Review

Ixabepilone: targeting βIII-tubulin expression in taxane-resistant malignancies

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Abstract

Microtubule-targeting agents, such as taxanes and epothilones, block mitosis and cell proliferation by targeting the dynamics of the cytoskeleton. The taxanes are widely used for treatment of various malignancies, but primary and acquired resistance to chemotherapy remains a significant clinical concern. Class I, II, III, IV, and V β-tubulin isotypes are expressed in human tumors. Overexpression of the βIII-tubulin isotype is one mechanism that can render tumor cells resistant to taxanes. The relative expression of βIII-tubulin correlates with clinical outcomes in several tumor types, including breast cancer, non–small cell lung cancer, and ovarian cancer. A novel analogue of epothilone B, ixabepilone, has recently been approved in combination with capecitabine for the treatment of patients with anthracycline- and taxane-resistant locally advanced or metastatic breast cancer and as monotherapy in patients whose tumors are resistant or refractory to an anthracycline, a taxane, and capecitabine. The significant antitumor activity of ixabepilone in taxane-resistant tumors may be related to its preferential suppression of the dynamic instability of α/βIII-microtubules in cells expressing high levels of βIII-tubulin. [Mol Cancer Ther 2009;8(1):17–25]

Introduction

Despite clinical response to taxane-containing chemotherapy regimens seen in many tumor types, the majority of patients treated with these regimens relapse over time. Because an increasing number of patients are being treated with taxanes in the adjuvant and first-line metastatic setting, the development of resistance is a clinically important problem. There is an urgent need to understand the mechanisms by which tumor cells become refractory to chemotherapy and to implement novel strategies for overcoming taxane resistance.

A well-characterized mechanism of resistance is overexpression of the *MDR-1* gene, which encodes P-glycoprotein, an efflux pump able to hamper retention of taxanes and other cationic drugs (1). Such a mechanism is widely seen in cultured tumor cells, but conclusive evidence is lacking in patients with solid tumors. After identification of the paclitaxel binding site in β-tubulin, Giannakakou et al. (2) identified point mutations in tubulin at the paclitaxel binding site that were responsible for resistance to microtubule binding agents. Although a strong correlation between tubulin point mutation and resistance to taxane-containing therapy was detected in lung cancer patients (3), other studies have failed to confirm the presence of tubulin point mutations in patients with taxane-resistant lung cancer and ovarian cancer or have found this to be a rare clinical occurrence (4–6).

A third mechanism of taxane resistance is the selective overexpression of β-tubulin. Kavallaris et al. (7) discovered that paclitaxel-resistant ovarian cancer cells overexpress class I, III, and IVa β-tubulin isotypes. Functionally, the presence of βIII-tubulin inhibits the assembly of β-tubulin subunits promoted by paclitaxel (8). In recent years, increased translational research in this area has provided substantial evidence that βIII-tubulin is overexpressed in various advanced tumors of patients treated with taxanes, suggesting that βIII-tubulin overexpression may be a common mechanism by which taxane resistance develops in patients with advanced malignancies (9, 10).

Epothilones are a novel class of microtubule inhibitor drugs that display antineoplastic activity by binding to and stabilizing microtubules (11). Ixabepilone is a novel analogue of epothilone B and has more potent antitumor activity than paclitaxel *in vitro* (12). Preclinical data indicate that ixabepilone may overcome taxane resistance by preferentially inhibiting the βIII-tubulin isotype (13). Ixabepilone has shown clinical activity in the treatment of anthracycline-resistant or pretreated and taxane-resistant metastatic breast cancer (MBC; refs. 14–16) and was recently approved in the United States for use in combination with capecitabine for the treatment of anthracycline- and taxane-resistant locally advanced or MBC and as monotherapy for the treatment of anthracycline-, taxane-, and capecitabine-resistant or refractory locally advanced or MBC.

Received 10/16/08; accepted 10/19/08.

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doi:10.1158/1535-7163.MCT-08-0986

This article will review emerging data on βIII-tubulin overexpression as an important mechanism of taxane resistance, its association with clinical outcomes, and the potential for ixabepilone as an emerging treatment strategy for overcoming taxane resistance associated with high βIII-tubulin expression.

Targeting the βIII-Tubulin Isotype in Microtubules

Microtubules are crucial for spindle formation during mitosis and for cellular proliferation. These dynamic components of the cytoskeleton are involved in the development and maintenance of cell shape, the transport of vesicles, mitochondria and other cellular components, and signaling. The microtubules are long, tube-shaped protein polymers composed of heterodimers of α-tubulin and β-tubulin. Microtubules display dynamic instability and treadmilling properties (17, 18). Dynamic instability is the switching of a microtubule between phases of growth and shortening, whereas treadmilling refers to the net growth of a microtubule at one end and shortening on the opposite end. Microtubule dynamics are critical to the proper alignment of chromosomes at the kinetochores, the movement of chromosomes during metaphase, and the segregation of chromosomes in anaphase and telophase (19–21).

Microtubule stability and dynamics can be affected by a variety of mechanisms, including cellular autoregulation of tubulin isotype expression, expression of mutated isotypes of tubulin, posttranslational modifications of tubulin, and altered levels of microtubule regulatory proteins (22). Owing to the critical functions of microtubules, particularly for cancer cells, drugs that target the stability and dynamic properties of microtubules have become an important class of chemotherapeutic agents. Inhibition of microtubule function impairs cell cycle progression by preventing proper chromosome capture, alignment, and segregation with subsequent cellular apoptosis (22, 23). Microtubule inhibitors interact with three major binding sites on the microtubules: the *Vinca* domain, the taxane site, and the colchicine site (22). Microtubule-destabilizing drugs include the *Vinca* alkaloids, estramustine, colchicine, and the combretastatins. Microtubule-stabilizing drugs include taxanes (paclitaxel, docetaxel, and albumin-bound paclitaxel) and epothilones, such as ixabepilone (22).

