Trimetrexate in Untreated and Previously Treated Patients With Metastatic Breast Cancer: A Cancer and Leukemia Group B Study

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Twenty-two patients with previously untreated metastatic breast cancer and nineteen patients with refractory metastatic breast cancer were treated with trimetrexate (TMTX). Patients received TMTX 8 mg/m^2/day if previously treated or 12 mg/m^2/day if previously untreated, both given by intravenous bolus days 1–5, every 21 days. None of the patients previously treated for metastatic disease responded to TMTX. There was one partial responder among the 22 patients with previously untreated metastatic disease. The primary toxicity was hematologic and occurred more frequently in patients with a pleural effusion, low serum protein or albumin, or poor performance status. There were three toxic deaths. The study for previously untreated patients required cyclophosphamide, doxorubicin, and 5-fluorouracil (CAF) after 4 cycles of TMTX. This study design for previously untreated patients allows the Cancer and Leukemia Group B (CALGB) to prospectively evaluate the activity of new agents in "chemotherapy-sensitive" metastatic breast cancer.

Key words: carcinoma, breast, trimetrexate

INTRODUCTION

Breast cancer is the most common neoplasm and the second leading cause of cancer death in women [1]. Recurrent or metastatic cancer is incurable with a median survival of only 2 years. Although many current chemotherapeutic agents have activity in breast cancer, their use alone or in combination is primarily palliative with a modest effect on overall survival [2].

Single agent methotrexate (MTX) yields responses in approximately 30% of patients with metastatic breast cancer who have not received prior chemotherapy [3]. Trimetrexate (TMTX), a nonclassical folate antagonist, is sufficiently different from MTX to warrant clinical trials to determine its efficacy in breast cancer. TMTX has demonstrated a broader spectrum of antitumor activity than MTX in preclinical trials and is active against human and murine tumor cell lines resistent to MTX, based on impaired cell membrane transport, but not when resistance is due to dihydrofolate reductase gene amplification [4]. Unlike MTX, the clearance of TMTX is primarily nonrenal, occurring by biotransformation and elimination in the liver [5]. Here we report on two multiinstitutional clinical trials conducted by the CALGB evaluating the efficacy of TMTX in previously treated and untreated metastatic breast cancer.

PATIENTS AND MATERIALS

All patients were treated on 1 of 2 CALGB protocols. Nineteen women with previously treated advanced breast cancer were enrolled on CALGB study 8742, a phase II study, between June 1987 and August 1987. Twenty-two women with previously untreated advanced breast cancer were randomly assigned to receive the TMTX arm on CALGB study 8642 between December 1986 and August 1987. This study compares "upfront" phase II agents for 2 to 4 cycles followed by standard chemotherapy with cyclophosphamide, doxorubicin, and 5-fluorouracil (CAF) with initial CAF. Analysis of the crossover data

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from CALGB study 8642 will be the subject of a future report.

All patients were women with histologically documented Stage IV breast carcinoma. The untreated women had received no prior chemotherapy for metastatic disease. Six of the women previously untreated for metastatic disease had received prior adjuvant chemotherapy. Previously treated patients had received only 1–2 prior chemotherapy regimens for metastatic disease, excluding TMTX. All patients were >12 months since adjuvant chemotherapy, >3 weeks since hormonal therapy, >2 weeks since surgery, and >3 weeks since radiation therapy. Required laboratory values, unless documented to be due to disease, were granulocytes >1,800/μl, platelet count >100,000/μl, hemoglobin >10 g/dl, BUN <1.5 times normal, creatinine <1.5 mg/dl, bilirubin <1.5 times normal, transaminases <1.5 times normal. Untreated patients could not have visceral crisis, defined as lymphangitic spread to lungs, carcinomatous meningitis, bone marrow replacement, or significant liver disease. However, a screening bone marrow biopsy and spinal fluid cytology were not required. No previous or concomitant malignancy, or other serious medical or psychiatric illness, was permissible. All patients gave informed consent and the protocols were approved by the local Institutional Review Boards.

Doses of TMTX were based on phase I clinical trial recommendations [6,7]. Previously untreated patients received TMTX at a dose of 12 mg/m^2 per day intravenously (iv) over 5 to 15 min, days 1–5 every 21 days. Patients were evaluated after 2 cycles of TMTX. If patients had achieved a complete or partial response or stable disease, TMTX was continued for 2 more cycles. After 4 cycles of TMTX, all patients then received standard CAF chemotherapy. Patients who progressed prior to receiving 4 cycles of TMTX, changed their therapy to CAF at that time.

