Phase I Study of Escalating Doses of Edatrexate in Combination with Paclitaxel in Patients with Metastatic Breast Cancer

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ABSTRACT

Motivated by the observation of preclinical synergy, a Phase I dose escalation study of edatrexate in combination with a 3-h paclitaxel infusion was performed in patients with advanced breast cancer to determine the maximum tolerated dose (MTD) of edatrexate and the toxicities associated with this combination and to report preliminary observations of efficacy with this novel combination.

Thirty-six patients were enrolled in this Phase I trial. Thirty-five eligible patients were treated every 21 days in cohorts of at least three patients and were assessable for toxicity. One patient was ineligible due to hyperbilirubinemia. Stepwise dose escalations of edatrexate were administered until grade >3 nonhematological dose-limiting toxicities were reported. The initial dose level of edatrexate was 180 mg/m²; subsequent cohorts were treated with escalating doses of edatrexate (210, 240, 270, 300, 350, and 400 mg/m²). Edatrexate was administered by i.v. infusion over 1 h. Paclitaxel was administered 24 h later at a fixed dose of 175 mg/m² as a 3-h infusion with standard dexamethasone, diphenhydramine, and cimetidine premedication.

The MTD of edatrexate was reached at the 350 mg/m² level in this study. Grade 3 diarrhea was seen in one patient at the 300 and 400 mg/m² dose levels, requiring dose reductions. Two patients experienced grade 4 stomatitis at the 400 mg/m² dose level and also required dose reduction, establishing the MTD as 350 mg/m². Grade 3 nausea and vomiting were noted in two of three patients at the highest dose level. Of 35 patients, 4 patients reported grade 3 myalgias and 1 patient reported grade 3 neurosensory complaints, which were seen mostly at the 350 and 400 mg/m² dose levels; however, 1 patient reported grade 3 myalgias at 180 mg/m². No cumulative neurotoxicity was observed, and no patient experienced an allergic reaction to paclitaxel.

In 23 patients with bidimensionally measurable disease, there were four complete (17%) and seven partial responses, with an overall response rate of 48% (95% confidence interval, 27–69%). All of the responses were seen in patients who had not received prior chemotherapy for stage IV disease. The median duration of response was not assessable because many responding patients went on to receive high-dose chemotherapy treatment with stem cell support.

The combination of edatrexate and paclitaxel for treatment of metastatic breast cancer is a feasible and safe regimen. The MTD of edatrexate was 350 mg/m² when combined with a 3-h infusion of paclitaxel (175 mg/m²) given 24 h later. Activity was noted even among patients who had relapsed shortly after receiving methotrexate- and/or doxorubicin-containing adjuvant regimens. Additional studies evaluating the sequences and dosing schema for this combination are warranted to improve the response proportion and define the duration of the response.

INTRODUCTION

Despite recent chemotherapeutic advances, metastatic breast cancer treatment remains a challenging area of research. Standard first-line chemotherapy produces complete or partial responses in 35–65% of patients, without resulting in a major prolongation of overall survival. Methotrexate has significant activity in breast cancer, and a wide range of dosages and schedules have been studied. Although single-agent response rates for standard-dose methotrexate have ranged between 25–35% (1, 2), methotrexate combinations, including the standard CMF combination used primarily in breast cancer, have shown somewhat higher responses of 20–50% (3). Efforts to increase clinical activity and expand the spectrum of response among solid neoplasms have led to the development of a new folate analogue, 10-ethyl-10-deaza-aminopterin (edatrexate). Edatrexate, like methotrexate, interferes with DNA synthesis by inhibiting the enzyme dihydrofolate reductase. It has been shown that compared to methotrexate, this novel 4-amino folate analogue demonstrates a higher affinity for the one-carbon-reduced folate transporter and the enzyme polyglutamate synthetase in tumors, thus resulting in a greater intracellular accumulation of this analogue as longer chain polyglutamates (4–6). Lower levels of edatrexate polyglutamates compared to methotrexate polyglutamates accumulate in normal proliferative tissues, resulting in reduced host toxicity and an enhanced therapeutic index (7). This superior antitumor activity compared to methotrexate was seen in a number of murine tumor models and human tumor xenografts including MX-I mammary carcinoma, leading to Phase I and Phase II studies of edatrexate that showed...
activity in various human malignancies (8). Because classical folate analogues like edatrexate are not cross-resistant to anthracyclines (9) and activity has been seen in anthracycline-resistant metastatic breast cancer by several groups, edatrexate represents an interesting candidate for further development in this disease (10–12). Stomatitis is the most common DLT seen with edatrexate administration; however, reports of diarrhea, leukopenia, thrombocytopenia, rash, and elevations of serum glutamic-oxaloacetic transaminase have also been noted (13).

The significant antitumor activity of paclitaxel against metastatic breast cancer is well described (14). The dose-effect relationships and infusion schedule effects have been studied in vitro and in vivo for both paclitaxel alone and paclitaxel in combination with other agents (15, 16). Preclinical combination studies using simultaneous pulse dosing of edatrexate and paclitaxel with SKBR-3 and ZR-75-1 human breast adenocarcinoma cell lines showed marked synergism. When cell lines were pretreated with edatrexate followed by paclitaxel, an increase in the synergism was noted, whereas the reverse sequence showed antagonism (17). However, subsequent studies of this combination of agents against the P388 tumor in mice showed that simultaneous administration and sequential (edatrexate before paclitaxel) administration were approximately equivalent and were markedly more efficacious than methotrexate with paclitaxel.

Motivated by these studies and prior clinical studies showing the efficacy and safety of single-agent paclitaxel (18–22) and edatrexate (10–12) in metastatic breast cancer and of the sequential combination of edatrexate and paclitaxel in advanced solid tumors, the clinical trial reported here was performed.

PATIENTS AND METHODS

Eligibility. All patients were required to have histologically proven metastatic breast cancer. Patients were allowed to have received only one prior regimen for the treatment of their metastatic cancer, with no history of prior taxane therapy. Prior methotrexate as an adjuvant treatment was allowed. Patients were required to have adequate bone marrow (total WBC count ≥ 3,000/mm³ and platelet count ≥ 150,000/mm³), hepatic (bilirubin < 1.0 mg/dl), and renal function (creatinine level < 1.2 mg/dl or creatinine clearance > 50 ml/min) with a Karnofsky performance status of >70%. No radiation or chemotherapy was to be given for at least 3 weeks (6 weeks for nitrosoureas) before starting this study, and patients were to have recovered from the major toxic effects of prior therapy. No clinical congestive heart failure, active angina, or unstable heart rhythms were allowed. Patients were ineligible if they had clinically significant edema, ascites, or pleural effusions. Pregnant or lactating women were excluded; patients were required to be at least 18 years of age, and all patients were counseled to practice effective contraception during this Phase I study. All patients gave informed consent to participate in this study as approved by our center’s institutional review board.

Treatment Plan/Dose Escalation. Patients were treated in the ambulatory clinic with edatrexate administered i.v. over 1 h, every 3 weeks. Paclitaxel was given approximately 24 h later as a 3-h infusion at 175 mg/m² via a nonpolyvinylchloride delivery system with a micropore filter. Premedication for paclitaxel consisted of dexamethasone (20 mg, p.o.) at 14 and 7 h before treatment and cimetidine (300 mg, i.v.) and diphenhydramine hydrochloride (50 mg, i.v.) 30 min before the paclitaxel infusion. The study was initially designed to escalate the doses of edatrexate in cohorts of five patients/dose level. If one of five patients experienced any grade 3/4 nonhematological toxicity, two more patients were accrued to that dose level. If another episode of grade ≥3 toxicity occurred, the dose escalation was to cease, and the dose level below was to be declared the MTD. Because of the surprising lack of toxicity with the combination, the protocol was later amended to enter only three patients/dose level, beginning with dose level 6 (see Table 2). If one of three patients experienced any grade 3/4 nonhematological toxicity, two more patients were added. If another episode of grade ≥3 toxicity occurred, the dose escalation would cease, and the dose level below would be declared the MTD. The starting dose of edatrexate was 180 mg/m² and was escalated to 210, 240, 270, 300, 350, and 400 mg/m². There was no intrapatient dose escalation, and a minimum of three patients were treated per dose level. Patients were continued on study if complete remission, partial remission, or stable disease was demonstrated, and patients were removed from study if disease progression or unacceptable toxic effects were noted. Sufficient patient accrual to each dose level was accomplished to estimate the MTD. Patients with early disease progression with insufficient follow-up for toxicity were replaced.

Patient Response and Toxicity Evaluation. Patients were evaluated for adverse events between cycles and at the time of each treatment. Complete blood counts were obtained at the time of each treatment with edatrexate/paclitaxel. Tumor evaluations by chest radiographs and/or computed tomography occurred at least every 8 weeks or more frequently at the discretion of the primary physician. Standard criteria of response for measurable and evaluable indicator lesions were used (20).

Based on Phase I studies of edatrexate and paclitaxel, the anticipated possible toxicities were myelosuppression, stomatitis, nausea, vomiting, diarrhea, alopecia, peripheral neuropathy, epistaxis, hypersensitivity reactions, arrhythmias, fatigue, and interstitial pneumonitis. Toxicities were graded on a scale of 0–4 using the Common Toxicity Criteria of the United States National Cancer Institute.

RESULTS

Thirty-six patients were enrolled into this Phase I clinical trial. Thirty-five patients were eligible and were treated according to the study design; all were assessable for toxicity. Twenty-three patients had measurable disease and were assessable for response. The characteristics of these patients can be found in Table 1.

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3 H. Skipper and F. M. Schabel, personal communication.

4 F. M. Sirotnak, unpublished results.

During this trial, 217 cycles of chemotherapy were administered. The median number of cycles per patient was 4 (range, 2–33 cycles). Six patients received 2 cycles of chemotherapy, and 16 patients received more than 5 cycles. The MTD for edatrexate was 350 mg/m² when combined with paclitaxel at 175 mg/m². Although grade 3/4 neutropenia was seen at all dose levels with this combination, hematological toxicity was not dose limiting. There were no treatment delays due to hematological toxicity; the use of growth factors was unnecessary, and no patient required blood product transfusion while on study.

The patients were entered in a dose-escalating fashion and monitored for toxicity and response. Once a grade 3/4 toxicity was seen, two more patients were entered at that dose level. At the second dose level (210 mg/m²), one patient with disease progression experienced grade 3 stomatitis, thus two more patients were accrued. No other toxicities were seen, and escalation continued. Grade 3 diarrhea was seen in one patient at the 300 and 400 mg/m² dose levels, respectively, requiring dose reductions. Grade 4 stomatitis was seen in two patients at the 400 mg/m² dose level, also requiring dose reductions and defining the MTD as 350 mg/m². Grade 3 nausea and vomiting were seen in two of three patients at the highest dose level. Of 35 patients, 4 patients reported grade 2 myalgias and 1 patient reported a grade 2 neurosensory complaint; toxicities were seen mostly at the 350 and 400 mg/m² dose levels; however, 1 patient reported grade 2 myalgias at 180 mg/m². No febrile neutropenic events or cumulative neurotoxicity was seen on this trial, and no patient experienced an allergic reaction to paclitaxel. Overall, the treatment was well tolerated, with no hospitalizations, delays, or treatment-related deaths reported. The nonhematological toxicities encountered per dose level are outlined in Table 2.

Although not a primary end point of this Phase I trial, the observed clinical activity of this combination was encouraging. Eleven responses were seen (Table 3), with antitumor activity noted throughout all dose levels. Four complete responses and seven partial responses were reported among the 23 patients with bidimensionally measurable disease, for an overall response rate of 47.8% (exact 95% confidence interval, 27–69%). Most responses were seen in soft tissue and chest wall disease; however, two patients had partial responses in liver, and one patient had a complete response in bone, with resolution of the lytic lesion seen previously on plain radiograph and nuclear bone scintigraphy. None of the 11 patients with clinical responses had received prior chemotherapy for metastatic disease. Two had received hormonal agents as stage IV treatment. Nine of the 11 responding patients had received prior adjuvant chemotherapy: (a) 7 of 9 had been treated with a doxorubicin-containing regimen; and (b) 6 of 9 had been treated with a methotrexate-containing regimen. The median duration from adjuvant therapy to treatment on protocol was 22 months (range, 1–68 months) for the 25 patients who had received adjuvant therapy before study entry. The mean time from adjuvant chemotherapy to this paclitaxel/edatrexate regimen for the responding patients was 26.7 months.

### Table 1: Patient Characteristics

<table>
<thead>
<tr>
<th>No. of patients</th>
<th>35</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients assessable for toxicity</td>
<td>35</td>
</tr>
<tr>
<td>No. of patients evaluable for response</td>
<td>35</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>Median 49, Range 32–69</td>
</tr>
<tr>
<td>No. of females</td>
<td>35</td>
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<tr>
<td>Postmenopausal</td>
<td>25</td>
</tr>
<tr>
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<td>10</td>
</tr>
<tr>
<td>Karnofsky performance status</td>
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<tr>
<td>Prior treatment</td>
<td>None 4, Adjuvant chemotherapy only 23, Chemotherapy for stage IV disease 5, Hormone therapy only 4</td>
</tr>
<tr>
<td>Metastatic sites of disease</td>
<td>Lung 15, Lymph node 12, Liver 9, Bone 8, Chest wall 4, Adrenal 1</td>
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</table>

**DISCUSSION**

The development of new chemotherapeutic combinations is sometimes empiric but is often based on the principles of combining optimal doses of drugs with single-agent activity that are not cross-resistant and have nonoverlapping toxicities. Paclitaxel, a microtubular stabilizing agent, and edatrexate, a classical antifolate, have demonstrated significant single-agent activity in advanced breast cancer (10, 11, 23). The combination of these two agents in preclinical studies has shown significant synergy (17), DLTs do not overlap, and the mechanisms of resistance differ. Moreover, anthracycline-resistant tumors are not cross-resistant to folate analogues. Paclitaxel resistance may be attributable in part to p-glycoprotein-mediated pleiotropic drug resistance, with clinical responses correlating with low levels of MDRI gene expression (24), although other mechanisms involving alterations in tubulin (25–28) are likely to exist. Tumors develop resistance to folate analogues by one of a variety of alterations involving folate transport and intracellular metabolism (7). In light of these observations, we performed this Phase I study of a novel folate analogue, edatrexate, and standard-dose paclitaxel for the treatment of metastatic breast cancer. In this study, we demonstrated that clinically relevant doses of edatrexate and of paclitaxel, in a 3-h infusion, can be given together safely without significant toxicity. The MTD achieved with the combination in this study was 350 mg/m² higher than was expected based on prior studies, with the DLT manifesting as stomatitis and diarrhea (similar to the DLT of single-agent edatrexate). Prior studies of edatrexate in combination with paclitaxel in varying schedules demonstrated lower MTDs. A Phase I study of biweekly edatrexate and 24 h paclitaxel with granulocyte colony-stimulating factor support in solid tumors showed the MTD of edatrexate to be 125 mg/m² (12). A similar paclitaxel schedule combined with methotrexate also showed profound granulocytopenia without granulocyte colony-stimulating factor support (29). Single-agent paclitaxel, when given as a 24-h infusion, has been shown to have significant myelosuppression; therefore, it is not surprising to see myelosuppression as the major DLT in these studies. A second study...
paclitaxel doses were escalated to 250 mg/m². The higher MTD patients, the response rates for 3-h paclitaxel infusions ranged ever, it should be noted that in various studies of comparable observed were seen in those patients with minimal or no prior tumor activity was noted in this Phase I trial, the responses mide rational and appealing to use after doxorubicin/cyclophosphamide. If important activity and good tolerability are con-
doxorubicin-resistant tumors should not be cross-resistant to combination to study in Phase II/III trials, particularly because Phase I toxicity study, these results, coupled with the tolerability 

of the combination of paclitaxel and edatrexate, performed at our institution, combined biweekly edatrexate with biweekly paclitaxel in solid tumor patients. The study design was to escalate edatrexate to 120 mg/m²; however, when a MTD was not reached, the edatrexate dose was fixed at 120 mg/m², and paclitaxel doses were escalated to 250 mg/m². The higher MTD achieved with the combination of edatrexate and paclitaxel with this schedule was surprising and exciting, suggesting nonlinear pharmacokinetics. In our experience, there was no suggestion of increased incidence of neurotoxicity associated with the addition of edatrexate to paclitaxel. Hematological toxicity was seen throughout all dose levels but was not dose limiting and was consistent with the toxicity profile seen with shorter paclitaxel infusion schedules. Grade 3 or 4 leukopenia and neutropenia consistent with the toxicity profile seen with shorter paclitaxel intervals. Although one cannot accurately estimate efficacy from a Phase I toxicity study, these results, coupled with the tolerability and feasibility of this combination, make it a promising doublet will translate to a superior combination regimen in this clinical setting.

REFERENCES

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**Table 2** Leukopenia, stomatitis, and diarrhea by dose level for all courses of treatment

<table>
<thead>
<tr>
<th>Dose (mg/m²)</th>
<th>Leukopenia</th>
<th>Stomatitis</th>
<th>Diarrhea</th>
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<td></td>
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<td>NCI grade</td>
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<td>1  0  3  4</td>
<td>1  0  3  4</td>
<td>1  0  3  4</td>
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<td>1  2  3  4</td>
<td>1  2  3  4</td>
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<tr>
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<td>1  2  3  4</td>
<td>1  2  3  4</td>
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<td>1  2  3  4</td>
<td>1  2  3  4</td>
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<tr>
<td>300 175</td>
<td>1  2  3  4</td>
<td>1  2  3  4</td>
<td>1  2  3  4</td>
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<tr>
<td>350 175</td>
<td>1  2  3  4</td>
<td>1  2  3  4</td>
<td>1  2  3  4</td>
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<tr>
<td>400 175</td>
<td>1  2  3  4</td>
<td>1  2  3  4</td>
<td>1  2  3  4</td>
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</tbody>
</table>

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**Table 3** Phase I paclitaxel + edatrexate activity in measurable patients

<table>
<thead>
<tr>
<th>Overall response rate</th>
<th>CR rate</th>
<th>PR rate</th>
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<tr>
<td>47.8% n = 11/23 (95% CI, 27–69%)</td>
<td>17.4% n = 4/23</td>
<td>30.4% n = 7/23</td>
</tr>
</tbody>
</table>

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a EDX, edatrexate; PTX, paclitaxel; NCI, National Cancer Institute.


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