Six α-tubulin and seven β-tubulin isotypes have been identified (24). The isotypes of β-tubulin are differentially expressed in a variety of tissues and cell types (25, 26). In mammals, the βI and βIVb isotypes are constitutively expressed in all tissues, whereas classes III, IVa, and II are primarily expressed in the brain and neurons (27–29). Although expression of βV-tubulin was originally identified only in the chicken, the protein seems to be expressed at low levels in mammalian cells (30). Elevated expression of βV-tubulin has been shown to disrupt microtubule assembly and cause resistance to paclitaxel (30), suggesting that this protein may confer resistance to selected chemotherapeutic agents. However, the clinical significance of these findings is unknown. The βVI isotype is specifically expressed in blood cells and hematopoietic tissues (29, 31).

βIII-tubulin is different from the other isotypes in its amino acid sequence and posttranslational modifications, which may include phosphorylation and polyglutamylation (27). Therefore, the interaction of paclitaxel with βIII-tubulin is different from its interaction with other tubulin isotypes (8, 32).

Several groups have shown that high levels of βIII-tubulin are associated with taxane resistance in human cancer cell lines [lung cancer (29), ovarian cancer (7), prostate cancer (33), breast cancer (29), and pancreatic cancer (34)]. Expression of βIII-tubulin in transfected Chinese hamster ovary cells led to a dose-dependent reduction in microtubule formation and conferred resistance to paclitaxel (35). Although overexpression of βIII-tubulin did not affect the inherent dynamic stability of microtubules in Chinese hamster ovary cells, the effects of paclitaxel on microtubule dynamics were reduced in βIII-tubulin–overexpressing Chinese hamster ovary cells compared with those overexpressing βI-tubulin (32). The importance of βIII overexpression in tumors resistant to taxanes was further recognized in a study that used antisense oligodeoxynucleotides against the *Hβ4* gene, which encodes the βIII-tubulin protein (36). The antisense oligodeoxynucleotide treatment led to a 40% to 50% decrease in Hβ4 mRNA levels in human paclitaxel-resistant non–small cell lung cancer (NSCLC) cells (A549-T24). A similar trend was observed in protein levels. The decreased βIII-tubulin expression in antisense oligodeoxynucleotide-treated A549-T24 cells resulted in a 39% increase in the sensitivity to paclitaxel (36). βIII-tubulin may also play a role in resistance to other chemotherapeutic agents, such as DNA-targeting agents. The use of small interfering RNA to decrease the expression of βIII-tubulin in the human NSCLC cell lines, H460 and Calu6, resulted in increased sensitivity to cisplatin, doxorubicin, and VP-16 *in vitro* (37). Similarly, down-regulation of βIII-tubulin expression in H460 and Calu6 cells increased sensitivity to the microtubule inhibitors paclitaxel and vincristine (37).

Correlation of βIII-Tubulin Overexpression and Clinical Outcomes

An association between βIII-tubulin overexpression and worse prognosis has been reported in several human tumors, including advanced malignancies that are routinely treated with taxanes [e.g., breast cancer (38), lung cancer (9), and ovarian cancer (10, 39)] and malignancies that inherently respond poorly to taxanes [e.g., renal cell carcinoma (40)].

Breast Cancer

Overexpression of the βIII-tubulin isoform is associated with taxane resistance in breast cancer cell lines, and some clinical studies support a relationship between poor response to taxanes and high βIII-tubulin levels (38, 41, 42). Although the distribution of β-tubulin isotypes in breast cancer before therapy versus normal breast tissue

