The Effect of Ketoconazole on the Pharmacokinetics and Pharmacodynamics of Ixabepilone: A First in Class Epothilone B Analogue in Late-Phase Clinical Development

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Abstract

Purpose: To determine if ixabepilone is a substrate for cytochrome P450 3A4 (CYP3A4) and if its metabolism by this cytochrome is clinically important, we did a clinical drug interaction study in humans using ketoconazole as an inhibitor of CYP3A4.

Experimental Design: Human microsomes were used to determine the cytochrome P450 enzyme(s) involved in the metabolism of ixabepilone. Computational docking (CYP3A4) studies were done for epothilone B and ixabepilone. A follow-up clinical study was done in patients with cancer to determine if 400 mg/d ketoconazole (inhibitor of CYP3A4) altered the pharmacokinetics, drug-target interactions, and pharmacodynamics of ixabepilone.

Results: Molecular modeling and human microsomal studies predicted ixabepilone to be a good substrate for CYP3A4. In patients, ketoconazole coadministration resulted in a maximum ixabepilone dose administration to 25 mg/m² when compared with single-agent therapy of 40 mg/m². Coadministration of ketoconazole with ixabepilone resulted in a 79% increase in AUC0-t. The relationship of microtubule bundle formation in peripheral blood mononuclear cells to plasma ixabepilone concentration was well described by the Hill equation. Microtubule bundle formation in peripheral blood mononuclear cells correlated with neutropenia.

Conclusions: Ixabepilone is a good CYP3A4 substrate in vitro; however, in humans, it is likely to be cleared by multiple mechanisms. Furthermore, our results provide evidence that there is a direct relationship between ixabepilone pharmacokinetics, neutrophil counts, and microtubule bundle formation in PBMCs. Strong inhibitors of CYP3A4 should be used cautiously in the context of ixabepilone dosing.

Ixabepilone (BMS-247550; Bristol-Myers Squibb; IXEMPRA) is a semisynthetic analogue of epothilone B that interacts with microtubules and induces them to form stable bundles in cells (1–3). Epothilones have some similarities to taxanes in targeting and stabilizing microtubules but also have important differences; the class is structurally unrelated to taxanes, and unlike taxanes and anthracyclines, has low susceptibility to multiple mechanisms of drug resistance (reviewed in ref. 1). Promising phase I/II studies have led to a completion of a phase III study in breast cancer and approval by the United States Food and Drug Administration for use in the treatment of breast cancer.

We and others have shown that ixabepilone has moderately variable interpatient pharmacokinetics due in part to variations in drug metabolism (2, 4, 5). Because related epothilone analogues serve as cytochrome P450 3A4 (CYP3A4) substrates, it was hypothesized that ixabepilone would also undergo metabolism by liver cytochromes (e.g., CYP3A4; ref. 6).

To determine the effect of cytochrome P450s on ixabepilone biotransformation in vitro, a series of studies with liver microsomes were done together with molecular dynamic
simulations of CYP3A4 ixabepilone docking within the context of data derived from published crystal structures of this enzyme (7–9). Finally, a phase lb study was designed to assess the effects of ketoconazole (a strong CYP3A4 inhibitor) on ixabepilone pharmacokinetics and pharmacodynamics (10).

### Materials and Methods

**In vitro studies**

**Enzyme kinetics.** Under linear conditions, [14C]ixabepilone was incubated in triplicate with pooled human liver microsomes (BD Biosciences; n = 20) at drug concentrations of 0.1, 0.3, 1, 2.5, 5, 10, and 25 μmol/L and expressed CYP3A4 (at drug concentrations of 0, 0.3, 1, 2.5, 5, 10, 25, 50, and 100 μmol/L) in the presence of NADPH (1 mmol/L). Incubations (0.5 mL) in 0.1 mmol/L phosphate buffer containing 0.5 mmol/L of MgCl2 (pH 7.4) were carried out at 37°C for 5 or 10 min and stopped with ice-cold acetonitrile (1:1, v/v). The protein concentration of human liver microsomes was 0.24 mg/mL (for 0.1 and 0.3 μmol/L ixabepilone concentrations) or 0.5 mg/mL (for 1–25 μmol/L ixabepilone concentrations). The CYP3A4 concentration was 25 pmol/mL (for 0.1 and 0.3 μmol/L ixabepilone concentrations) and 50 pmol/mL (for 1–100 μmol/L ixabepilone concentrations). For both human liver microsomes and CYP3A4, the incubation time was 5 min for incubations at 0.1 to 2.5 μmol/L ixabepilone concentrations and 10 min for 5 to 100 μmol/L ixabepilone concentrations. After quenching with acetonitrile (0.5 mL), the samples were centrifuged for 5 min at 3,000 rpm. The supernatants were then analyzed by liquid chromatography tandem mass spectrometry (LC-MS/MS) to determine the concentration of ixabepilone (see Bioanalytical Methods).