Previously treated patients received TMTX at 8 mg/m^2 day iv bolus days 1–5, every 21 days. Patients were to receive a minimum of 2 courses unless rapid progression or extraordinary medical circumstances occurred. After 2 cycles of TMTX, if the patient had a complete or partial response or stable disease, TMTX was to be continued until progression.

During treatment, complete blood counts were obtained weekly. A history and physical examination with measurement of all palpable lesions, blood chemistries to include liver and renal function studies, and urinalysis were obtained on the day of treatment. Lesions measurable only on X-ray or scan were accessed every two cycles of treatment.

A complete response required disappearance of all measurable or evaluable metastatic disease. A partial response required a reduction of >50% in the sum of the product of the perpendicular diameters of all measurable lesions lasting >4 weeks. Stable disease was defined as <50% reduction or <25% increase in the sum of the products of the two perpendicular diameters of all measured lesions. Progression was defined as a 25% increase in the product of two perpendicular diameters for any measured lesion since entry on study, or for responders, the size at time of maximum regression. The appearance of new areas of malignant disease was considered evidence of progression.

Dose escalations and reductions were based on the highest toxicity grade of the previous course. For grade 0 or 1 toxicity, the dose was increased 33%. For grade 2 toxicity, the dose was not changed. For grade 3 or 4 toxicity, the dose was reduced by 50%. Toxicity was graded using standard CALGB criteria [8]. The association of several patient characteristics to degree of hematologic toxicity was evaluated using a one-sided Fisher's exact test.

RESULTS

The accrual of patients was cut short of the respective goals of 25 previously untreated patients and 40 previously treated patients (20 with and 20 without prior methotrexate) due to several presumed toxic deaths on both trimetrexate studies. Eighteen of the 19 previously treated patients were evaluable for response. One patient was determined to be ineligible postmortem, due to 4 prior chemotherapy regimens for metastatic disease. All 19 previously treated patients were evaluable for toxicity. All 22 patients previously untreated for metastatic disease were evaluable for both response and toxicity. On-study characteristics for eligible patients are shown in Table I.

No previously treated patient responded to trimetrexate. Of the 18 eligible patients, 4 patients had stable disease, 12 patients had progressive disease, and 2 patients had no response due to early death. One of the 22 previously untreated patients responded to TMTX. A partial response in the lung was noted after 4 cycles of TMTX. Thus the observed response rate for previously untreated patients is 4.5%, the exact binomial 95% confidence interval is 0.0 to 18.5%. Twelve patients had stable disease, 6 patients had progressive disease, 2 patients had no response due to early death, and 1 patient had no response due to a change to CAF on day 2 on study. This patient had anaphylaxis and shock after one dose of TMTX.

All patients, both previously treated and previously untreated for metastatic disease, were evaluable for toxicity. Toxicity due to treatment with TMTX is shown in Table II. Two early deaths occurred among eligible previously treated patients during treatment with TMTX. One of these is considered a toxic death. At the time of initiation of therapy, this patient had acute pancreatitis, liver metastases with an elevated SGOT 1.5 times

TABLE I. Patient Characteristics

	Untreated	Previously treated
No. of cases entered/eligible	22/22	19/18
Median age	60	58
Menopause status pre/peri/post	5/1/16	1/1/16
Estrogen receptor (ER) status[a] negative/positive/unknown	10/10/2	7/10/1
Progesterone receptor (PgR) status[a] negative/positive/unknown	9/8/5	8/6/4
Prior therapy		
Hormonal	9	10
Chemotherapy	6[b]	18
Radiation	9	12
Immunotherapy	0	1
Dominant metastatic site		
Visceral	15	9
Osseous	2	4
Soft tissue	5	5
Performance status[c]		
0 (normal)	11	6
1 (ambulatory)	9	4
2 (<50% time in bed)	2	8
Response to TMTX		
Partial response	1	0
Stable disease	12	4
Progressive disease	6	12
Early death	2	2
Unevaluable[d]	1	0

[a]ER and/or PgR positive = ≥7 fmol/mg protein.
[b]Adjuvant chemotherapy >12 months prior.
[c]Eastern Cooperative Oncology Group (ECOG) criteria.
[d]Treatment changed on day 2 of study due to anaphylaxis to TMTX.