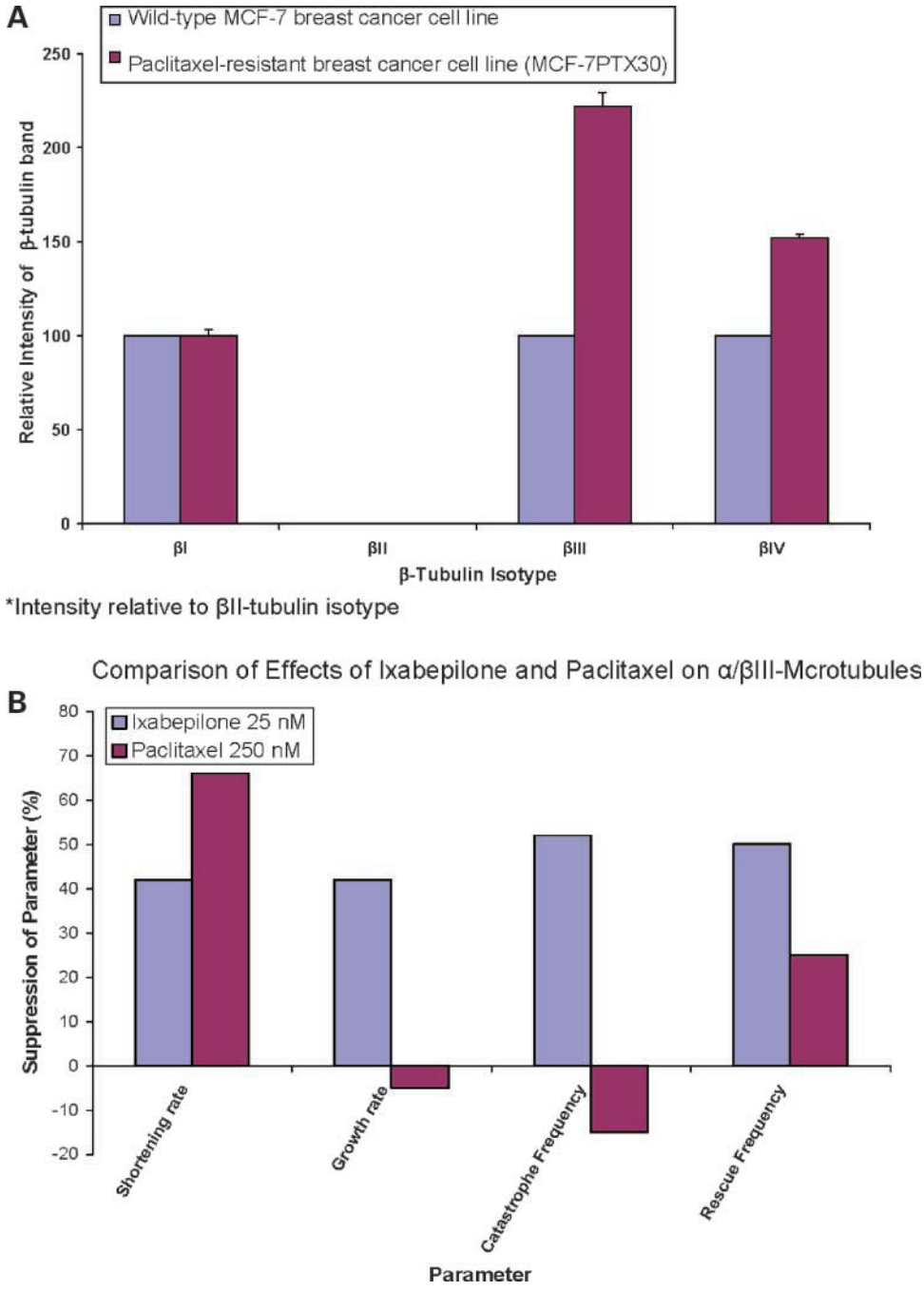

Figure 1. **A,** expression of β-tubulin isotypes in the MCF-7 breast cancer cell line and paclitaxel-resistant breast cancer cells, MCF-7^{PTX30}. All expression levels were set relative to the expression of βII-tubulin. Paclitaxel-resistant cells, MCF-7^{PTX30}, have a higher expression of βIII- and βIV-tubulin. Data adapted from Banerjee (44). **B,** comparison of the effects of ixabepilone and paclitaxel on the dynamics of α/βIII-microtubules. Ixabepilone (25 nmol/L) preferentially suppressed the dynamic instability of α/βIII-microtubules, whereas paclitaxel (250 nmol/L) had little effect on the growth rate and catastrophe frequency of α/βIII-microtubules. Adapted from Jordan (13).

shows no substantial differences (43), the distribution of β-tubulin isotypes may be affected by therapy. For example, analysis of wild-type and paclitaxel-resistant MCF-7 breast cancer cells showed no difference in βI-tubulin and no expression of βII-tubulin in either cell line, but did show a 2.5-fold increase in βIII-tubulin and a 1.5-fold increase in βIV-tubulin in the paclitaxel-resistant MCF-7 cells compared with wild-type MCF-7 cells (Fig. 1A; ref. 44). In another study, βIII-tubulin expression was increased 2.9-fold in docetaxel-exposed MCF-7 cells compared with the parental cell line (45). *In vitro*, altered expression of β-tubulin isotypes is associated with acquired docetaxel resistance (46).

Clinically, βIII-tubulin overexpression may be associated with more aggressive disease. Backus et al. examined 83 cases of breast cancer by immunohistochemistry for βIII-tubulin expression. Tumors were graded according to the Nottingham score. βIII-tubulin staining was scored for

intensity (scale, 0–3) and proportion of cells stained (scale, 0–5). The authors found that pathologic grade 3 tumors had a mean βIII-tubulin score of 4.88 versus 0.73 for pathologic grade 1 tumors ($P < 0.01$; ref. 47).

Expression of tubulin isotypes has been analyzed in breast cancer specimens from patients receiving taxanes (38, 48). In a small study of 41 patients with breast cancer who received docetaxel chemotherapy, class II, III, and IV β-tubulin isotypes were expressed in 56%, 65%, and 82% of samples, respectively. Of the 41 patients, 22 (54%) had a partial response to docetaxel. The authors concluded that no clear association was observed between response to docetaxel and the level of expression of βIII-tubulin (48). Paradiso et al. explored tumor biomarkers predictive of paclitaxel sensitivity in 72 patients with advanced breast cancer. βIII-tubulin was expressed in 84% of tumors obtained from the 70 patients analyzed. Approximately 59% and 41% of patients had low and high levels of βIII-tubulin, respectively. Only 2% of the patients with low βIII-tubulin expression had disease progression while receiving paclitaxel versus 38% of those with high βIII-tubulin expression ($P = 0.000$ by χ^2). Univariate analysis showed that βIII-tubulin was the only factor found to be predictive of response to paclitaxel (38). In another study of 92 patients treated with first-line paclitaxel-containing chemotherapy, 35% of the patients with tumors that expressed high βIII-tubulin experienced disease progression after paclitaxel versus 7% of patients with tumors expressing low levels of βIII-tubulin ($P < 0.002$; ref. 49). These data suggest that βIII-tubulin immunohistochemical expression analysis could assist in predicting response to paclitaxel in patients with advanced breast cancer.

Non–Small Cell Lung Cancer

High expression of βIII-tubulin has been associated with a lower probability of response to taxane- or vinorelbine-containing regimens and with a worse prognosis in NSCLC