**K**<sub>m</sub> and **V**<sub>max</sub> values for ixabepilone metabolism in human liver microsomes and CYP3A4 were determined by fitting the rates of parent drug disappearance in the presence of ixabepilone to the Michaelis-Menten equation ([**V** = (**V**<sub>max</sub> / **Km**+S)] using a nonlinear regression analysis in Sigmaplot (version 8).

**Metabolite identification.** Metabolites were identified by LC-MS/MS after analyzing samples from a [14C]ixabepilone (20 μmol/L) incubation with pooled human liver microsomes. The results from these analyses showed that ixabepilone was mainly metabolized to oxidative metabolites. In addition to parent drug (P), the identified metabolites included P+16, P+14, and P-2 metabolites (11).

**cDNA-expressed CYP enzymes.** Human cDNA-expressed enzymes (BD Biosciences; CYP1A2, CYP2A6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4) were incubated with ixabepilone. The incubation mixtures, in duplicate, consisted of 100 pmol/mL of CYP enzyme, 0.5 μmol/L ixabepilone, 1 mmol/L NADPH, and 0.1 μmol/L phosphate buffer containing 0.5 mmol/L of MgCl2 (pH 7.4). The final mixtures consisted of 0.5 mg/mL human liver microsomes (preincubated with monoclonal antibody), 0.5 μmol/L ixabepilone, 1 mmol/L NADPH, and 0.1 μmol/L phosphate buffer (pH 7.4) that were incubated for 10 min at 37°C with shaking. The reaction was stopped by addition of 0.5 mL of acetonitrile. The samples were centrifuged for 10 min at 3,000 rpm, and aliquots of the supernatant were analyzed by LC-MS/MS (see Bioanalytical Methods). Positive control incubations were carried out in the absence of antibody solution as described above. An incubation without NADPH and without antibody served as negative controls. In addition, another control incubation was carried out in the same manner described above but the incubation mixture contained anti–egg white protein antibody to assess nonspecific reactions.

**Computational methods for substrate docking.** A detailed description of the molecular dynamic simulations done and metabolism site predictions are presented in Supplementary Information.

### Clinical ketoconazole interaction study

**Patient eligibility.** Advanced cancer patients unresponsive to standard therapeutic interventions were enrolled in this open label phase I study (10). Additional criteria included an Eastern Cooperative Oncology Group performance score of 0 to 1 and no more than three prior chemotherapeutic regimens. The remaining criteria were identical to previously published reports (1, 2, 4, 13–23).

**Study design, treatment administration, and dose escalation.** This was a study administering ketoconazole, a nanomolar inhibitor of CYP3A4, with ixabepilone. In cycle 1 (3 wk), ixabepilone was coadministered in escalating doses per treatment cohort with a fixed dose of ketoconazole. In cycle 2, ixabepilone was administered as a fixed dose (40 mg/m²) without administering ketoconazole.

During cycle 1, patients received a 3-h i.v. infusion of ixabepilone on day 1 at a starting dose of 10 mg/m². Ketoconazole (400 mg/d) was given orally with a meal on day -1 (24 h before the infusion of ixabepilone), on day 1 (2 h before the infusion of ixabepilone), and on days 2 to 5 (six doses). The dose of ixabepilone in cycle 1 was increased to 20, 30, and 40 mg/m² for subsequent cohorts of subjects based on safety evaluations. However, the dose escalation increment could be <10 mg/m² based on observed events in the preceding dose level and the pharmacokinetic and pharmacodynamic data obtained at prior dose levels. Based on a standard minimum enrollment design of three subjects at each dose level, any dose level could be expanded to at least six patients based on the observation of one or more dose-limiting toxicities (DLT). The maximum tolerated dose was exceeded when defined by the dose at which at least 33% of six patients had DLT(s). One dose level below this, the maximum tolerated dose was fully explored in terms of toxicities observed in at least 6 patients. For each dose level at the maximum tolerated dose or below, additional subjects could be enrolled to obtain additional safety data.

During cycle 2 (3 wk), based on cycle 1 toxicities, patients received a 3-h i.v. infusion with a maximum of 40 mg/m² of ixabepilone on day 1. The toxicity-based dose reductions and DLT criteria for ixabepilone were followed as published in prior studies (2, 13). Similarly, prophylaxis regimens for ixabepilone were strictly adhered to as previously published (2, 13).
**Inhibition of Ketoconazole Metabolism**

**Pretreatment and posttreatment investigations.** Standard physical, objective antitumor response, and laboratory assays were carried out as previously described (2, 13). In addition to standard protocol tests, the [14C]erythromycin breath test was administered before protocol treatment to assess for hepatic CYP3A activity (Metsol).

**Toxicity assessment.** Because toxicity was the major end point of this clinical study, clinical and laboratory assessments were done weekly. In certain patients, especially those with abnormal laboratory variables, repeat laboratory assessments were done twice within the same week and continued as clinically indicated.

