NOVEL CYTOTOXIC AGENTS: EPOTHILONES*

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ANABSTRACT

The epothilones are a new class of non-taxane, microtubule-stabilizing agents with efficacy in taxane-resistant, metastatic breast cancer. Epothilones have a unique binding mechanism to β tubulin that differs from the taxanes, reducing the risk of resistance. They are also more potent antitumor agents than taxanes, requiring lower concentrations.

Currently, 6 epothilones are in clinical trials. Ixabepilone, a semisynthetic analog of epothilone B, is being investigated as a neoadjuvant and metastatic treatment option in breast cancer and was recently approved for marketing as a treatment for metastatic breast cancer in the United States. Phase II trials demonstrate effectiveness in taxane-resistant and taxane-naïve patients with metastatic breast cancer, including those whose tumors are triple negative. There is some indication that biologic tumor activity, specifically in levels of glutamate acid-terminated and acetylated α tubulin, may provide markers of activity. A phase III trial comparing ixabepilone plus capecitabine to capecitabine alone in patients with metastatic breast cancer demonstrated that the combination was superior to the single agent, regardless of hormone-receptor status.

An ongoing phase II trial is investigating combinations of ixabepilone and bevacizumab in human epidermal growth factor receptor 2-positive metastatic breast cancer. In the neoadjuvant setting, ixabepilone demonstrated complete response rates that compare favorably to the other single chemotherapy agents. The compound also may prove particularly effective in patients with estrogen receptor-negative tumors. In conclusion, the epothilones, particularly ixabepilone, appear to offer a new option in the treatment of taxane-resistant metastatic breast cancer.

(Many treatments for metastatic breast cancer are based on tumor phenotype, whether hormone receptor status or human epidermal growth factor receptor 2 (HER2) amplification (Figure 1). New pathways identified in breast cancer oncogenic signaling, such as the PI3K/PTEN/Akt pathway, will lead to new classes of drugs and new methods of identifying the most appropriate therapy for patients, potentially increasing long-term survival.

One such class is the epothilones. Epothilones are a new class of nontaxane microtubule-stabilizing agents. They are derived from the fermentation of the mycobacteria Sorangium cellulosum and were discovered in soil bacteria from southern Africa in 1987.1

Epothilones target cellular microtubules and tubulin. Microtubules are filaments formed with the polymerization of heterodimeric α/β-tubulin subunits. They are a major structural part of the cell found in the cytoskeleton and play a fundamental role in cellu-
lar functions, such as cell division, signaling, shape, and movement. Microtubules undergo polymerization and depolymerization, leading to the formation and functioning of the mitotic spindle. Agents such as the taxanes and vinca alkaloids that target tubulin and microtubules interfere with the function of the mitotic spindle, blocking cells at the metaphase/anaphase junction. These agents are divided into 2 classes, destabilizing or stabilizing, based on their effects on microtubule polymerization and the mass of microtubules at high drug concentrations.

Destabilizing agents decrease the mass of cellular microtubules and inhibit tubulin polymerization, whereas stabilizing agents increase the polymer mass, stabilize microtubules, and induce the formation of microtubule bundles in cells. Both prevent the cell from moving into the final stages of division.

Although the taxanes and vinca alkaloids are thought to inhibit microtubule dynamics, the epothilones arrest the cell cycle at the Gap 2/mitosis phase, preventing mitosis through tubulin polymerization and inducing apoptosis. They do this by binding specifically and uniquely to β tubulin (Figure 2) through a methylthiazole side chain that occupies a region not occupied by the taxanes. They also have a binding mode distinct from the taxanes, sharing just 1 polar contact point (C7-OH). Thus, the epothilones provide an option for taxane-resistant patients.

Epothilones are more potent antitumor agents than the taxanes, with IC50 values in the sub- or low-nanomolar range. Thus, a lower concentration is required to arrest cellular division and growth. Epothilones are also less susceptible to resistance than the taxanes given their different sites of activity. This has been confirmed through in vitro and in vivo tumor models that are naturally resistant to or acquire resistance to paclitaxel.

Mechanisms of potential resistance are tubulin mutation, overexpression of the βIII tubulin isotype or efflux pump proteins, P-glycoprotein (Pgp)-mediated resistance, substrates of multidrug transports, or overexpression of cell membrane transporters. However, in the epothilones, overexpression of Pgp minimally affects the cytotoxicity, and point mutations in β tubulin do not necessarily confer resistance.