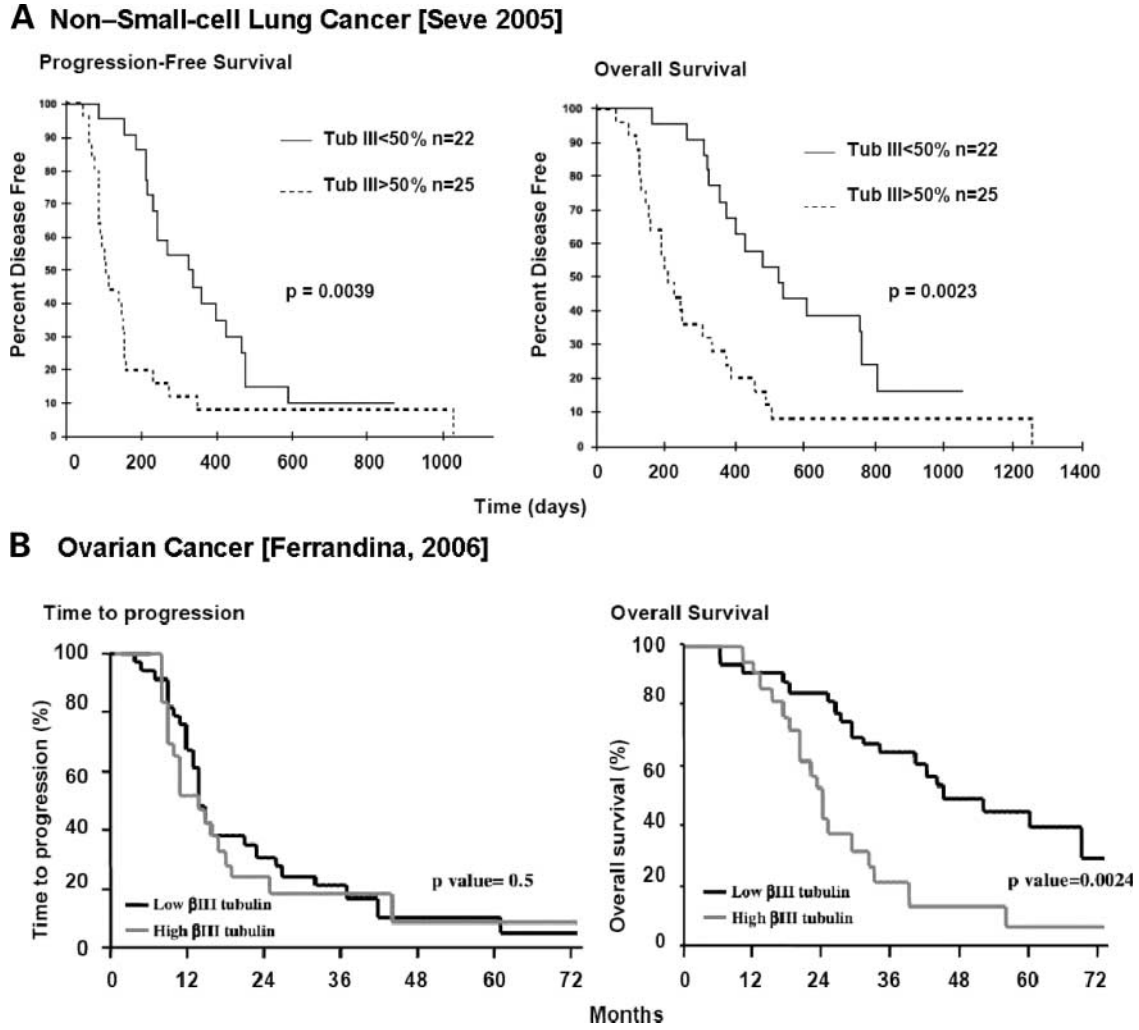

Figure 2. Correlation of βIII-tubulin expression with worse progression-free survival and overall survival in NSCLC (**A**) and ovarian cancer (**B**; refs. 9, 13). Reprinted with permission.

patients (42, 50, 51). Among 19 patients with NSCLC receiving taxane-based regimens, all patients were positive for βI-tubulin; βII- and βIII-tubulin were expressed in 83% and 89% of patients, respectively (50). The patients whose tumors overexpressed βIII-tubulin had a median progression-free survival of 41 days compared with 288 days for patients with lower levels of βIII-tubulin ($P = 0.02$). These data suggest that overexpression of βIII-tubulin in NSCLC may be associated with worse prognosis in patients receiving taxane-based chemotherapy.

It is often difficult to discriminate whether the prognostic value of a tumor marker resides in its ability to predict response to a specific therapy, or as a marker of intrinsic tumor aggressiveness independent of the type of therapy. Seve et al. addressed this question by correlating the expression of βIII-tubulin in tumors of NSCLC patients with patient outcome. Patients were treated with paclitaxel-based regimens ($n = 47$) or gemcitabine-based regimens ($n = 44$; ref. 9). Among patients receiving paclitaxel, those whose tumors expressed low levels of βIII-tubulin had significantly better response rate ($P < 0.001$), longer progression-free survival ($P < 0.004$), and better overall survival ($P < 0.002$). This variable was not predictive in patients receiving gemcitabine-based chemotherapy (Fig. 2A). Multivariate analysis confirmed that low levels of βIII-tubulin expression independently correlated with progression-free survival ($P = 0.003$) and overall survival ($P = 0.003$). These results suggest that expression level of βIII-tubulin is predictive of response to therapy and overall clinical outcome in patients with NSCLC receiving paclitaxel-containing regimens (9).

In another study, Okuda et al. investigated the expression levels of βIII-tubulin in patients with NSCLC who had undergone complete resection. Among 50 patients who received platinum and paclitaxel, those with tumors negative for βIII-tubulin ($n = 23$) had a better prognosis, with significantly longer survival ($P = 0.03$) than the βIII-tubulin–positive patients ($n = 27$; ref. 52). Univariate analysis showed that βIII-tubulin expression was the only statistically significant prognostic factor ($P = 0.03$) associated with overall survival of NSCLC patients treated with platinum and paclitaxel. Other factors analyzed included smoking status, pathologic stage, epidermal growth factor receptor mutations, and expression of excision repair cross-complementation group 1 (ERCC1). Pathologic stage and ERCC1 expression were independent prognostic factors for overall survival of patients receiving platinum-containing regimens (52).

Ovarian Cancer

Clinical response rates of ~80% are achieved with first-line taxane-containing regimens in women with advanced ovarian cancer, but the majority of these patients relapse and do not respond successfully to further treatment. A direct comparison of three known mechanisms of paclitaxel resistance showed that overexpression of class III β-tubulin is predominant in patients with ovarian cancer versus P-glycoprotein overexpression or βIII-tubulin mutation. A significant increase in the expression of the βIII-tubulin isotype was detected at both the mRNA and protein expression levels in paclitaxel-resistant versus paclitaxel-sensitive tumors ($P < 0.001$; ref. 10).