**Pharmacokinetic and pharmacodynamic samples.** Blood samples (4 mL per sample) were collected from an indwelling catheter or by direct venipuncture using tubes containing K3EDTA as the anticoagulant. Every reasonable attempt was made to obtain a dedicated peripheral catheter on the opposite arm or side of body from that used for drug infusion. For the purposes of assessing ixabepilone blood concentrations, the collection times were predose, at 90 and 180 min used for drug infusion. The factors in the analysis were patient and cycle. The analysis of ixabepilone was conducted against an eight-point standard curve ranging from 4 to 10,000 nmol/L. The standard curve was fitted with a linear regression weighted by reciprocal concentration squared (1/x²).

**Standard curves.** Standard curves and quality control samples defining the dynamic range of the bioanalytical method were prepared in blank microsomes processed in the same manner as the test samples. The analysis of ixabepilone was conducted against a four-point standard curve ranging from 4 to 10,000 nmol/L. The standard curve was fitted with a linear regression weighted by reciprocal concentration squared (1/x²).

**Statistical analysis.** To assess the effect of ketoconazole on the pharmacokinetics of ixabepilone, two-way ANOVA was done on dose-normalized log(C_{max}) and log(AUC_{0-∞}) for all patients who had values for cycles 1 and 2. The factors in the analysis were patient and cycle. Because a 40 mg/m² maximum tolerated dose from cycle 1 was not achieved, C_{max} and AUC_{0-∞} from cycle 1 were dose normalized to a 40 mg/m² dose. Point estimates and 90% confidence intervals for means and differences between means on the log scale were exponentially factored to obtain estimates for geometric means and ratios of geometric means on the original scale. The ratio of population geometric means of C_{max} and AUC_{0-∞} for ixabepilone given in combination with ketoconazole (cycle 1) to ixabepilone given alone (cycle 2), along with respective 90% confidence intervals, was reported.

**Results**

**In vitro studies.** The consumption of ixabepilone by human liver microsomes and human cDNA-expressing CYP3A4 can be characterized by monophasic Michaelis-Menten kinetics. The K_{m} values for the oxidative metabolism of ixabepilone were 8.2 and 4.3 μmol/L in human liver microsomes and CYP3A4, respectively. V_{max} values were 1,399 nmol/mg protein/min (~13 nmol/pmol CYP3A4 in human liver microsome/min) and 17.9 nmol/pmol CYP/min for human liver microsomes and CYP3A4, respectively (Fig. 1A and B).
The major metabolic pathways have yet to be identified, but preliminary analysis shows that beyond its two degradants (adiol and oxazine derivative), several P+16, P+14, and P-2 metabolites have been characterized (11). Using cDNA-expressed CYP enzymes, ixabepilone was completely metabolized by CYP3A4 and remained almost unchanged (90%) by other CYP enzymes (1A2, 2A6, 2C8, 2C9, 2C19, and 2D6).

When ixabepilone was incubated with human liver microsomes at a clinically relevant concentration (clinical Cmax = 0.6 μmol/L following a single 70 mg of i.v. administration; ref. 24) of 0.5 μmol/L, 64.5% of ixabepilone was consumed under these conditions (Table 1). When ixabepilone was incubated with human liver microsomes in the presence of ketoconazole (1 μmol/L), an inhibitor of CYP3A4, the oxidative metabolism of ixabepilone was inhibited by ~90%. The inhibitors of the other CYP enzymes (furafylline for CYP1A2, tranylcypromine for CYP2A6, montelukast for CYP2C8, sulfaphenazole for CYP2C9, benzylnirvanol for CYP2C19, and quinidine for CYP2D6) did not significantly inhibit the oxidative metabolism of ixabepilone (inhibition in the range of 5-29%).

Consumption of ixabepilone in human liver microsomes was almost entirely inhibited by anti-CYP3A4 monoclonal antibodies (83%; Table 2). No significant inhibition was observed in the presence of other CYP inhibitory antibodies (anti-1A2, anti-2C8, anti-2C9, anti-2C19, and anti-2D6).

Computational methods for substrate docking. Detailed results are described in Supplementary Information. The dynamic docking of epothilone B to CYP3A4 active site shows