TABLE II. Grade 3 to 5 Toxicity With Trimetrexate*

Adverse event	No. (%) of patients ($N = 41$)
Leukopenia[a]	10 (25)
Thrombocytopenia[a]	9 (22)
Anemia[a]	4 (10)
Infection	4 (10)
Allergy	4 (10)
Hepatic	3 (7)
Stomatitis	3 (7)
Nausea/vomiting	2 (5)
Diarrhea	2 (5)
Genitourinary	2 (5)
Skin	2 (5)
Alopecia[b]	2 (5)
Bone/muscle	2 (5)
Pulmonary	1 (2)
Neurologic-CNS	1 (2)
Fever without infection	1 (2)

*Toxicity graded using CALGB standard criteria.
[a]$N = 40$. One patient excluded due to early death without nadir hematologic values.
[b]$N = 39$. Two patients exluded due to baseline alopecia.

normal, and a pleural effusion. Her serum total protein and albumin were normal. After receiving 5 days of TMTX at 8 mg/m^2, she developed grade 4 pancytopenia, exfoliative dermatitis, and stomatitis. No bacteremia was documented, but she was persistently febrile from day 6, despite broad spectrum antibiotics, and died on day 16. A second eligible patient died during treatment with TMTX, but this was not considered treatment related. This patient had extensive liver metastases with markedly abnormal hepatic function, low serum albumin, and ascites prior to treatment. She received 5 days of TMTX at 8 mg/m^2 and died on day 6. This death was attributed to hepatic disease and not toxicity as there were no apparent complications due to treatment except for occasional nausea and vomiting. An additional toxic death occurred in a patient ineligible due to 4 prior chemotherapy regimens. The patient had extensive metastases to liver, lung, bone, and skin with massive upper extremity edema. Liver function tests were normal, but the serum albumin was decreased to 1.9 g/dl. The patient had no pleural effusion or ascites. After the patient received TMTX at 8 mg/m^2 for 5 days, she developed life-threatening myelosuppression, stomatitis, and severe infection. As this patient was ineligible, her data were excluded from response, but not toxicity tabulations.

Among the patients previously untreated for metastatic disease, there were 2 deaths during treatment with TMTX. One patient died at home in her sleep on day 12 of unknown cause. This patient had borderline renal function with a creatinine clearance <40 ml/min. A toxic death occurred in a second patient with extensive liver metastases with ascites, extensive pulmonary metastases with a large pleural effusion, hypoproteinemia (6.1 g/dl), and hypoalbuminemia (2.8 g/dl). The patient died on day 16 on study from presumed septic shock.

Although severe or life-threatening toxicity was not always predictable, certain clinical features were more common in patients with significant myelotoxicity. These were the presence of a pleural effusion, a performance status of 2 or worse and a low serum total protein <6.5 g/dl or serum albumin <3.5 g/dl. The presence of liver metastases, abnormal liver function tests, and prior chemotherapy were not associated with increased myelotoxicity (Table III). No patient had significant impairment of renal function to allow assessment of this variable.

Of the 18 eligible previously treated patients, 15 were treated until disease progression, 2 patients stopped due to death, and 1 patient with stable disease withdrew consent after 2 cycles due to lack of tumor regression on treatment. Two patients progressed after 1 cycle, 10 patients after 2 cycles, and 1 each after 3, 5, and 6 cycles. Two patients had the dose decreased (50 and 75%) due to toxicity and 8 patients had their dose escalated (range: 33

TABLE III. Hematologic Toxicity Based on Patient Characteristics

Characteristic	No. of patients[a]	No. of patients grade 3 to 5 toxicity	*P* value[b]
Liver metastases			
Yes	15	3	0.328
No	25	8	
Pleural effusion			
Yes	10	6	0.014
No	30	5	
Total protein <6.5 g/liter or albumin <3.5 g/liter[c]			
Yes	9	5	0.041
No	28	5	
Performance status			
0–1	32	6	0.024
2	8	5	
SGOT or SGPT >50 IU/liter			
Yes	14	4	0.595
No	26	7	
Alk Phos >100 IU/liter			
Yes	21	7	0.305
No	19	4	
Prior chemotherapy			
Yes	18	7	0.135
No	22	4	

[a] $N = 40$. One patient excluded from all toxicity tabulations due to early death without hematologic nadir values.
[b] One-sided *P*-values. Fisher's exact test.
[c] $N = 37$. Baseline serum protein and albumin not obtained in three patients.

to 77%) due to absence of toxicity. For the 22 previously untreated patients, 10 patients completed 4 cycles of TMTX. Twelve patients had their TMTX stopped early, 2 due to toxicity, 2 early death, and 8 progressive disease (2 after 3 cycles, 4 after 2 cycles, and 2 after 1 cycle).

DISCUSSION

In this study no significant activity of TMTX was found in either patients with previously treated or previously untreated metastatic breast cancer. Leiby [9] in 28 patients with refractory breast cancer reported partial responses in 3 of 20 patients [15%; (95% confidence interval 0.0,30.6)]. Similar to our previously treated population, TMTX was given as a short iv infusion on days 1 to 5 of a 21 day treatment cycle. All three patients had received prior methotrexate. Robert et al. [10] using this same treatment regimen, reported partial response in 2 of 29 patients with previously treated metastatic breast cancer. Both patients had prior methotrexate exposure. Exposure to prior methotrexate was not an influencing factor in our study, with 61% of refractory patients and 75% of patients previously untreated for metastatic disease having received no prior methotrexate. Given the small sample sizes of our study and those of both Leiby [9] and Robert et al. [10], these response rates may not be different. Nonetheless, this minimal response rate, especially in patients with no prior therapy where drug efficacy is best assessed, indicates that TMTX has a limited role in the treatment of breast cancer.

Hematologic toxicity was significant and often unpredictable. However, certain clinical features were significantly more common in our patients with severe or life-threatening myelotoxicity. Eisenhauer et al. [11] found that the predictors of severe and life-threatening toxicities from TMTX were the presence of liver metastases and low pretreatment serum protein levels (<6.5 g/dl). Grem et al. [12] in a review of 272 patients treated with TMTX on phase I trials, reported a significant increase in severe or life-threatening toxicity in patients with hypoalbuminemia (≤3.5 g/dl) or hypoproteinemia (≤6.0 g/dl). They also reported that doses given by short infusions daily for 5 days or weekly for 3 weeks were generally more toxic than those given by every other week short infusions or continuous infusion schedules. They did not find performance status or prior therapy to be predictive of toxic effects. In contrast to Eisenhauer et al., we found no association between the presence or absence of liver metastases and significant toxicity in our study. In addition, although TMTX is 90% cleared by hepatic metabolism, elevated liver function tests were also not associated with increased toxicity. However, low

serum total protein or low albumin was associated with a significant increase in hematologic toxicity. Fanucchi et al. [13] in a phase I and clinical pharmacokinetic study of TMTX showed that the magnitude of thrombocytopenia correlated with the amount of exposure to TMTX. At any given dose level, the total body clearance varied widely and was independent of dose. Patients with pretreatment hypoalbuminemia had a reduced total body clearance of TMTX and hence increased drug exposure. These investigators [13] judged that this hypoalbuminemia reflected impaired hepatic synthetic function. They reported 2 toxic deaths among 28 patients, both in patients who had severe thrombocytopenia and hypoalbuminemia. One of these patients also had a large pleural effusion. Fanucchi et al. [13] did not find total body clearance to be correlated with pretreatment SGOT or creatinine clearance.

Six of 10 of our patients with a pleural effusion developed grade 3 or 4 myelosuppression, whereas 5 of 30 patients without an effusion had such toxicity. TMTX may be similar to MTX, which is known to be sequestered into third spaces, with consequential prolongation of drug exposure and increased toxicity [14]. Data from phase I trials suggest that prolonged exposure to high TMTX concentrations resulting from low drug clearance may lead to increased toxicity [15]. In a recent report, Allegra et al. [16] found the median end-infusion TMTX levels to be 2.5- to 3-fold higher ($P < 0.05$) in patients with Grade 3 to 5 gastrointestinal and/or myelotoxicity compared to patients with lesser toxicity. In this phase I clinical trial, TMTX was given as a 24-hr infusion with an initial dose of 16 mg/m^2 and a maximum-tolerated dose of 200 mg/m^2.

Poor performance status was also associated with increased toxicity. However, the independent prognostic value of performance status is uncertain as all patients with a performance status of 2 or worse had either a low total serum protein or albumin or a pleural effusion.

In summary, in our study, TMTX demonstrated little activity against metastatic breast cancer in patients with either prior or no prior chemotherapy. The primary, and occasionally lethal, toxicity was myelosuppression. In our patients this toxicity was associated with the presence of a pleural effusion, a low total protein or albumin, and a poor performance status. The failure to detect an association between liver metastases, liver dysfunction and significant myelosuppression may reflect too small a sample size.

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