Ferrandina et al. (39) specifically addressed the role of βIII-tubulin overexpression in predicting clinical outcome in ovarian carcinoma. Among patients with unresectable ovarian cancer treated with taxane-containing chemotherapy, those with tumors with high levels of βIII-tubulin experienced a shorter median overall survival compared with those with a low βIII-tubulin content (25 versus 46 months; $P = 0.002$; Fig. 2B; ref. 39). Multivariate analysis showed that high βIII-tubulin content was independently associated with worse prognosis. This study did not detect a significant correlation with response to treatment or time to progression, suggesting that overexpression of βIII-tubulin might indicate intrinsic biological aggressiveness of the tumor rather than predict drug resistance in this population (39).

The expression of βIII-tubulin in four different histologic types of epithelial ovarian cancer was examined in a study involving 80 chemonaive patients (53). The histotypes were serous carcinoma ($n = 24$), mucinous carcinoma ($n = 10$), endometrioid carcinoma ($n = 11$), and clear cell carcinoma ($n = 35$). Immunohistochemistry detected higher βIII-tubulin in clear cell (86%) and mucinous (80%) carcinomas compared with endometrioid (45%) and serous (21%) carcinomas (53). Patients with measurable lesions after the first operation ($n = 19$) were treated with taxane-based regimens. Of these 19 patients, those with high βIII-tubulin levels ($n = 10$) had no response to chemotherapy. Those with low levels of βIII-tubulin ($n = 9$) had an overall response rate of 56% (53).

In a recent study, Raspaglio et al. showed that hypoxia increases the expression of βIII-tubulin in A2780 human ovarian cancer cells and confers resistance to paclitaxel. An element within the 3′ flanking region of the *βIII-tubulin* gene mediated hypoxia-induced increase in βIII-tubulin expression through a hypoxia-inducible factor-1α–dependent mechanism (54), whereas methylation of this 3′ hypoxia-inducible factor-1α–binding element abrogated induction (54). These data suggest that βIII-tubulin may play a prominent role in resistance to chemotherapy also in other tumor types, where the hypoxic tumor microenvironment *in vivo* may lead to sustained overexpression of the protein such as in lung cancer.

Melanoma and Renal Cell Carcinoma

Mhaidat et al. (55) examined the role of βIII-tubulin in resistance to docetaxel in melanoma cell lines. Docetaxel-resistant cell lines were shown to express higher levels of βIII-tubulin, which was incorporated into microtubules. Abrogation of βIII-tubulin expression using small interfering RNA sensitized melanoma cells to docetaxel-induced apoptosis, confirming the functional role of the protein in acquisition of the taxane-resistant phenotype (55).

The presence of mutations in the βI-tubulin and the distribution of βI and βIII isotypes were studied in patients with renal cell cancer and healthy subjects (40). There was an increase in the expression of the βI-tubulin isotype in

patients with renal cell cancer, but no specific mutations were found. In cell lines, there was no correlation between expression of βI-tubulin and drug sensitivity, but a significant correlation between class III β-tubulin expression and vinblastine sensitivity was observed (40). Investigation of βIII-tubulin expression as it relates to prognosis in patients with renal cell carcinoma is warranted.

Antitumor Activity of Epothilones—Mechanisms of Action

Despite an overall lack of structural similarity, molecular modeling studies suggest that the epothilones and taxanes may have a common binding site on microtubules, and *in vitro* studies show competitive inhibition between epothilone B and the taxanes (11, 56). Considering the common binding site of tubulin for epothilones and taxanes, they may be expected to exhibit similar resistance profiles. There are, however, significant differences in the mechanism of action that enable epothilones to overcome taxane resistance (12, 13, 57).

The antitumor activity of ixabepilone is mediated by mitotic arrest of cells at G_2-M (12) followed by p53- and BAX-dependent apoptosis (58). In contrast, the antitumor effects of paclitaxel, assessed as absolute growth delay, correlated with paclitaxel-induced apoptosis ($P = 0.001$) but not with mitotic arrest ($P = 0.124$; ref. 59). In MCF-7 breast cancer cells, the effects of epothilone B and paclitaxel on microtubule dynamics were found to be similar, particularly inhibition of microtubule growth rate, growth length, shortening rate, shortening length, and overall dynamicity (60).

In preclinical studies, ixabepilone activity is not substantially affected by overexpression of P-glycoprotein or mutations in β-tubulin, both of which have been linked to resistance to taxanes, although tubulin mutations do not seem to be relevant to clinical resistance (12). The effects of epothilone A, epothilone B, and paclitaxel were compared in various paclitaxel-sensitive and paclitaxel-resistant cell lines, including SW620 colon carcinoma cells, SW620AD-300 cells resistant to paclitaxel due to overexpression of P-glycoprotein, 1A9 ovarian cancer cells, and 1A9 (PTX22) ovarian cancer cells resistant to paclitaxel due to expression of a modified tubulin (57). In both parental cell lines, epothilone B was more efficacious than either epothilone A or paclitaxel. Furthermore, the epothilones exhibited significant activity in the paclitaxel-resistant cell lines despite the presence of P-glycoprotein or tubulin mutations (57).

Ixabepilone in βIII-Tubulin–Overexpressing Cells

Although tumor resistance to certain microtubule inhibitors may be related to the overexpression of βIII-tubulin, it does not seem to affect sensitivity to ixabepilone. Ixabepilone suppressed the dynamic instability of purified α/βIII-microtubules, whereas paclitaxel was less potent in suppressing the growth rate and catastrophe frequency of purified α/βIII-microtubules (Fig. 1B; ref. 13).

Pat-21 cells are a paclitaxel-resistant breast cancer model developed from a patient with breast cancer who did not respond to a treatment regimen consisting of paclitaxel and dexverapamil (multidrug resistance reversal agent). Before receiving this regimen, the patient had been treated with doxorubicin, cyclophosphamide, methotrexate, and 5-fluorouracil (13). Pat-21 tumor cells do not overexpress the efflux transporters P-glycoprotein or multidrug resistance protein 1. However, Western blot analyses revealed a high βIII-tubulin expression level and relatively little expression of βII and βV.[4] Despite the prominent expression of βIII-tubulin, ixabepilone showed significant activity in Pat-21 cells *in vitro* and *in vivo* (13). Figure 3 shows the superior efficacy of ixabepilone in this resistant breast cancer model versus paclitaxel, docetaxel, and vinorelbine (61).