There are 6 categories of epothilones. Each differs based on the methylthiazole side chain. Epothilones A, B, E, and F are the epoxides and epothilones C and D are the olefins. Four epothilone B compounds are currently in trials: ixabepilone (BMS-247550), patupilone (EPO-906), BMS-310705 (a water-soluble semisynthetic epothilone), and ZK-EPO. Two epothilone D compounds are in trials: KOS-862 and KOS-1584.

**IXABEPILONE**

Ixabepilone is a semisynthetic analog of epothilone B. It is a potent inducer of microtubule stabilization and has demonstrated efficacy in taxane-resistant tumors as well as various cell lines in preclinical stud-
The major difference between ixabepilone and epothilone B is the side chain of NH instead of O (Figure 3). In October 2007, ixabepilone became the first epothilone approved for marketing in the United States. It is indicated for the treatment of metastatic or locally advanced breast cancer in combination with capecitabine in patients who failed an anthracycline and a taxane, and as a monotherapy for the treatment of metastatic or locally advanced breast cancer in patients who failed an anthracycline, a taxane, and capecitabine. The product information carries a black box warning that ixabepilone in combination with capecitabine is contraindicated in patients with aspartate aminotransferase or alanine aminotransferase greater than 2.5 x upper limit of normal (ULN) or bilirubin greater than 1 x ULN due to increased risk of toxicity and neutropenia-related death.

In phase I trials to establish a recommended dosage, the maximum tolerated dose for ixabepilone was 50 mg/m² intravenously (IV) every 3 weeks over 1 hour. Dose-limiting toxicities were neuropathy and neutropenia. Increasing the infusion duration to 3 hours to minimize neurotoxicities resulted in increased myelosuppression and mucositis toxicities. Thus, the recommended dosage was reduced to 40 mg/m².

Other phase I dose-defining trials gave ixabepilone daily for 5 days every 3 weeks at 6 mg/m² IV over 1 hour, resulting in neuropathy and fatigue. These same toxicities occurred in weekly dosing of 25 mg/m² on a 21-day schedule, or 20 mg/m² on a 28-day schedule over 1 hour. Thus, although the epothilones represent a new class of anticancer agents, they appear to have similar toxicities to existing agents.

Phase I trials also demonstrated activity in cervical, breast, ovarian, renal cell carcinomas, and in mesothelioma and basal cell carcinomas (Table 1).

### IXABEPILONE PHASE II TRIALS IN METASTATIC BREAST CANCER

Four phase II trials have been published (Table 2). Low et al evaluated ixabepilone IV at 6 mg/m²/day for 5 days every 3 weeks in 37 patients with metastatic or locally advanced breast cancer who had received paclitaxel and/or docetaxel as prior neoadjuvant, adjuvant, or metastatic therapy.

Baseline tumor biopsies were performed, and levels of glutamate acid (glu)-terminated and acetylated α-tubulin, markers of microtubule stabilization, were detected by Western blot and immunohistochemistry in a subset of matched pre- and posttreatment tumor biopsies. In all, 37 patients received 153 cycles of ixabepilone. In taxane-resistant patients, there was 1 complete response, 7 partial responses (19%), 13 patients with stable disease (35%), and 16 patients with progressive disease (43%).

Grade 3 and 4 toxicities included neutropenia (35%), febrile neutropenia (14%), fatigue (14%), diarrhea (11%), nausea/vomiting (5%), myalgia/arthralgia (3%), and sensory neuropathy (3%). Two patients were removed from the study because of prolonged grade 2 or 3 neurotoxicity, and 3 patients for other grade 3 and 4 nonhematologic toxicities. In

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#### Table 1. Phase I Trials: Ixabepilone IV

<table>
<thead>
<tr>
<th>Timing</th>
<th>Response</th>
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<tbody>
<tr>
<td>1 h, 5 d, Q 21 d (n = 27)</td>
<td>Objective responses in patients with cervical, breast, and basal cell cancer</td>
</tr>
<tr>
<td>1 h, 1 d, Q 21 d (n = 25)</td>
<td>Objective partial responses in paclitaxel-refractory ovarian cancer (n = 2) and breast cancer (1 taxane naïve, 1 taxane refractory)</td>
</tr>
<tr>
<td>1 h, 3 d, Q 21 d (n = 26)</td>
<td>Disease-limiting toxicity: neutropenia. Prolonged stable disease in patients with mesothelioma, ovarian cancer, and renal cell carcinoma</td>
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</table>

IV = intravenous.

Data from Abraham et al; Mani et al; and Zhuang et al.
addition, 3% of patients experienced grade 3/5 sensory peripheral neuropathy, with baseline neurologic function tests predicting grade 2 or higher peripheral neuropathy.

Compared with baseline, a biopsy conducted after the second cycle of ixabepilone showed increased levels of glu-terminated and acetylated α tubulin, which may serve as an indication of tumor response (Figure 4).14

Denduluri et al evaluated a diverse group of patients (African American, Asian, white, and Hispanic) with taxane-naïve metastatic breast cancer. Patients received ixabepilone 6 mg/m²/day IV for 5 days every 3 weeks until unacceptable toxicity or disease progression occurred. Six patients underwent baseline and posttreatment (cycle 2, day 2) tumor biopsies, which were analyzed for acetylated α tubulin, tau-1, and p53 expression.15

In all, 23 patients received 210 cycles with a median of 8 cycles per patient. Thirteen patients (57%) had partial responses, 6 (26%) had stable disease for at least 6 weeks, and 4 (17%) had progressive disease. Median time to progression was 5.5 months; duration of response was 5.6 months. Overall response rates (25%–68%) were comparable with studies evaluating initial taxane therapy.

Grade 3 or 4 toxicities included neutropenia (22%), fatigue (13%), anorexia (9%), and motor neu-

### Table 2. Phase II Trials in Ixabepilone

<table>
<thead>
<tr>
<th>Author</th>
<th>Dose</th>
<th>Inclusion Criteria</th>
<th>Results</th>
<th>Toxicities (grade 3/4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low et al</td>
<td>6 mg/m²/d, 5 d, Q 3 wk</td>
<td>Taxane-resistant metastatic disease</td>
<td>N = 37</td>
<td>Neutropenia (35%), febrile neutropenia (14%), fatigue (14%), diarrhea (11%), nausea/vomiting (5%), myalgia/arthritis (3%), and sensory neuropathy (3%)</td>
</tr>
<tr>
<td>Dendaluri et al</td>
<td>6 mg/m²/d, d 1–5 Q 3 wk</td>
<td>Taxane-naïve metastatic disease</td>
<td>N = 23</td>
<td>Neutropenia (22%)</td>
</tr>
<tr>
<td>Thomas et al</td>
<td>3-h infusion of 40 mg/m² Q 3 wk</td>
<td>Metastatic disease experienced while receiving or within 4 mo of taxane therapy (6 mo if adjuvant taxane only), already received an anthracycline, and had taxane as last regimen.</td>
<td>N = 49</td>
<td>Neutropenia (12%), Fatigue (13%), Anorexia (9%), Motor neuropathy (4%)</td>
</tr>
<tr>
<td>Perez et al</td>
<td>40 mg/m², 3-h IV infusion on d 1 of 21-d cycle; maximum of 16 cycles or until evidence of DP</td>
<td>Metastatic disease resistant to anthracycline, taxane, and capecitabine</td>
<td>N = 113</td>
<td>Peripheral sensory neuropathy (14%), fatigue/asthenia (13%), myalgia (8%), and stomatitis/mucositis (6%)</td>
</tr>
</tbody>
</table>

CR = complete response; DP = disease progression; IV = intravenous; PD = progressive disease; PR = partial response; SD = stable disease.

Data from Low et al; Denduluri et al; Thomas et al; and Perez et al.
ropathy (4%). The 6 patients with paired biopsies all exhibited increases in tumor \(\alpha\)-tubulin acetylation after treatment; however, acetylated \(\alpha\) tubulin, tau-1, or p53 expression did not correlate with clinical response.\(^{15}\)

Thomas et al assessed ixabepilone in patients with metastatic breast cancer who had experienced disease progression while receiving or within 4 months of taxane therapy (6 months if adjuvant taxane only), who had already received an anthracycline, and who had a taxane as their last regimen. Eighteen patients (37%) were triple negative. Those with grade 2 or higher neuropathies were excluded.\(^{16}\)

Intravenous ixabepilone was administered as a 3-hour infusion of 40 mg/m\(^2\) every 3 weeks. Of the 49 patients treated with 40-mg/m\(^2\) ixabepilone for 3 hours, 6 (12%; 95% confidence interval [CI], 4.7%–26.5%) experienced a partial response and 20 (41%) experienced stable disease. Median time to progression was 2.2 months (95% CI, 1.4–3.2 months) and median survival was 7.9 months. In responders, the median response duration was 10.4 months.

The most common adverse events were fatigue and sensory neuropathy, which were primarily grade 1/2. This study demonstrated a lack of cross-resistance between taxanes and ixabepilone.

Perez et al evaluated ixabepilone in patients with metastatic breast cancer resistant to anthracycline, taxane, and capecitabine. Sixty-five patients were estrogen receptor (ER) positive and 51 were ER negative. Forty-two patients (33%) were triple negative. Ixabepilone 40 mg/m\(^2\) IV was administered over 3 hours on day 1 of a 21-day cycle with a maximum of 16 cycles or until evidence of disease progression. One hundred and twenty-six patients were treated and 113 were assessable for response by an independent radiology facility.\(^{17}\)

The objective response rate was 11.5% (95% CI, 6.3%–18.9%), with 13 of 113 patients experiencing a partial response. Fifty percent of patients achieved stable disease, and 15 patients had stable disease for at least 6 months. The median duration of response and progression-free survival was 5.7 and 3.1 months, respectively, and median overall survival was 8.6 months.

Grade 3/4 treatment-related events included peripheral sensory neuropathy (14%), fatigue/asthenia (13%), myalgia (8%), and stomatitis/mucositis (6%). Resolution of grade 3/4 peripheral sensory neuropathy occurred after a median period of 5.4 weeks, primarily through dose reduction.

Phase II trials evaluating ixabepilone and targeted therapies are ongoing. One is evaluating trastuzumab in 28 metastatic patients with HER2-positive tumors who either received only hormonal therapy for metastatic breast cancer or prior chemotherapy including trastuzumab. All will receive ixabepilone 40 mg/m\(^2\) IV over 3 hours every 3 weeks and trastuzumab 6 mg/kg every 3 weeks.\(^{18}\)

Another phase II trial is evaluating patients with metastatic breast cancer who had not received any previous chemotherapy once the cancer metastasized. This ongoing trial will compare outcomes in patients randomly assigned to 1 of 3 cohorts: ixabepilone 16 mg/m\(^2\) over 1 hour weekly for 3 weeks with 1 week off; ixabepilone 40 mg/m\(^2\) over 3 hours every 3 weeks and bevacizumab 15 mg/kg every 3 weeks; or paclitaxel 90 mg/m\(^2\) over 1 hour every week for 3 weeks with 1 week off and bevacizumab 10 mg/kg every 2 weeks.\(^{19}\)

**IXABEPILONE PHASE III TRIALS IN METASTATIC BREAST CANCER**

In the single published phase III trial, ixabepilone plus capecitabine was compared to capecitabine alone in patients with advanced breast cancer who had previously been treated with an anthracycline and a taxane. Patients were randomized to ixabepilone (40 mg/m\(^2\) IV over 3 hours every 3 weeks) plus capecitabine (1000 mg/m\(^2\) orally twice daily for 14 days) or capecitabine (1250 mg/m\(^2\) orally twice daily for 14 days) alone.\(^{20}\)

A median of 5 ixabepilone and capecitabine cycles, and 4 capecitabine-only cycles were administrated to
752 randomized patients, 84% of whom had visceral disease. Ixabepilone plus capcitabine demonstrated superiority to capcitabine alone, with significant benefit maintained across patients with ER-negative and progesterone receptor (PR)-negative tumors, and HER2-negative tumors.

Grade 3/4 adverse events included neuropathy (ixabepilone + capcitabine 23% vs capcitabine 0%), hand-foot syndrome (18% vs 17%), and fatigue (9% vs 3%). Neuropathy was cumulative and reversible with a median time of 6 weeks to resolution of grade 3/4 neuropathies. The toxic death rate was 3% versus 1%, with a greater risk in patients with liver dysfunction.

IXABEPILONE IN THE NEOADJUVANT SETTING

A trial from Cussac et al investigated the role of ixabepilone in early stage breast cancer in the neoadjuvant setting. The trial protocol was conducted in patients with breast with TNM staging of T2 to T4, any N, M0, and at least 3 cm. A pre-treatment core biopsy of the tumor was obtained and patients received ixabepilone 40 mg/m² every 3 weeks for 4 cycles. Preliminary results on 96 patients identified a 19% pathologic clinical response in the breast and a 12% pathologic response in the breast and nodes.21

Patients who are stable or responding with acceptable toxicities will complete the neoadjuvant therapy, then undergo surgical resection with a sample retained for RNA profiling. Those who experience a more than 2-week delay for toxicity, or a grade 3 neurotoxicity or progressive disease, will discontinue ixabepilone and switch to an anthracycline combination, then undergo surgical resection followed by radiation and an adjuvant anthracycline combination. A biopsy sample will be obtained during surgery for RNA profiling.21

Preliminary data involved 161 patients with stage IIA and IIB breast cancer who underwent 4 cycles of IV ixabepilone treatment at 40 mg/m² over 3 hours every 3 weeks. Twenty-nine patients (18%) had a complete response in the breast tumor; 17 (11%) had a complete response in breast and axilla. In patients who are triple negative (n = 42), 11 patients (26%) obtained a complete response in the breast and 8 (19%) in the breast and axilla.22 Trials comparing ixabepilone to other single-agent therapies (eg, doxorubicin, cyclophosphamide, docetaxel, and paclitaxel) in the neoadjuvant setting demonstrated complete response rates after 4 cycles that compared favorably to response rates reported for studies of single-agent taxanes.21

There is also evidence that low ER levels may be a predictive marker for ixabepilone response in the neoadjuvant setting, although measurement by fluorescence in situ hybridization, not immunohistochemistry, is required to provide an adequate predictor of response.21 Meanwhile, clinical studies with other epothilones continue (Table 3).22-27

CONCLUSIONS

In conclusion, epothilones, particularly ixabepilone, appear likely to offer an additional treatment option for patients with metastatic breast cancer who have failed taxanes, with the possibility that this class of drugs may be used even earlier in the metastatic or adjuvant setting.

DISCUSSION

Dr Zamboni: Is the peripheral neuropathy that occurs with ixabepilone occurring in the same patients who experienced it with a taxane? In other words, could it be a flare or is it a completely different occurrence?

Dr Adek: There is some evidence that those who experienced some neuropathies with taxane were more prone to neuropathies with ixabepilone.

Dr Ignoffo: Is there a dose-toxicity response curve with neuropathy?
Dr Adel: There seemed to be in the phase I trials. I think in the future, with more experience, we will learn more about the best dose in the practice setting, particularly in the neoadjuvant setting.

Mr Solimando: Are allergic reactions an issue with ixabepilone as it is with paclitaxel?

Dr Adel: In phase I studies, patients were required to receive pretreatment with steroids and antihistamines. Now, given what we know from the phase II trials, the only requirement is pretreatment with antihistamines.

Dr Kuhn: Can you tell us more about the pharmacology? How are epothilones metabolized, and are they metabolized differently from the taxanes? Will there be reactions with other medications?

Dr Adel: Although it is not emphasized in any of the trials, I am sure that they are metabolized through the CYP 3A4 substrate.

Dr Kuhn: That can be an issue today because our patients are living longer. Once all else fails and the can’t be very debilitating to patients and is sometimes the primary reason they come off these compounds.

Also, we are discussing grade 3/4 neurotoxicities. My biggest question is, what are we going to do about it?

Dr Adel: There really is no agent that we are aware of that would inhibit or help with the neurotoxicities.

Dr Kuhn: And if you use gabapentin or pregabalin, you really have to titrate the patient up. But there are small but randomized trials of vitamin E and acetyl carnitine.

Dr Almuete: The other thing to point out about epothilones is the fatigue. I think in the future, with more experience, we will learn more about the best dose in the practice setting, particularly in the neoadjuvant setting.

Dr Kuhn: That can be an issue today because our patients are living longer. Once all else fails and the can’t be very debilitating to patients and is sometimes the primary reason they come off these compounds.

REFERENCES


