388 pemetrexed concentration results from 252 patients in 10 phase II single-agent pemetrexed studies (25). A previously established open two-compartment population pharmacokinetic model parameterized in terms of clearance (CL), central volume of distribution (V₁), intercompartmental clearance (Q), and peripheral volume of distribution (V₂) with calculated creatinine clearance estimated by the Cockcroft-Gault method (CrCLCG,std) as a covariate with respect to CL and body surface area as a covariate with respect to V₁ were included in the model (25). Potential differences in pemetrexed pharmacokinetics between the two populations were identified as those which resulted in a significant decrease in the minimum value of the NONMEM objective function (≥10.828 points for 1 degree of freedom; P < 0.001) on inclusion of a dichotomous variable as a covariate differentiating the current study population from the reference population. Plots of individual empirical Bayesian estimates of model variables (e.g., CL and V₁; obtained using the POSTHOC option in NONMEM) by dose were used to identify potential dose-related alterations in pemetrexed pharmacokinetics.

To examine possible pharmacodynamic relationships, pemetrexed total systemic exposure [area under concentration curve (AUC)] was estimated based on individual empirical Bayesian estimates of CL. The relationship between pemetrexed AUC, cyclophosphamide total dose administered, and occurrence of any grade 3 or 4 events and grade 3 or 4 neutropenia was summarized graphically (scatter plots and associated box plots) to identify any exposure-toxicity/response relationship.

**Results**

**Patient demographics.** A total of 65 patients, all female, signed informed consent and were registered into the study at one of three study centers in Austria and the Czech Republic. Of these 65 patients, 8 patients were not entered in the study: 5 patients failed to meet inclusion criteria, 1 patient had a personal conflict, 1 patient revised her previous decision, and 1 patient experienced a hypersensitivity to the folic acid or vitamin supplementation required by protocol. Thus, a total of 57 patients finally received treatment according to the protocol between January 2001 and September 2004 (Table 1). The patients ranged in age from 32 to 81 years (median, 55 years), with a median WHO performance status of 0 (range, 0-2). More than three quarters of patients had at least two sites of metastatic disease at baseline. Liver, lung, and bone metastases were each present in about half of the patients. In general, the patients were heavily pretreated; two thirds had already received chemotherapy in the metastatic setting, with about half of them receiving two lines or more. The majority of the patients had received a prior anthracycline-, cyclophosphamide-, and/or taxane-based therapy.

**Table 1. Patient characteristics (N = 57)**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age (range), y</td>
<td>55 (32, 81)</td>
</tr>
<tr>
<td>WHO performance scale, n (%)</td>
<td>0 42 (73.7) 1 13 (22.8) 2 2 (3.5)</td>
</tr>
<tr>
<td>No. metastatic sites, n (%)</td>
<td>1 12 (21.1) 2 18 (31.6) 3 17 (29.8) 4 10 (17.5)</td>
</tr>
<tr>
<td>Location of metastases, n (%)</td>
<td>Liver 29 (50.9) Lung 27 (47.4) Bone 32 (56.1)</td>
</tr>
<tr>
<td>Estrogen/progesterone receptors, n (%)</td>
<td>+/+ 25 (43.9) +/− 6 (10.5) U/U 19 (33.3) +/N 3 (5.3) +/U 2 (3.5) U/− 1 (1.8)</td>
</tr>
<tr>
<td>Prior therapy, n (%)</td>
<td>Surgery 53 (93.0) Chemotherapy 52 (91.2) Anthracycline 51 (89.5) Cyclophosphamide 45 (78.9) Taxane 39 (68.4) Trastuzumab 8 (14.0) Adjuvant setting 36 (63.2) Neoadjuvant setting 9 (15.8) Locally advanced setting 1 (1.8) Metastatic setting 39 (68.4) 1 line of therapy 8 (14.0) 2 lines of therapy 18 (31.6) ≥3 lines of therapy 13 (22.8) Radiotherapy 47 (82.5) Hormonal therapy 39 (68.4) Immunotherapy 2 (3.5)</td>
</tr>
</tbody>
</table>

Abbreviations: N, not assessed; U, unknown.
342 cycles of therapy, there were 48 cycles delayed (14.0%) for clinically significant reasons; these included 25 delays due to neutropenia (52.1% of delays). Although neutropenia caused some dose reductions and delays, there was no evidence of cumulative hematologic toxicity. Other causes of cycle delays were transient increases in ALT or AST (13 incidents, 27.1% of delays), transient decreases in creatinine clearance (4 incidents, 8.3% of delays), insufficient prophylactic dexamethasone and nasopharyngitis (each 2 incidents, each 4.2% of delays), and infection and cough (each 1 incident, each 2.1% of delays). Patient compliance with daily oral folic acid supplementation was reported as 100% throughout the study.