<table>
<thead>
<tr>
<th>Treatment/inhibitor Enzyme inhibited</th>
<th>Ixabepilone remaining, nmol/L* (% †)</th>
<th>Ixabepilone metabolized (%)</th>
<th>% Inhibition ‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive control</td>
<td>88.6 (35.5)</td>
<td>64.5</td>
<td>—</td>
</tr>
<tr>
<td>Heat killed</td>
<td>249.5 (100†)</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>No NADPH</td>
<td>234.7 (100†)</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>Furafylline CYP1A2</td>
<td>117.4 (47.1)</td>
<td>52.9</td>
<td>17.9</td>
</tr>
<tr>
<td>Tranylcypromine CYP2A6</td>
<td>111.9 (44.9)</td>
<td>55.1</td>
<td>14.5</td>
</tr>
<tr>
<td>Montelukast CYP2C8</td>
<td>135.6 (54.4)</td>
<td>45.6</td>
<td>29.2</td>
</tr>
<tr>
<td>Sulfaphenazole CYP2C9</td>
<td>110.1 (44.1)</td>
<td>55.9</td>
<td>13.4</td>
</tr>
<tr>
<td>Benzylnirvanol CYP2C19</td>
<td>96.3 (38.6)</td>
<td>61.4</td>
<td>4.8</td>
</tr>
<tr>
<td>Quinidine CYP2D6</td>
<td>100.7 (40.4)</td>
<td>59.6</td>
<td>7.5</td>
</tr>
<tr>
<td>Ketoconazole CYP3A4</td>
<td>234 (93.8)</td>
<td>6.2</td>
<td>90.4</td>
</tr>
</tbody>
</table>

*Average of triplicates.
†% Remaining was calculated as follows: nmol of ixabepilone in the incubation mixture with inhibitor/nmol of ixabepilone in the negative control (heat killed) > 100 (nanomole values were determined from LC/MS analysis).
‡% Inhibition = (% ixabepilone remaining - % ixabepilone in positive control) / (% ixabepilone in negative control - % ixabepilone in positive control).
§ The amount of ixabepilone in the negative control samples was designated as 100% as the peak area ratio of the parent drug to internal standard in the incubations was designated as 100%.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ixabepilone remaining, nmol/L* (% †)</th>
<th>Ixabepilone metabolized (%)</th>
<th>% Inhibition ‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive control</td>
<td>110.7 (47.2)</td>
<td>52.8</td>
<td>—</td>
</tr>
<tr>
<td>No NADPH</td>
<td>234.7 (100†)</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>Control-with antibody against hen egg white</td>
<td>93.8 (40.0)</td>
<td>60.0</td>
<td>NI*</td>
</tr>
<tr>
<td>With anti-CYP3A4</td>
<td>213.1 (90.8)</td>
<td>9.2</td>
<td>82.6</td>
</tr>
<tr>
<td>With anti-CYP1A2</td>
<td>87.1 (37.1)</td>
<td>62.9</td>
<td>NI*</td>
</tr>
<tr>
<td>With anti-CYP2C8</td>
<td>96.0 (49.0)</td>
<td>59.1</td>
<td>NI*</td>
</tr>
<tr>
<td>With anti-CYP2C9</td>
<td>93.6 (39.9)</td>
<td>60.1</td>
<td>NI*</td>
</tr>
<tr>
<td>With anti-CYP2C19</td>
<td>88.0 (37.5)</td>
<td>62.5</td>
<td>NI*</td>
</tr>
<tr>
<td>With anti-CYP2D6</td>
<td>99.0 (42.2)</td>
<td>57.8</td>
<td>NI*</td>
</tr>
</tbody>
</table>

Abbreviation: NI, no inhibition.
* Average of triplicates.
†% Remaining was calculated as follows: nmol ixabepilone in the incubation mixture with inhibitor / nmol ixabepilone in the negative control (heat killed) > 100 (nanomole values were determined from LC/MS analysis).
‡% Inhibition = (% ixabepilone remaining - % ixabepilone in positive control) / (% ixabepilone in negative control - % ixabepilone in positive control).
§ The amount of ixabepilone in the negative control samples was designated as 100% as the peak area ratio of the parent drug to internal standard in the incubations was designated as 100%.
* Control incubation contained a monoclonal antibody against hen egg white lysozyme to assess for nonspecific reactions.
*% Ixabepilone remaining was less than or equal to the % ixabepilone in the positive control incubation sample.
that only one position is possible with high interaction energy (-81.0 kcal; Supplementary Fig. S2A). In contrast, for ixabepilone, two distinct final positions are possible (-90.0 and -96.3 kcal; Supplementary Fig. S2B and S2C, respectively). The latter positioning (Supplementary Fig. S2C) is characterized by two hydrogen bond networks and the higher interaction energies are consistent with higher number of specific interactions between substrate and the protein.

### Clinical ketoconazole interaction study

#### Demographics and treatment compliance.

Twenty-nine patients were enrolled into this study, of which 2 (7%) were never treated. The median age of enrollment was 62 years (range, 33-84 years) and 18 patients (67%) were female. Two (7%) patients had genotype CYP3A5*1/*3 (see Supplementary Information for Methods). The Eastern Cooperative Oncology Group performance score was either 0 or 1 (n = 4) or 1 (n = 26). Twenty-four patients (89%) had extensive (metastatic) disease and 26 (96%) had undergone chemotherapy previously. The number of chemotherapy regimens used were two and three in 10 (37%) and 13 (48%) patients, respectively. The most common diagnosis was gynecologic malignancy (carcinoma of ovaries, cervix, endometrium) followed by prostatic adenocarcinoma. Liver metastases were observed in 2 patients (~7%) and the median (range) pretreatment bilirubin (mg/dL), alanine aminotransferase (IU/L), and albumin (g/dL) levels were 22 (14-43), 19 (8-60), and 3.8 (2.8-4.3), respectively. The main reason for discontinuation of treatment was disease progression/relapse (12 patients, 44%).