Other βIII-tubulin–overexpressing cell lines, including H1155 (human lung cancer), LX-1 (human lung cancer), and DU4475 (human breast cancer), were also sensitive to ixabepilone *in vitro* and *in vivo* (61). For all βIII-tubulin–overexpressing cell lines tested, the log cell kill values for ixabepilone (1.6–4.2) were higher than those for docetaxel and vinorelbine (0.1–0.9; Fig. 3B). Docetaxel and vinorelbine were ineffective in these cell lines (61).

Ixabepilone in Taxane-Resistant Breast Cancer

Two dosing schedules of ixabepilone have been evaluated for the treatment of MBC: 40 mg/m^2 once every 3 weeks (14, 15, 62) and 6 mg/m^2/d on days 1 to 5 every 3 weeks (63, 64). Ixabepilone has shown clinical efficacy and acceptable tolerability, as monotherapy and in combination with capecitabine, in patients with MBC, including those with disease resistant to anthracyclines, taxanes, and/or capecitabine (14–16, 62–65). In phase II clinical trials, response rates ranged from 57% in patients with MBC previously untreated with taxanes (62) to 11.5% in patients with anthracycline-, taxane-, and capecitabine-resistant disease (14). Resistance was defined as disease progression during treatment for metastatic disease or recurrence ≤6 months of adjuvant or neoadjuvant therapy.

In a phase III trial in patients who had progressed after anthracycline- and taxane-based therapy, the combination of ixabepilone plus capecitabine significantly increased median progression-free survival relative to capecitabine monotherapy (5.8 versus 4.2 months, respectively), with a 25% decrease in the risk of disease progression (hazard ratio, 0.75; 95% confidence interval, 0.64–0.88; $P = 0.0003$; ref. 16). Tumor biopsies from patients in the phase III trial were analyzed by immunohistochemistry for βIII-tubulin expression. Figure 3 shows images from patients with negligible, moderate, and high levels of βIII-tubulin (61). The combination of ixabepilone plus capecitabine was well tolerated with manageable and reversible toxicities consistent with the safety profile of each drug. The most frequently occurring grade 3/4 adverse events observed

[4] Weinberg, Jordan, Himes, and Wilson, unpublished data.

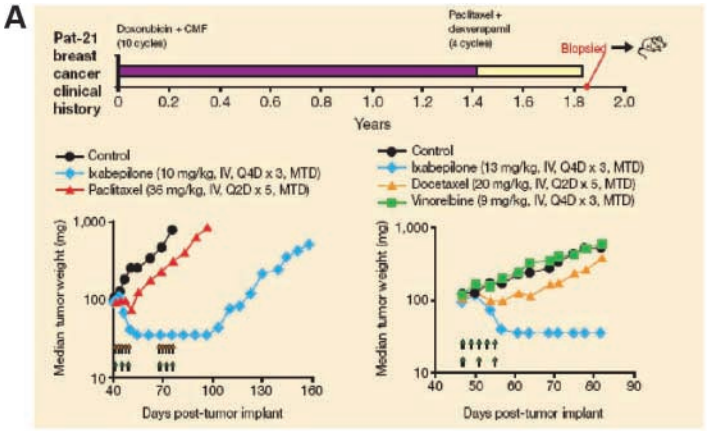

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Table 1. Antitumor Activity of Microtubule Inhibitors in βIII-tubulin Overexpressing Human Tumor Xenografts

Tumors	Antitumor Activity (log cell kill)		
	Ixabepilone	Docetaxel	Vinorelbine
Breast			
DU4475	2.6	0.9	0.2
Pat-21	1.6	0.3	0.3
Lung			
H1155	4.2	0.2	0.1
LX-1	2.6	0.5	0.1

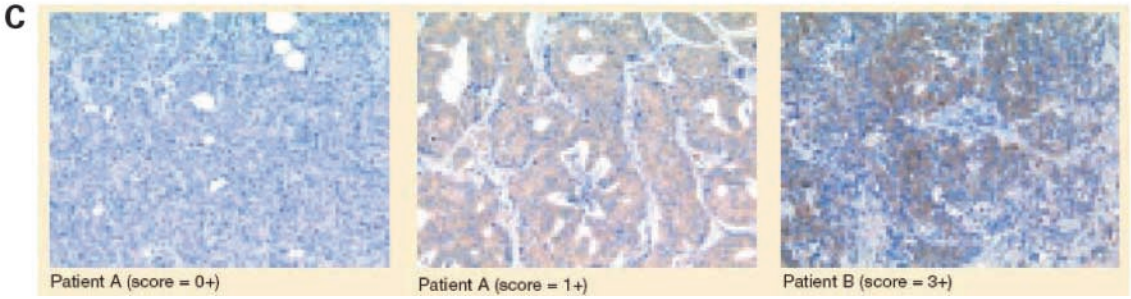

Figure 3. **A,** description of the Pat-21 breast cancer model, in which ixabepilone shows superior activity to paclitaxel, docetaxel, and vinorelbine (13). **B,** log cell kill activity of ixabepilone in the βIII-tubulin – overexpressing tumor xenografts (59). **C,** βIII-tubulin expression in patients with MBC. Scoring was based on a 0 to 3 scale, where 0 = no, 1 = light, 2 = moderate, and 3 = strong staining. Samples with <10% staining received a score of 0 (59).

with the combination were neutropenia (67.5%), peripheral sensory neuropathy (21.1%), hand-foot syndrome (18.1%), fatigue (8.9%), myalgia (7.9%), and arthralgia (2.7%). Of note, the combination therapy achieved response rates of 33% versus 14% with capecitabine monotherapy in patients with primary resistance to taxanes, defined as progressive disease as best response to taxane-based chemotherapy (16).

Conclusions

Microtubule-inhibiting agents, such as the taxanes, are an important class of chemotherapy agents that are used to treat numerous types of tumors. Unfortunately, the majority of patients develop resistance over time, limiting the continued use of this class of chemotherapeutic agents. Although many potential mechanisms may be involved, overexpression of the βIII-tubulin isotype is emerging as a critical event in the development of resistance to paclitaxel and docetaxel in a variety of tumor types. βIII-tubulin overexpression may also be a characteristic of more aggressive or difficult-to-treat disease. The antitumor activity of ixabepilone in taxane-resistant cancers may be correlated with its effective suppression of the dynamic instability of α/βIII-microtubules, as ixabepilone has shown substantial activity in βIII-tubulin–overexpressing cells *in vitro* and in human xenograft models. Studies are ongoing to determine whether ixabepilone activity is truly independent of βIII-tubulin expression. The clinical efficacy of ixabepilone in patients with taxane-resistant advanced breast cancer supports the unique activity of this microtubule inhibitor compared with other chemotherapeutic agents. Based on its clinical profile, ixabepilone has been approved in the United States as monotherapy and in combination with capecitabine for the treatment of MBC resistant to anthracyclines, taxanes, and/or capecitabine.

Disclosure of Potential Conflicts of Interest

F.F.Y. Lee: employee of Bristol-Myers Squibb Pharmaceutical Research Institute. No other potential conflicts of interest were disclosed.

References

1. Horwitz SB, Lothstien L, Manfredi JJ, et al. Taxol: mechanisms of action and resistance. Ann N Y Acad Sci 1986;466:733 – 44.

2. Giannakakou P, Sackett DL, Kang YK, et al. Paclitaxel-resistant human ovarian cancer cells have mutant β-tubulins that exhibit impaired paclitaxel-driven polymerization. J Biol Chem 1997;272:17118 – 25.

3. Monzo M, Rosell R, Sanchez JJ, et al. Paclitaxel resistance in non-small-cell lung cancer associated with β-tubulin gene mutations. J Clin Oncol 1999;17:1786 – 93.

4. Tsurutani J, Komiya T, Uejima H, et al. Mutational analysis of the β-tubulin gene in lung cancer. Lung Cancer 2002;35:11 – 6.

5. Šale S, Sung R, Shen P, et al. Conservation of the class I β-tubulin gene in human populations and lack of mutations in lung cancer and paclitaxel-resistant ovarian cancers. Mol Cancer Ther 2002;1:215 – 25.

6. Lamendola DE, Duan Z, Penson RT, Oliva E, Seiden MV. β Tubulin mutations are rare in human ovarian carcinoma. Anticancer Res 2003;23:681 – 6.

7. Kavallaris M, Kuo DYS, Burkhart CA, et al. Taxol-resistant epithelial ovarian tumors are associated with altered expression of specific β-tubulin isotypes. J Clin Invest 1997;100:1282 – 93.

8. Lu Q, Luduena RF. Removal of β-III isotype enhances taxol induced microtubule assembly. Cell Struct Funct 1993;18:173 – 82.

9. Seve P, Mackey J, Isaac S, et al. Class III β-tubulin expression in tumor cells predicts response and outcome of patients with non-small cell lung cancer receiving paclitaxel. Mol Cancer Ther 2005;4:2001 – 7.

10. Mozzetti S, Ferlini C, Concolino P, et al. Class III β-tubulin overexpression is a prominent mechanism of paclitaxel resistance in ovarian cancer patients. Clin Cancer Res 2005;11:298 – 305.

11. Bollag DM, McQueney PA, Zhu J, et al. Epothilones, a new class of microtubule-stabilizing agents with a taxol-like mechanism of action. Cancer Res 1995;55:2325 – 33.

12. Lee FY, Borzilleri R, Fairchild CR, et al. BMS-247550: a novel epothilone analog with a mode of action similar to paclitaxel but possessing superior antitumor efficacy. Clin Cancer Res 2001;7: 1429 – 37.

13. Jordan MA, Miller H, Ray A, et al. The Pat-21 breast cancer model derived from a patient with primary Taxol resistance recapitulates the phenotype of its origin has altered β-tubulin expression and is sensitive to ixabepilone. Presented at the 97th AACR Annual Meeting, April 1 – 5, 2006, Washington, District of Columbia, Late breaking poster session, Abstract LB-280.

14. Perez EA, Lerzo G, Pivot X, et al. Efficacy and safety of ixabepilone (BMS-247550) in a phase II study of patients with advanced breast cancer resistant to an anthracycline, a taxane, and capecitabine. J Clin Oncol 2007;25:3407 – 14.

15. Thomas E, Tabernero J, Fornier M, et al. Phase II clinical trial of ixabepilone (BMS-247550), an epothilone B analog, in patients with taxane-resistant metastatic breast cancer. J Clin Oncol 2007;25: 3399 – 406.

16. Thomas ES, Gomez HL, Li RK, et al. Ixabepilone plus capecitabine for metastatic breast cancer progressing after anthracycline and taxane treatment. J Clin Oncol 2007;25:5210 – 7.

17. Mitchison T, Kirschner M. Dynamic instability of microtubule growth. Nature 1984;312:237 – 42.

18. Rodionov VI, Borisy GG. Microtubule treadmilling *in vivo*. Science 1997;275:215 – 8.

19. Rusan NM, Fagerstrom CJ, Yvon AM, Wadsworth P. Cell cycle-dependent changes in microtubule dynamics in living cells expressing green fluorescent protein-α tubulin. Mol Biol Cell 2001;12:971 – 80.

20. Saxton WM, Stemple DL, Leslie RJ, Salmon ED, Zavortink M, McIntosh JR. Tubulin dynamics in cultured mammalian cells. J Cell Biol 1984;99:2175 – 86.

21. Zhai Y, Kronebusch PJ, Simon PM, Borisy GG. Microtubule dynamics at the G_2/M transition: abrupt breakdown of cytoplasmic microtubules at nuclear envelope breakdown and implications for spindle morphogenesis. J Cell Biol 1996;135:201 – 14.

22. Jordan MA, Wilson L. Microtubules as a target for anticancer drugs. Nat Rev Cancer 2004;4:253 – 65.

23. Jordan MA, Wendell K, Gardiner S, Derry WB, Copp H, Wilson L. Mitotic block induced in HeLa cells by low concentrations of paclitaxel (Taxol) results in abnormal mitotic exit and apoptotic cell death. Cancer Res 1996;56:816 – 25.

24. Verdier-Pinard P, Wang F, Martello L, Burd B, Orr GA, Horwitz SB. Analysis of tubulin isotypes and mutations from taxol-resistant cells by combined isoelectrofocusing and mass spectrometry. Biochemistry 2003; 42:5349 – 57.

25. Roach MC, Boucher VL, Walss C, Ravdin PM, Luduena RF. Preparation of a monoclonal antibody specific for the class I isotype of β-tubulin: the β isotypes of tubulin differ in their cellular distributions within human tissues. Cell Motil Cytoskeleton 1998;39:273 – 85.

26. Dumontet C, Sikic BI. Mechanisms of action of and resistance to antitubulin agents: microtubule dynamics, drug transport, and cell death. J Clin Oncol 1999;17:1061 – 70.

27. Katsetos CD, Legido A, Perentes E, Mörk SJ. Class III β-tubulin isotype: a key cytoskeletal protein at the crossroads of developmental neurobiology and tumor neuropathology. J Child Neurol 2003;18: 851 – 66.

28. Lewis SA, Lee MG, Cowan NJ. Five mouse tubulin isotypes and their regulated expression during development. J Cell Biol 1985;101:852 – 61.

29. Burkhart CA, Kavallaris M, Horwitz SB. The role of β-tubulin isotypes in resistance to antimitotic drugs. Biochim Biophys Acta 2001;1471:1 – 9.

30. Bhattacharya R, Cabral F. A ubiquitous β-tubulin disrupts microtubule assembly and inhibits cell proliferation. Mol Biol Cell 2004;15:3123 – 31.

31. Wang D, Villasante A, Lewis SA, Cowan NJ. The mammalian β-tubulin repertoire: hematopoietic expression of a novel, heterologous β-tubulin isotype. J Cell Biol 1986;103:1903 – 10.

32. Kamath K, Wilson L, Cabral F, Jordan MA. βIII-tubulin induces paclitaxel resistance in association with reduced effects on microtubule dynamic instability. J Biol Chem 2005;280:12902 – 7.

33. Ranganathan S, Benetatos CA, Colarusso PJ, Dexter DW, Hudes GR. Altered β-tubulin isotype expression in paclitaxel-resistant human prostate carcinoma cells. Br J Cancer 1998;77:562 – 6.

34. Liu B, Staren ED, Iwamura, T, Appert H, Howard J. Mechanisms of taxotere-related drug resistance in pancreatic carcinoma. J Surg Res 2001;99:179 – 86.

35. Hari M, Yang H, Zeng C, Canizales M, Cabral F. Expression of class III β-tubulin reduces microtubule assembly and confers resistance to paclitaxel. Cell Motil Cytoskeleton 2003;56:45 – 56.

36. Kavallaris M, Burkhart CA, Horwitz SB. Antisense oligonucleotides to class III β-tubulin sensitize drug-resistant cells to Taxol. Br J Cancer 1999; 80:1020 – 5.

37. Gan PP, Pasquier E, Kavallaris M. Class III β tubulin mediates sensitivity to chemotherapeutic drugs in non small cell lung cancer. Cancer Res 2007;67:9356 – 63.

38. Paradiso A, Mangia A, Chiratti A, et al. Biomarkers predicative for clinical efficacy of taxol-based chemotherapy in advanced breast cancer. Ann Oncol 2005;16:iv14 – 9.

39. Ferrandina G, Zannoni GF, Martinelli E, et al. Class III β-tubulin overexpression is a marker of poor clinical outcome in advanced ovarian cancer patients. Clin Cancer Res 2006;12:2774 – 9.

40. Ferguson RE, Taylor C, Stanley A, et al. Resistance to the tubulin-binding agents in renal cell carcinoma: no mutations in the class I β-tubulin gene but changes in tubulin isotype protein expression. Clin Cancer Res 2005;11:3439 – 45.

41. Hasegawa S, Miyoshi Y, Egawa C, et al. Prediction of response to docetaxel by quantitative analysis of class I and III β-tubulin isotype mRNA expression in human breast cancers. Clin Cancer Res 2003;9:2992 – 7.

42. Seve P, Dumontet C. Is class III β-tubulin a predictive factor in patients receiving tubulin-binding agents? Lancet Oncol 2008;9:168 – 75.

43. Dozier JH, Hiser L, Davis JA, et al. β Class II tubulin predominates in normal and tumor breast tissues. Breast Cancer Res 2003;5:R157 – 69.

44. Banerjee A. Increased levels of tyrosinated α-, β(III)-, and β(IV)-tubulin isotypes in paclitaxel-resistant MCF-7 breast cancer cells. Biochem Biophys Res Commun 2002;293:598 – 601.

45. Shalli K, Brown I, Heys SD, Schofield AC. Alterations of β-tubulin isotypes in breast cancer cells resistant to docetaxel. FASEB J 2005;19: 1299 – 301.

46. Noguchi S. Predictive factors for response to docetaxel in human breast cancers. Cancer Sci 2006;97:813 – 20.

47. Backus SR, Blankenship J, Luduena R, Yeh I-T. Expression of β III tubulin in breast cancer: correlation with tumor grade. FASEB J 2007;21: 708.4.

48. Bernard-Marty C, Terilleux I, Dumontet C, et al. Microtubule-associated parameters as predictive markers of docetaxel activity in advanced breast cancer patients: results of a pilot study. Clin Breast Cancer 2002;3:341 – 5.

49. Tommasi S, