Phase II Trial of Ixabepilone, an Epothilone B Analog, in Patients With Metastatic Breast Cancer Previously Untreated With Taxanes

Neelima Denduluri, Jennifer A. Low, James J. Lee, Arlene W. Berman, Janice M. Walshe, Ujala Vatas, Catherine K. Chow, Seth M. Steinberg, Sherry X. Yang, and Sandra M. Swain

ABSTRACT

Purpose
Ixabepilone is an epothilone B analog that binds to microtubules and results in microtubule stabilization and mitotic arrest. Ixabepilone was evaluated for efficacy and safety in a phase II clinical trial for women with metastatic breast cancer.

Patients and Methods
Patients were eligible if they had not previously received treatment with a taxane and had measurable metastatic breast cancer. Ixabepilone was administered at 6 mg/m²/d intravenously days 1 through 5 every 3 weeks until unacceptable toxicity or disease progression. Patients underwent pretreatment and post-treatment tumor biopsies, and tissues were analyzed for acetylated α-tubulin, tau-1, and p53 expression when possible.

Results
Twenty-three patients received 210 cycles with a median of eight cycles (range, two to 22 cycles) per patient. Thirteen patients (57%; exact 95% CI, 34.5% to 76.8%) had partial responses, six patients (26%) had stable disease, and four patients (17%) had progressive disease. Median time to progression and duration of response were 5.5 and 5.6 months, respectively. Four patients required dose reductions for neutropenia, neuropathy, or fatigue. Grade 3 or 4 toxicities included neutropenia (22%), fatigue (13%), anorexia (9%), and motor neuropathy (4%). Thirty-nine percent of patients experienced grade 1, 13% experienced grade 2, and none experienced grade 3/4 sensory neuropathy. The six patients with paired biopsies all had increases in tumor α-tubulin acetylation after treatment. Baseline or cycle 2 acetylated α-tubulin, tau-1, or p53 expression did not correlate with clinical response.

Conclusion
Women with metastatic breast cancer previously untreated with taxanes have a meaningful durable response to single-agent ixabepilone therapy. Minimal hematologic toxicity and no grade 3 sensory neuropathy were noted.

INTRODUCTION

Breast cancer is estimated to account for more than 40,910 deaths in 2007 in the United States. Taxanes, potent microtubule stabilizers, have remained the mainstay of cytotoxic therapy for patients with metastatic breast cancer during the last decade. Adjuvant therapy with taxanes is the standard of care for patients with node-positive breast cancer. Although taxanes are efficacious, routine steroid premedication use, neuropathy, and bone marrow suppression continue to be problematic. In addition, increasing use of taxanes in the adjuvant setting has led to development of resistance and decreased response rates with single-agent use in the metastatic setting. This highlights the need for development of novel agents with greater or equal efficacy and improved adverse effect profiles.

Microtubules are comprised of αβ-tubulin heterodimers. Dynamic equilibrium of αβ-tubulin polymerization and depolymerization is necessary for adequate microtubule function and normal cell division. Polymerized α-tubulin undergoes post-translational modifications such as acetylation, a marker for microtubule stabilization. Several microtubule-associated proteins, including tau-1, play a role in microtubule stabilization. Taxanes induce mitotic arrest by inhibiting depolymerization of the microtubules. The epothilones are a new class of microtubule-stabilizing chemotherapeutic agents.
agents derived from the myxobacterium Sorangium cellulosum. Epothilones are poor substrates for P-glycoprotein, and exhibit activity in paclitaxel-resistant cancer cell lines.\textsuperscript{8,9} Ixabepilone is a semisynthetic analog of epothilone B. Ixabepilone binds to β-tubulin, stabilizes tubulin, disrupts microtubule homeostasis, and induces G2/M cell cycle arrest and apoptosis.\textsuperscript{9,10}

Promising preclinical data prompted evaluation of ixabepilone in multiple phase I studies. A phase I study completed at the National Cancer Institute established that ixabepilone administered at 6 mg/m\textsuperscript{2}/d for 5 days every 3 weeks was the maximum tolerated dose.\textsuperscript{11} The dose-limiting toxicity was neutropenia in heavily pretreated patients. Objective responses were noted in patients with breast cancer. Neurotoxicity was noted, but seemed to be decreased on this administration schedule compared with schedules in other phase I trials. This trial also established that corticosteroid premedication is not required with ixabepilone, in contrast to treatment with paclitaxel or docetaxel.\textsuperscript{11}

Because of encouraging results and tolerable toxicity profiles in phase I studies, a phase II trial with two cohorts was initiated at our institution to evaluate efficacy of ixabepilone in the metastatic breast cancer population. The cohort of patients previously treated with taxanes before ixabepilone treatment has been published.\textsuperscript{12} We present the clinical efficacy, safety, and correlative studies in the cohort of patients that did not receive taxanes in the adjuvant or metastatic setting. Although microtubule function and mechanisms of microtubule-stabilizing agents are well understood, biomarkers of tumor response or tumor resistance to microtubule-stabilizing agents are yet to be fully elucidated. In tumor biopsies obtained before and during treatment with ixabepilone, we evaluated p53, tau-1 expression, and α-tubulin acetylation to determine whether baseline or cycle 2 levels and changes between these time points were associated with clinical response to ixabepilone.

### Eligibility

Eligible patients had a diagnosis of metastatic breast adenocarcinoma that was confirmed pathologically; an Eastern Cooperative Oncology Group performance status of 0, 1, or 2; and measurable disease by Response Criteria in Solid Tumors criteria.\textsuperscript{13} Patients could not have received a taxane in the adjuvant or metastatic setting, but there were no other limits for prior therapy. Laboratory values required a total bilirubin ≤ 1.5× institutional upper limit of normal, ALT and AST ≤ 2.5× institutional upper limit of normal, creatinine within normal institutional limits, an absolute neutrophil count of ≥ 1.5 × 10\textsuperscript{9}/L, and platelet count of ≥ 100 × 10\textsuperscript{9}/L. Because of possible interference of cytochrome P450 3A4 activity by ixabepilone, patients were excluded from the study had a 70% probability of early termination.

### Response Assessment

Measurable disease was assessed by computed tomography imaging using Response Criteria in Solid Tumors guidelines.\textsuperscript{15} Patients underwent baseline imaging within 4 weeks of enrollment, and were scanned before every other cycle.

### Statistical Considerations

This protocol was designed to evaluate efficacy of ixabepilone, and was conducted using a two-stage optimal design to rule out a low 5% response rate in favor of a 30% response rate.\textsuperscript{13} With 10% probabilities each of accepting a poor agent and rejecting a good agent, an early stopping rule would be implemented if no responses were seen in the first seven patients. Three or more responses in 21 patients were considered consistent with an active agent worthy of additional development. Under the null hypothesis (5% response probability), the study had a 70% probability of early termination.

Progression-free survival probabilities as a function of time were calculated using the Kaplan-Meier method with censoring at last follow-up (without progression), or removal from study for toxicity. Duration of response was calculated actuarially for 13 patients who had confirmed partial responses, and was calculated from the day of first radiologic partial response until the date of progression. An exact Wilcoxon rank sum test was performed to compare continuous parameters between responders (partial response) and nonresponders (stable and progressive disease).\textsuperscript{12} P values are two tailed, and are presented without adjustment for multiple comparisons.

### Correlative Studies

Patients with tumors that could be biopsied under local anesthesia had core or punch biopsies obtained at baseline and on cycle 2 day 2, approximately 18 to 24 hours after the cycle 2 day 1 dose. If a tumor biopsy was not feasible at baseline, tissue blocks from prior tumor biopsy were obtained.

Immunohistochemistry on tissue sections of formalin-fixed paraffin-embedded samples was performed using a standard avidin-biotin-peroxidase complex indirect immunoperoxidase procedure. Mouse monoclonal antibodies to p53 (Vector Laboratories Inc, Burlingame, CA), acetylated α-tubulin (Sigma, St Louis, MO), and tau-1 (Chemicon International Inc, Temecula, CA) were applied. Immunohistochemistry staining signal was analyzed quantitatively with assistance of the Automated Cellular Imaging System (Chroma Vision Medical Systems Inc, San Juan Capistrano, CA). Six areas of each tumor were scored using a free-scoring or 40× tool to generate an average percentage and intensity of stained tumor cells. Staining index was calculated by multiplying the percentage of positively stained cells by average staining intensity after subtracting machine readouts of the corresponding negative control for each marker. An average labeling percentage was reported for p53 and staining index was reported for acetylated α-tubulin and tau-1.

### Results

#### Patients

Twenty-three patients were enrolled between June 2002 and September 2005, two of whom were enrolled due to a registration error. The baseline characteristics and patients’ prior therapy for breast cancer are summarized in Table 1. Sixteen of 23 patients received prior

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**Table 1. Baseline Characteristics and Prior Therapy for Breast Cancer**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
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<tr>
<td>Age, median (range)</td>
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</tr>
<tr>
<td>Performance status</td>
<td>0 (0-2)</td>
</tr>
<tr>
<td>Tumor site</td>
<td>22 (22)</td>
</tr>
<tr>
<td>Prior therapy</td>
<td>21 (21)</td>
</tr>
<tr>
<td>Prior taxane</td>
<td>6 (6)</td>
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**Denduluri et al**
chemotherapy, and 11 of 18 patients with hormone receptor–positive breast cancer were treated with prior hormonal therapy. Twelve patients received prior anthracycline therapy. Patients received a median of eight cycles (range, two to 22 cycles). Of 23 patients enrolled, 17 patients were removed from study because of disease progression, four patients were removed because of toxicity, and two patients remain on study (with treatment times of 9 and 10 months each).

**Efficacy**

The objective response rate was 57% (95% CI, 34.5% to 76.8%), with 13 partial responses. Stable disease for at least 6 weeks was the best response for 26% (six patients) of patients. The median time to progression for all patients was 5.5 months (Fig 1). For 13 patients who achieved a partial response, median duration of response from date of first documentation of response was 5.6 months. The best response in each patient’s target tumor measurements from baseline are presented in Figure 2. Measurable responses were observed in visceral sites such as lung and liver, soft tissue masses, and lymph nodes. Improvements were also seen in nontarget lesions, including breast, skin, and bone. Of five patients who received ixabepilone as initial therapy for their metastatic breast cancer, three had partial responses and one patient each had stable disease and progressive disease. Of the 18 patients with hormone receptor–positive disease, 12 (67%) had partial responses, four patients (22%) had stable disease, and two patients (11%) had progressive disease. One of five patients with hormone receptor–negative disease achieved a partial response.

**Toxicities**

A total of 210 cycles of therapy were administered. Toxicity information was collected for all patients. The worst toxicity grades are summarized in Table 2. No patients experienced febrile neutropenia. Only one patient required filgrastim. The most notable and frequent nonhematologic toxicities were fatigue, neuropathy, and nail changes. Four patients were removed from the study because of toxicity. Reasons for removal from the study included the following: one patient because of grade 3 weight loss, one patient with grade 3 motor neuropathy, one patient because of prolonged autonomic neuropathy, and one patient because of grade 2 fatigue. Four patients required dose reductions while on study, and two of these four patients required two...
dose reductions. The dose was reduced due to prolonged neutropenia in one patient, neuropathy in three patients, and fatigue in two patients. The patient with prolonged neutropenia received filgrastim for subsequent cycles. Eight patients required antinausea medication for one cycle, and two patients required antinausea medication for two cycles.

Neuropathy

Five of 23 patients had grade 1 baseline neuropathy before treatment with ixabepilone. Three of five patients with baseline grade 1 sensory neuropathy developed worsening neuropathy: two patients had grade 2 sensory neuropathy, and one patient had grade 2 motor neuropathy. Of 18 patients with no baseline neuropathy, nine patients developed grade 1, and two patients developed grade 2 sensory neuropathy. One of 18 patients developed grade 3 motor neuropathy. In total, three patients developed at least grade 2 sensory neuropathy, one patient developed grade 2 motor neuropathy, and one patient developed grade 3 motor neuropathy. The patient who developed grade 3 motor neuropathy had severe rheumatoid arthritis and did not regain function of her fingers due to both ixabepilone and worsening rheumatoid arthritis.

Correlative Studies

Three markers (p53, tau-1, acetylated α-tubulin) were examined in available biopsies for at least one time point in 13 patients. Biopsies at both baseline and during cycle 2 were available from six patients for acetylated α-tubulin and five patients for both acetylated α-tubulin and p53 (four patients had partial response, and one patient had progressive disease). Increased acetylation of α-tubulin was seen (Fig 3) in all six paired biopsies regardless of clinical response. Notably, two tumor samples showed increased abnormal expression of p53 at baseline. Baseline, cycle 2, or changes from baseline to post-treatment biopsies in tau-1 and p53 expression and acetylation of α-tubulin were not associated with response to ixabepilone (Table 3).

Table 2. Toxicities

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>CTC Toxicity Grade</th>
<th>No. of Patients</th>
<th>%</th>
<th>No. of Patients</th>
<th>%</th>
<th>No. of Patients</th>
<th>%</th>
<th>No. of Patients</th>
<th>%</th>
<th>No. of Patients</th>
<th>%</th>
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<td>13</td>
<td>57</td>
<td>2</td>
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<td>3</td>
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<td>17</td>
<td>2</td>
<td>9</td>
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<td>Constipation</td>
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<td>13</td>
<td>10</td>
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<td>5</td>
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<td>Fatigue</td>
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<td>6</td>
<td>26</td>
<td>3</td>
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<tr>
<td>Nail changes</td>
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<td>6</td>
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<td>4</td>
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<tr>
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<tr>
<td>Neuropathy, sensory</td>
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<td>9</td>
<td>39</td>
<td>3</td>
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<td>35</td>
<td>4</td>
<td>17</td>
<td>2</td>
<td>9</td>
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<tr>
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<td>6</td>
<td>26</td>
<td>3</td>
<td>13</td>
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<td>0</td>
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Abbreviation: CTC, National Cancer Institute Common Toxicity Criteria.

DISCUSSION

Our data demonstrate that ixabepilone administered daily for 5 days every 3 weeks is an efficacious agent in metastatic breast cancer. The high partial response rate of 57% and 5.6-month duration of response in a taxane-naïve patient population are encouraging. Of 12 patients who received prior anthracyclines on our study, seven (58%) achieved a partial response. A trial evaluating ixabepilone 40 mg/m² as a single dose every 3 weeks in a taxane-naïve metastatic breast cancer population that was treated with anthracyclines reported a response rate of 34%.16 Patient populations treated with ixabepilone exposed to prior taxanes achieved response rates between 11.5% and 31%,12,17-19

Our response rate compares favorably to response rates (25% to 68%) in studies that evaluated docetaxel or paclitaxel in patient populations that did not previously receive taxanes.20-26 In a trial in which 21% of patients received adjuvant chemotherapy, the response rate was 29% and the median time to progression was 5.3 months after paclitaxel therapy.27 Patients treated with docetaxel after anthracycline therapy achieved a 30% response rate and a 4.25-month time to progression.24 Patients treated with ABI-007 (American BioScience, Santa Monica, CA), a taxane that does not require corticosteroid premedication, experienced a 33% response rate and a 5.75-month time to progression.28 The median time to progression of 5.5 months on our trial is similar to that of these agents.

Bone marrow suppression, an issue with taxanes and other schedules of ixabepilone, was not predominant in our patients.20,26,27,29 Only one patient had neutropenia requiring growth factor support and delay in ixabepilone therapy. Our rates of neuropathy with this schedule were less frequent than in other trials using ixabepilone during shorter administration schedules or taxanes including ABI-007.16,21,22,25,30 Relatively low rates of grade 2 and 3 neuropathy (13% grade 2 sensory neuropathy, 0% grade 3 sensory neuropathy, 4% grade 2 motor neuropathy, 4% grade 3 motor neuropathy) on
Shown are acetylated es.11,12,31 Increased duration of peak concentrations of microtubule-ixabepilone may be due to daily administration with lower peak doses. The increase in acetylation confirms that ixabepilone stabilized microtubules in target tissue, and similar findings were observed in patients with breast and renal cell cancer treated with ixabepilone.12,31 Acetylation of α-tubulin should be evaluated in large randomized studies to assess if changes correlate with clinical response.

Our data did not show any association with p53 expression and response to ixabepilone treatment. Nine patients with tissue available had p53 levels in tumor consistent with wild-type p53. Six of these patients achieved partial responses, and three patients achieved stable disease. Two patients on our study with inflammatory breast cancer had increased expression of p53 in the tumor samples, suggesting a mutated p53 phenotype.36 One of these patients achieved a partial response; the other patient had progressive disease. These results are consistent with prior evidence that tumor response to microtubule-stabilizing agents is indifferent to intact p53 status.37 There is no consensus whether tau-1 expression inhibits or enhances cytotoxicity to microtubule-stabilizing agents. Our data did not show association between tau-1 expression and clinical response to ixabepilone. Similarly, tau-1 expression did not correlate with pathologic complete response to ixabepilone in a pharmacogenomic study.35 However, low tumor tau-1 expression did correlate with response to paclitaxel.38-40

An absence of significant trends in the biomarkers in our study may be due to small numbers of patients with available biopsies and few patients with progressive disease. Another possible reason we were unable to detect significant trends is that we assessed each biomarker using immunohistochemistry. Immunohistochemistry shows an effect in target tissue, but the degree of expression is not necessarily quantitative and may not be an ideal method to predict response to cytotoxic therapy. Future studies to validate predictive markers of response to ixabepilone may be more fruitful with microarray analyses.35,39

In conclusion, our study establishes that ixabepilone has efficacy in patients previously untreated with taxanes similar to response rates seen with docetaxel or paclitaxel in the first- or second-line metastatic setting.21,23-26,29 Most patients tolerated ixabepilone well, with low hematologic toxicity and minimal nausea, vomiting, and diarrhea. Unlike paclitaxel and docetaxel, ixabepilone does not require corticosteroid premedication. Neurotoxicity, a major concern for microtubule-stabilizing drugs, was relatively mild on this schedule. Continued evaluation of potential predictors of response to ixabepilone should be pursued. The favorable efficacy and tolerability seen in our trial and other trials discussed must be confirmed. We await data from two phase III trials examining capecitabine and ixabepilone...
compared with capcitabine alone in approximately 2,000 patients who have been treated previously with anthracyclines and taxanes. If these trials are positive, additional evaluation of ixabepilone in randomized phase III studies in the first-line metastatic and adjuvant settings would be warranted.

**AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST**

The author(s) indicated no potential conflicts of interest.

**CONCEPT AND DESIGN**

Neelima Denduluri, Jennifer A. Low, James J. Lee, Seth M. Steinberg, Sandra M. Swain

**REFERENCES**


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Ixabepilone Treatment for MBC

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