The erythromycin breath test, a measure of hepatic CYP3A activity, was administered to subjects before treatment for exploratory evaluation as a marker of the rate of clearance of ixabepilone. Percent of [14C]ixabepilone metabolized per hour ranged from 0.96 to 4.72, a 4.9-fold range. Genomic DNA from 24 subjects was analyzed for the CYP3A5 and CYP2D6 genotype. Nine and three patients had one or two CYP3A5 alleles that coded for active enzyme, respectively, based on a lack of either the *3 or *6 variant. Seven patients were homozygous wild-type for CYP2D6 (see Supplementary Information for Methods).

All patients received ketoconazole administered by study personnel on-site and after administration of ketoconazole; a mouth check was done to ensure that the subjects had swallowed the dose. A total of two patients (7%) missed at least 1 day of ketoconazole dosing in cycle 1. In addition, treatment compliance was monitored by drug accountability, medical record, and case report forms. In terms of concomitant medicines, there were no xenobiotics used that were known to interfere with CYP3A4- or CYP3A5-directed metabolism.

#### Pharmacokinetics and interaction effect analysis.

The exposure and clearance of ixabepilone was affected by coadministration of ketoconazole (Tables 3 and 4). Specifically, ixabepilone clearance decreased in 19 of 22 patients when coadministered with ketoconazole. As shown in Table 4, coadministration of ketoconazole with ixabepilone resulted in an increase of ~7% in ixabepilone $C_{\text{max}}$. However, the 90% confidence interval indicates that this effect is not statistically different from the null hypothesis (i.e., no difference in effect). Coadministration of ketoconazole with ixabepilone resulted in a 79% increase in $AUC_{0-\infty}$. The 90% confidence interval indicates that this effect is statistically different from the null hypothesis. The mean plasma ixabepilone concentration versus time for all patients by dose is shown in Supplementary Fig. S4.

The clearance of ixabepilone did not correlate with CYP3A4 activity as measured by the erythromycin breath test (Supplementary Fig. S5). Furthermore, the mean clearance of eight subjects with at least one CYP3A5 allele that coded for active enzyme did not differ from that of 10 subjects without active enzyme (data not shown).

### Table 3. Summary statistics for ixabepilone pharmacokinetic variables

<table>
<thead>
<tr>
<th>Cycle</th>
<th>Ixabepilone (mg/m²)</th>
<th>Pharmacokinetic variables</th>
<th>$C_{\text{max}}$ (ng/mL)</th>
<th>$AUC_{0-\infty}$ (ng h/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10 (n = 4)</td>
<td>$C_{\text{max}}$ (ng/mL)</td>
<td>54.3 (14)</td>
<td>996.0 (22)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$AUC_{0-\infty}$ (ng h/mL)</td>
<td>48.3 (26.3)</td>
<td>70.3 (31.3)</td>
</tr>
<tr>
<td>2</td>
<td>20 (n = 12)</td>
<td>$C_{\text{max}}$ (ng/mL)</td>
<td>97.1 (44)</td>
<td>1,694.1 (76)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$AUC_{0-\infty}$ (ng h/mL)</td>
<td>66.0 (29.7)</td>
<td>81.6 (40.7)</td>
</tr>
<tr>
<td>3</td>
<td>25 (n = 7)</td>
<td>$C_{\text{max}}$ (ng/mL)</td>
<td>200.3 (61)</td>
<td>2,740.8 (52)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$AUC_{0-\infty}$ (ng h/mL)</td>
<td>55.0 (11.4)</td>
<td>66.2 (13.7)</td>
</tr>
<tr>
<td>4</td>
<td>30 (n = 4)</td>
<td>$C_{\text{max}}$ (ng/mL)</td>
<td>246.9 (49)</td>
<td>3,144.6 (56)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$AUC_{0-\infty}$ (ng h/mL)</td>
<td>93.9 (27.4)</td>
<td>100.7 (38.2)</td>
</tr>
<tr>
<td>5</td>
<td>40 (n = 20)</td>
<td>$C_{\text{max}}$ (ng/mL)</td>
<td>206.1 (38)</td>
<td>1,848.8 (46)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$AUC_{0-\infty}$ (ng h/mL)</td>
<td>53.2 (25.7)</td>
<td>51.9 (23.9)</td>
</tr>
</tbody>
</table>