**MTD and toxicity.** As detailed in Table 2, DLTs occurred in six patients at dose levels 1, 6, 13, and 14. At dose level 14, the first patient experienced grade 4 neutropenia associated with grade 4 infection at cycle 1, as DLT. This patient subsequently died due to streptococcal sepsis on day 12 of cycle 1, an event considered possibly related to study drug. In addition, the patient experienced grade 1 stomatitis and vomiting during cycle 1. Because of the DLT, an additional cohort of up to three patients was to be enrolled. Another patient at this dose level also experienced a DLT grade 3 diarrhea at cycle 1; this patient also had grade 3 vomiting. Because of the two DLTs observed at this dose level (grade 4 neutropenia and associated infection in one patient, grade 3 diarrhea in another patient), the possibly study drug-related death, the mucosal-related toxicities experienced by two of the patients, and the substantial toxicities experienced at the previous dose level, enrollment was closed, and dose level 14, pemetrexed at 2,400 mg/m² and cyclophosphamide at 600 mg/m², was designated the MTD. The dose formally to be recommended for future phase II studies, defined prospectively as the next lower dose level, was therefore pemetrexed at 2,100 mg/m² and cyclophosphamide at 600 mg/m².

As previously mentioned, there is evidence that high baseline plasma homocysteine levels are associated with increased toxicity in nonsupplemented patients (7). Therefore, pre-folic acid supplementation homocysteine levels were plotted against chemotherapy dose levels to assess whether there was any evidence of a difference in homocysteine levels across chemotherapy dose levels (Fig. 1). Although a slight trend to lower homocysteine levels (\( P \) not significant) was observed at higher dose levels, this trend seems insufficient to affect the MTD.

<table>
<thead>
<tr>
<th>Dose level</th>
<th>P/C (mg/m²)</th>
<th>Patients with DLTs/enrolled patients</th>
<th>DLTs</th>
<th>Other maximum grade 3 and 4 toxicities*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>400/400</td>
<td>1/6</td>
<td>G3 diarrhea</td>
<td>G3 ALT, G3 anemia, G3 ALT</td>
</tr>
<tr>
<td>2</td>
<td>500/400</td>
<td>0/3</td>
<td>None</td>
<td>G3 AST</td>
</tr>
<tr>
<td>3</td>
<td>500/600</td>
<td>0/3</td>
<td>None</td>
<td>G4 neutropenia, G3 neutropenia</td>
</tr>
<tr>
<td>4</td>
<td>500/800</td>
<td>0/7(^a)</td>
<td>None</td>
<td>G4 febrile neutropenia, G3 neutropenia</td>
</tr>
<tr>
<td>5</td>
<td>600/800</td>
<td>0/3</td>
<td>None</td>
<td>G4 neutropenia, G3 AST, G3 abdominal pain</td>
</tr>
<tr>
<td>6</td>
<td>800/600</td>
<td>1/6</td>
<td>G4 AST/ALT, G3 bilirubin</td>
<td>G3 neutropenia, G3 ALT</td>
</tr>
<tr>
<td>7</td>
<td>900/600</td>
<td>0/3</td>
<td>None</td>
<td>G4 neutropenia, G3 ALT, G3 neutropenia</td>
</tr>
<tr>
<td>8</td>
<td>1,000/600</td>
<td>0/3</td>
<td>None</td>
<td>G3 hypokalemia, G3 neutropenia, G3 anemia</td>
</tr>
<tr>
<td>9</td>
<td>1,100/600</td>
<td>0/4(^b)</td>
<td>None</td>
<td>G4 neutropenia, G3 ALT</td>
</tr>
<tr>
<td>10</td>
<td>1,300/600</td>
<td>0/3</td>
<td>None</td>
<td>G4 neutropenia, G3 weight loss</td>
</tr>
<tr>
<td>11</td>
<td>1,500/600</td>
<td>0/3</td>
<td>None</td>
<td>G4 neutropenia, G3 leukopenia, G3 infection</td>
</tr>
<tr>
<td>12</td>
<td>1,800/600</td>
<td>0/3</td>
<td>None</td>
<td>G3 neutropenia</td>
</tr>
<tr>
<td>13</td>
<td>2,100/600</td>
<td>2/6</td>
<td>G4 thrombocytopenia, G3 rash/desquamation</td>
<td>G4 leukopenia, G3 anemia</td>
</tr>
<tr>
<td>14</td>
<td>2,400/600</td>
<td>2/4(^c)</td>
<td>G4 neutropenia with G4 infection, G3 diarrhea</td>
<td>G3 fatigue, G3 vomiting</td>
</tr>
</tbody>
</table>

Abbreviations: C, cyclophosphamide; G3, grade 3; G4, grade 4; P, pemetrexed.

*Toxicities appearing on the same line occurred in the same patient.

\(^a\)Because one patient died from pneumonia 7 days after starting study drug therapy, an additional patient was enrolled. Of this four patient cohort, a second patient died (respiratory and heart failure, cycle 2). Three additional patients were subsequently enrolled, bringing the total to seven patients. Both deaths were unrelated to study drug.

\(^b\)Because one patient discontinued the study due to progressive disease 5 days after starting study drug therapy, an additional patient was enrolled.

\(^c\)Because of the DLTs and subsequent possibly study drug-related death (streptococcal sepsis) observed in the first patient enrolled, up to three additional patients were to be enrolled. However, the fourth patient experienced DLTs similar to the grade 1 mucosal toxicities reported in the first patient, so enrollment was stopped and this dose level was said to be the MTD.
Grade 3 or 4 neutropenia was the most frequent hematologic toxicity (a total of 16 incidences) occurring in at least one patient in every cohort from dose level 3 onwards (Table 2). Eight patients received RBC transfusions for grade 2 or 3 anemia, and one patient received platelet transfusions prophylactically for grade 4 thrombocytopenia to prevent bleeding.

Grade 3 and 4 nonhematologic toxicities were infrequent (Table 2). Grade 3 or 4 AST and ALT occurred most often in four (7.0%) and eight (14.0%) patients, respectively. The one patient who had both grade 4 AST and ALT also developed grade 3 bilirubin elevation.

Three patients were discontinued from the study (dose levels 5, 11, and 13) due to hematologic toxicities not defined as DLTs (two patients, neutropenia; one patient, leukopenia). There were no discontinuations due to study drug-related nonhematologic toxicities.

**Pharmacokinetic and pharmacodynamic evaluations.** A total of 96 pemetrexed determinations from 51 patients was evaluable for inclusion in the pharmacokinetic evaluations. As depicted in Fig. 2, dose-normalized concentrations of pemetrexed in plasma were generally within the range of the reference data (25), indicating that there were no overt alterations in pemetrexed pharmacokinetics in the current population. However, the dose-normalized concentrations seemed to be somewhat higher on average than those from patients given single-agent pemetrexed, which reflects a 40% decrease in pemetrexed clearance (CL) and central volume of distribution ($V_1$; $P < 0.001$ for both), as summarized in Table 3. These decreases in pemetrexed clearance and volume of distribution correspond to a 68% increase in AUC and a 56% increase in $C_{\text{max}}$ for the patients enrolled in the current study relative to single-agent administration of the same pemetrexed dose. The alterations in CL and $V_1$ are also reflected in a modest increase in half-life in this study population compared with the reference population of 252 patients (3.93 versus 3.38 hours).

As previously observed over a dose range of 126 to 838 mg/m$^2$ (25), pemetrexed pharmacokinetics also seem to be independent of pemetrexed dose within the current trial where doses ranged from 400 to 2,400 mg/m$^2$ as evidenced by the lack of a dose-related trend in CL, $V_1$, or $V_{SS}$ (Fig. 3). Therefore, $C_{\text{max}}$ and AUC are also expected to be dose proportional over this dose range. Furthermore, pemetrexed pharmacokinetics seemed to be independent of cyclophosphamide over a cyclophosphamide dose range of 400 to 800 mg/m$^2$ (data not shown).

Pharmacodynamic analysis did not identify a significant association between higher pemetrexed AUCs and the occurrence of grade 3 or 4 toxicities [Wilcoxon Ps for the comparisons, $P = 0.59$ (all toxicities) and $P = 0.18$ (neutropenia)].

**Antitumor activity.** Of the 57 enrolled patients, 50 (87.7%) were evaluable for response. Of the seven patients not evaluable for response, six were not followed for tumor evaluation at the discretion of the investigator because they did not have measurable lesions at baseline. One patient was not evaluable because the baseline tumor assessment was incomplete. Of the 50 evaluable patients, there were no complete responses and there were 13 (26%) confirmed partial responses at the following dose levels: DL1:1, DL3:2, DL4:2, DL5:1, DL6:1, DL7:1, DL9:1, DL10:1, DL12:2, and DL13:1. Responses occurred at most dose levels, with 8 of the 13 responders occurring at dose levels 1 to 7 ($n = 31$; pemetrexed dose range, 400-900 mg/m$^2$). Of the 37 nonresponders, 17 had SD at the following dose levels: DL1:3, DL2:1, DL5:2, DL6:3, DL8:3, DL9:1, DL10:1, DL11:1, and DL13:2.

**Discussion**

To identify non-anthracycline-based and non-taxane-based chemotherapy alternatives for patients with metastatic breast cancer, the combination of pemetrexed and cyclophosphamide was examined in a phase I dose escalation study. The primary objective of the study was the determination of the MTD of pemetrexed and cyclophosphamide combination therapy. The MTD was determined to be pemetrexed at 2,400 mg/m$^2$ and cyclophosphamide at 600 mg/m$^2$.

The designation of these dosages to represent the MTD for the drug combination under investigation was done despite the
addition, 9 of 31 patients at dose levels 1 to 7 and 8 of 26 levels 8 to 14 responded objectively with a partial remission. In 8 of 31 patients at dose levels 1 to 7 and 5 of 26 patients at dose study, but nevertheless, it was systematically recorded. Thus, increase in (therapeutic) effect.

dose reductions of all the agents with little resultant increase in cyclophosphamide is combined with these agents, requires toxicity (myelosuppression), which results when cyclophosphamide-methotrexate-5-FU (combination) therapy (30). The com-
cyclophosphamide-methotrexate-5-FU and cyclophospha-
States (28) and higher than in all dual-agent phase Istudies conducted in Japan (27) and in the United
the form of grade 1 mucositis in the patient who died, although possibly study drug-related death in one patient. In addition, was made because of the severity of toxicity, particularly the fact that the two patients’ DLTs were not the same as required by the protocol definition of MTD. Nevertheless, the decision was made because of the severity of toxicity, particularly the possibility study drug-related death in one patient. In addition, two patients developed mucosal-related toxicities: one in the form of grade 3 diarrhea, representing a DLT, and the other in the form of grade 1 mucositis in the patient who died, although not representing a DLT. The MTD of pemetrexed in combination with cyclophosphamide was much higher than had been found in single-agent pemetrexed studies conducted in Japan (27) and in the United States (28) and higher than in all dual-agent phase I studies (29). This contrasts with observations from studies of cyclophosphamide-methotrexate-5-FU and cyclophospha-
toxicity (myelosuppression), which results when cyclophosphamide is combined with these agents, requires dose reductions of all the agents with little resultant increase in overall dose intensity and therefore only a relatively moderate increase in (therapeutic) effect.

The assessment of clinical activity is not an aim of a phase I study, but nevertheless, it was systematically recorded. Thus, 8 of 31 patients at dose levels 1 to 7 and 5 of 26 patients at dose levels 8 to 14 responded objectively with a partial remission. In addition, 9 of 31 patients at dose levels 1 to 7 and 8 of 26 patients at dose levels 8 to 14 showed stabilization of their disease. These results obviously do not suggest the presence of a dose-response relationship for this drug combination.

The high MTD could not be explained by significantly lower initial homocysteine levels in the patients receiving the higher pemetrexed doses (Fig. 1) nor could it be explained by low homocysteine levels in the entire study population. Presupple-
ment homocysteine levels in this study cohort were comparable with those of patients with other cancers participating in phase I and II trials (31). Presupplementation homocysteine will be analyzed in the phase II study, and data from a larger number of patients will be available to assess the relationship between homocysteine level and pemetrexed dose. A secondary objective of this study was qualitative and quanti-ative assessment of the toxicities of the combination therapy. Toxicities associated with pemetrexed and cyclophosphamide in this study were found to be consistent with the known toxicity profiles of these agents. The combination was generally well tolerated, including no apparent cumulative hematologic toxicity; this suggests that future studies could administer a sufficient number of cycles with manageable toxicity.

The lower dose levels had fewer nonlaboratory toxicities and a lower incidence of hematologic toxicities, with the exception of neutropenia, which was distributed generally evenly across all dose levels except the lowest 2. Interestingly,
nonhematologic toxicities, primarily elevated AST and ALT, occurred more frequently at the lower dose levels.

Population pharmacokinetic analyses using two time points per patient from this study compared with reference single-agent data from 10 phase II studies (25) suggest that pemetrexed clearance and central volume of distribution were ~40% lower in the current study. The decrease in pemetrexed clearance and central volume of distribution is reflected in higher concentrations of pemetrexed. Increased pemetrexed concentrations should be associated with an increase in toxicity for the combination of pemetrexed and cyclophosphamide. Nevertheless, the latter seems not to be the case with this phase I study. As already detailed above, the MTD (2,400 mg/m²) was nearly twice as high as the MTD observed with single-agent pemetrexed (27, 28). Although no overt exposure-effect relationship between pemetrexed AUC or cyclophosphamide dose and the occurrence of grade 3 or 4 toxicities was observed, there was a trend toward more frequent grade 3 or 4 toxicities with higher pemetrexed AUC (P = 0.59 for all toxicities; P = 0.18 for neutropenia). Taken together, these observations support the decision to study a low and a high pemetrexed dose during the phase II study to determine definitively if a dose-effect relationship exists.

It is noteworthy that higher pemetrexed concentrations are evident for the end of the infusion time point [i.e., even before administration of cyclophosphamide (Fig. 2)]. This suggests that the difference in pharmacokinetics between the current study population and the reference population treated with single-agent pemetrexed may not be a reflection of cyclophosphamide administration. Cyclophosphamide pharmacokinetics were not evaluated in the phase I portion of this study. Intracellular and intratumoral concentrations of pemetrexed and cyclophosphamide are also unknown. A drug interaction between the two substances might be occurring that affects the functionality of pemetrexed and its metabolites. This might take the form of suppression of adequate transport of pemetrexed into the cell via inhibition of folate carrier or a reduction/inhibition of the degree of pemetrexed polyglutamation via folylpolyglutamate synthase. Alternatively, although not expected based on the differing elimination pathways for pemetrexed and cyclophosphamide, pemetrexed could be altering cyclophosphamide concentrations or functionality. More extensive pharmacokinetic analyses will be done in the subsequent phase II portion of the study to further evaluate the plasma pharmacokinetics of both pemetrexed and cyclophosphamide. Investigations of folate carrier activity or folylpolyglutamate synthase and the relative concentrations of their intermediate products (reduced pemetrexed, glutaminated pemetrexed) could also provide explanation for the observed MTD.

Following from the identification of the MTD, the protocol-specified dose recommended for the phase II study was the prior dose level; in this case, 2,100 mg/m² pemetrexed and 600 mg/m² cyclophosphamide. However, because DLTs (grade 4 thrombocytopenia and grade 3 rash/desquamation) were reported in two different patients at this dose level and because the pemetrexed dose would be double compared with that of the recommended dose of single-agent pemetrexed (as already detailed), it was decided not to carry this dose forward in the phase I setting. The phase II portion of the study will use a low pemetrexed dose of 600 mg/m² because this dose of pemetrexed has shown activity in phase II studies of metastatic disease (11–13) as well as in this phase I study (6 of 13 responses occurred at this or a lower dose). Pemetrexed at 1,800 mg/m², the dose level below the recommended dose, will be applied as the high dose in the phase II evaluation because no DLTs were reported at this dose level and even more tumor responses were observed (two of the three enrolled patients) compared with that of the protocol-defined recommended dose of 2,100 mg/m² (one of six enrolled patients). In addition, at the 1,800 mg/m² dose level, no grade 3 or 4 nonhematologic toxicities were seen and only one grade 3 neutropenia was reported.

Fig. 3. Pemetrexed pharmacokinetic variables (individual empirical Bayesian estimates) versus pemetrexed dose. To determine the relationship between pemetrexed pharmacokinetics and pemetrexed dose, the absolute dose of pemetrexed was plotted against plasma clearance (top), central volume of distribution (middle), and steady state volume of distribution (bottom).
In summary, this phase I study has reached its goal in identifying the MTD for pemetrexed and cyclophosphamide combination therapy in the treatment of patients with locally advanced or metastatic breast cancer (2,400 and 600 mg/m², respectively). As an immanent consequence, a prospective randomized phase II study is now under way that tests the combination of cyclophosphamide (600 mg/m²) and pemetrexed (600 or 1,800 mg/m²) to further elucidate whether there exists a dose dependency about tumor response, pharmacokinetics, and toxicities for this drug combination in patients with metastatic breast cancer in a multicenter setting.

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A Phase I Study of Pemetrexed (ALIMTA) and Cyclophosphamide in Patients with Locally Advanced or Metastatic Breast Cancer


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