#### Table 4. Statistics for ixabepilone dose normalized $C_{\text{max}}$ and $AUC_{0-\infty}$

<table>
<thead>
<tr>
<th>Pharmacokinetic variable (n = 22)</th>
<th>Adjusted geometric means</th>
<th>Ratio of geometric means</th>
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<tbody>
<tr>
<td></td>
<td>Treatment</td>
<td>Adjusted geometric means</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$C_{\text{max}}$ (ng/mL)</td>
<td>Cycle 1</td>
<td>231.49</td>
</tr>
<tr>
<td></td>
<td>Cycle 2</td>
<td>216.91</td>
</tr>
<tr>
<td>$AUC_{0-\infty}$ (ng h/mL)</td>
<td>Cycle 1</td>
<td>3,457.91</td>
</tr>
<tr>
<td></td>
<td>Cycle 2</td>
<td>1,930.43</td>
</tr>
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</table>
Drug target effect analysis. The formation of microtubule bundles caused by ixabepilone in PBMCs is a plasma concentration–dependent effect. For cycle 1 (ixabepilone and ketoconazole) and cycle 2 (ixabepilone alone), the percent of PBMCs with microtubule bundles was greatest just before the end of infusion at 3 hours, remained above baseline at 6 and 24 hours, and returned to approximately baseline at 48 hours (Supplementary Fig. S6). The percent of PBMCs with microtubule bundles also increased in the presence of ketoconazole and drug (Table 5). At 25 mg/m² (n = 6) of ixabepilone, the % (SE) bundle formation was 38.7 (±6.2) compared with that at 40 mg/m² (n = 17) of ixabepilone in the absence of ketoconazole (40.7 ± 8.6%). The relationship of microtubule bundle formation to plasma ixabepilone concentration after ixabepilone alone was well described by the Hill equation with effect at zero concentration (E₀) fixed to zero. The fitted variable values for the model are presented in Fig. 2A. The Hill equation with E₀ fixed at zero was the best model compared with the Hill equation with E₀ as a model variable; the Eₘₐₓ model with E₀ fixed to zero; the Eₘₐₓ model with E₀ as a model variable; the linear model or the log-linear model, based on the objective function, the Akaike criteria, and the Schwartz criteria. The plot relationship of microtubule bundle formation to plasma ixabepilone concentration (for patients dosed at 40 mg/m² only) is shown in Fig. 2A. The percent baseline tubulin bundles in cycle 1 was 0.8%, which was nearly identical to that observed in baseline values from our previous study (n = 49) and for baseline values in cycle 2 in this study, suggesting that ketoconazole itself had no influence on tubulin bundle formation in PBMCs (13). In addition, ixabepilone-mediated microtubule bundle formation is not inhibited by ketoconazole in HepG2 cells (Supplementary Fig. S1).

Clinical adverse events. Table 6 lists the cycle 1 DLT events following administration of ixabepilone and ketoconazole. During the administration of ketoconazole alone, there were no grade 2 or greater toxic events observed. Most patients complained of grade 1 abdominal discomfort (bloating/nausea) for the duration of this treatment period. The initial dose level of ixabepilone administered for cycle 1 was 10 mg/m² and subsequent cohorts of 20 and 30 mg/m² were opened. Based on observed DLTs and available pharmacokinetic and pharmacodynamic data, the 30 mg/m² cohort was closed and the 20 mg/m² cohort was expanded to 6 patients. On further safety data obtained at 20 mg/m², an intermediate dose level

Table 5. Percent of PBMCs with microtubule bundles observed in cycle 1 and 2 end-of-infusion

<table>
<thead>
<tr>
<th>Cycle 1 (with ketoconazole), mean % (±SD)</th>
<th>Cycle 2 (without ketoconazole), mean % (±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 mg/m² (n = 4)</td>
<td>22.5 (±9.4)</td>
</tr>
<tr>
<td>20 mg/m² (n = 8)</td>
<td>36.6 (±9.5)*</td>
</tr>
<tr>
<td>25 mg/m² (n = 6)</td>
<td>38.7 (±6.2)</td>
</tr>
<tr>
<td>30 mg/m² (n = 4)</td>
<td>56.5 (±4.1)</td>
</tr>
<tr>
<td>40 mg/m² (n = 18)</td>
<td>40.7 (±8.6)*</td>
</tr>
</tbody>
</table>

*P = 0.29.

Fig. 2. A, relationship of percent PBMCs with microtubule bundles and ixabepilone plasma concentration. B, relationship between percent PBMCs with microtubule bundles and nadir absolute neutrophil count (ANC). The line describes a negative linear fit equation with r² = 0.32 and curved lines represent 95% confidence intervals.
of 25 mg/m² was opened. Because 2 of 4 (50%) patients at 30 mg/m² had DLTs, and 2 of 7 (29%) at 25 mg/m² had DLTs, the latter dose was defined as the maximum tolerated dose by protocol criteria. The overall treatment-related adverse event profile of ixabepilone administered concomitantly with ketoconazole in cycle 1 was similar to that of ixabepilone administered alone beyond cycle 1. The most common treatment-related adverse events in cycle 1 across all dose cohorts were fatigue (n = 17 patients, 74%) and nausea (n = 12, 52%). Unexpected side effects occurred in two patients, both of whom developed acute renal failure while receiving the study medications (see Supplementary Information for details).

Peripheral neuropathy is a dose-limiting side effect after chronic dosing with ixabepilone. The incidence of treatment-related sensory peripheral neuropathy was consistent with previously published studies (reviewed in ref. 1). Peripheral neuropathy was primarily sensory and grade 1 (n = 6, 22%) or grade 2 (n = 7, 26%). Overall, only 2 patients (7%) reported grade 3 neuropathy and there were no drug discontinuations due to peripheral neuropathy.

Laboratory adverse events. Overall, the most frequent side effect in cycle 1 was hematologic and included neutropenia (n = 5; 19%), anemia (n = 4; 15%), thrombocytopenia (n = 4, 15%), and febrile neutropenia (n = 2; 7%), whereas the most common hematologic abnormality beyond cycle 1 was neutropenia (n = 7; 30%), thrombocytopenia (n = 2, 9%), and anemia (n = 1; 4%). Grade 4 neutropenia was reported in 3 patients (11%) in cycle 1 and in one patient (4%) beyond cycle 1. Grade 3 neutropenia were reported in 2 patients (9%) beyond cycle 1. Grade 3 thrombocytopenia and grade 3 febrile neutropenia was reported in 2 patients (7%) each in cycle 1. Grade 3 anemia was reported in 1 patient (4%) beyond cycle 1. Similarly, liver function tests (alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, and total bilirubin) during the ixabepilone administration phase remained grade 0 or 1 in severity. There was only one grade 2 alkaline phosphatase elevation and grade 3 increase in creatinine reported in one patient (4%). There was no grade 4 serum abnormality reported during this study and there was no dose-neutropenia relationship. Ixabepilone-associated neutropenia was not dependent on age, baseline absolute neutrophil count value, Eastern Cooperative Oncology Group performance score, or prior chemotherapy (two-way ANOVA, P > 0.2).

Microtubule bundles in PBMCs as a function of toxicity. As published previously, we showed a relationship between percent PBMCs with microtubule bundles at the end of infusion and absolute neutrophil count nadir (r² = 0.44, one phase exponential decay or r² = 0.32, linear relationship). Although the mono-exponential decay model gives a slightly better R² value, the linear model is the simplest model describing a relationship between both variables and gives variable estimates (e.g., slope at absolute neutrophil count values <2,000) that are similar to those obtained using the exponential decay model (Fig. 2B). Upon analyzing the data by neutropenic grade, there is a significant increase in maximum percent of PBMCs with microtubule bundles in patients with grade 2 or greater neutropenia compared with those with grade 1 or less toxicity (P = 0.002) and similarly for those with grade 0 to 2 neutropenia compared with those experiencing grade 3 to 4 neutropenia (includes febrile neutropenia) [mean (SE), 58 (14) versus 38 (10), respectively, P < 0.02] (figure not shown).

Antitumor response. Twenty-three patients (85%) had at least one target tumor lesion for assessment. Four patients (15%) had no measurable or evaluable target tumor lesions. Investigator assessment of tumor response indicated that 1 patient (4%) had a complete response (ovarian adenocarcinoma with abdominal metastases) based on serial positron emission tomography scans; 12 (44%) had stable disease; 10 (37%) had progressive disease; and response status was undetermined in 4 (15%) patients.

Discussion

The human microsomal studies clearly show that CYP3A4 plays a major role in the oxidative metabolism of ixabepilone at concentrations of 0.1 to 25 μmol/L in native human liver microsomes. The molecular modeling studies further validated these observations and showed the utility of various theoretical models in predicting CYP3A4 biotransformation. The clinical study validates the in vitro findings that inhibition of CYP3A4 by ketoconazole can significantly increase the exposure to the parent compound, ixabepilone, suggesting that inhibition of its metabolism directly alters its presence in blood. However, in vitro experiments cannot predict the extent of drug metabolism, as this is highly dependent on fraction metabolized (fₘ) by a given pathway. Unfortunately, due to degradation, the human Absorption, Distribution, Metabolism and Excretion study of ixabepilone did not allow an accurate measurement of fₘ. There might be other mechanisms for ixabepilone clearance, such as chemical degradation, which may not be accounted for in in vitro studies.

The modeling studies predicted a fairly tight docking into the CYP3A4 catalytic domain and based on docking alone it could be predicted that ixabepilone would likely be a substrate of CYP3A4 (compared with etoposide B). Because CYP3A4 is known to have homotropic and heterotropic substrate interactions, the docking with ixabepilone suggested that with this compound, such interactions are not likely to occur because the substrates are large enough to occupy the major part of the active

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Table 6. Cycle 1 DLTs

<table>
<thead>
<tr>
<th>Cycle 1 dose</th>
<th>No. patients</th>
<th>No. DLTs</th>
<th>DLT event</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 mg/m²</td>
<td>4</td>
<td>0</td>
<td>G3 fatigue and G4 neutropenia; G3 mucositis</td>
</tr>
<tr>
<td>20 mg/m²</td>
<td>12</td>
<td>2</td>
<td>G4 neutropenia and G3 diarrhea; febrile neutropenia</td>
</tr>
<tr>
<td>25 mg/m²</td>
<td>7</td>
<td>2</td>
<td>G3 mucositis; G3 fatigue, febrile neutropenia</td>
</tr>
<tr>
<td>30 g/m²</td>
<td>4</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>
site (volume of epothilone B has been estimated to be 709 Å³). However, a cooperative effect involving an external allosteric site cannot be excluded (7, 25). Furthermore, competitive effects are expected with ketoconazole as validated for other compounds. Finally, the interaction energies show that ixabepilone is likely to be a better substrate for CYP3A4 than epothilone B. The value of this novel docking strategy is that other clinically effective epothilone derivatives can be modeled in a similar manner.

The observation that ixabepilone is a good substrate for CYP3A4 catalysis in human liver microsomes alone cannot predict in vivo clearance (25–29). There are examples (e.g., sorafenib) where the in vitro studies predict that certain enzyme(s) metabolize drug(s) with high affinity, yet this effect is not observed clinically (30). Degradation of ixabepilone and the metabolites of degradants might also play a role in in vivo clearance. This underscores the importance of translating all observations seen in vitro to humans.

The clinical study with ketoconazole and ixabepilone clearly indicate that inhibition of CYP3A4 by ketoconazole significantly increases the exposure of ixabepilone in the blood and alters drug-target effects. These effects are reflected by increased toxicity when both drugs are coadministered. The implications of these findings are broad. First, although ketoconazole is one of the most potent inhibitors of CYP3A4, other clinically important inhibitors of CYP3A4 that are commonly used in cancer patients would be predicted to have significant drug interactions with ixabepilone (8). Second, inhibition of CYP3A4 by ketoconazole increases ixabepilone exposure in the blood, which directly correlates with increased formation of microtubule bundles in PBMCs. The latter is inversely correlated with nadir neutrophil counts. These results validate our previous findings in patients undergoing treatment with single-agent ixabepilone (2, 4). The implications are that by using our technically straightforward assay (approximately <8 hours performance time) to visually quantify tubulin bundles, one can predict both neutropenia and blood levels of ixabepilone. In our study, there was no clear relationship between blood concentrations of ixabepilone and neutrophil counts. However, based on a model describing exposure-response analyses of ixabepilone-induced neutropenia in patients with breast cancer (31), the absolute neutrophil count-time profile data were adequately described by the semimechanistic, nonlinear, mixed effects (“population”)

model of neutrophil dynamics, in which the inhibition of neutrophil progenitor production was related to the plasma concentration of ixabepilone by an inhibitory hyperbolic function. Together, this suggests that there is a direct relationship between ixabepilone pharmacokinetics, neutrophil counts, and microtubule bundles in PBMCs. Hence, a count of microtubule bundles in PBMCs serve as a surrogate for ixabepilone toxicity.

Third, comedications (e.g., dexamethasone) routinely used in cancer patients can serve as potent activators of the human pregnane X receptor. Pregnanes X receptor regulates CYP3A4 at the level of transcription, which could alter drug metabolism by inducing its expression (32). Ketoconazole can attenuate transcriptional regulation of CYP3A4 across the patient population (32–34). Furthermore, pregnane X receptor can also regulate tumor drug metabolism and resistance and ketoconazole may inhibit this process also (35–41). Together, coadministration of ketoconazole may be advantageous in protecting against a pregnane X receptor–mediated increased clearance of ixabepilone that may indeed lead to subtherapeutic drug levels and decreased antitumor effects. Supporting these findings are the observation that ketoconazole can augment cytotoxicity of drugs (e.g., nocodazole) in cancer cells (40, 42–44).

The clinical study was designed as both a dose escalation safety and tolerability assessment and as an evaluation of the pharmacokinetics of ixabepilone when administered with a strong CYP3A4 inhibitor such as ketoconazole. A dose of 25 mg/m² of ixabepilone with ketoconazole was tolerated by five of seven patients, whereas 40 mg/m² of ixabepilone is the recommended dose as a single agent. This result is consistent with the observed 79% increase in exposure. Based on these results, a reduced dose of 20 to 25 mg/m² of ixabepilone might be safely administered to a patient who must have continued therapy with a strong inhibitor of CYP3A4.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Acknowledgments
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References


The Effect of Ketoconazole on the Pharmacokinetics and Pharmacodynamics of Ixabepilone: A First in Class Epothilone B Analogue in Late-Phase Clinical Development

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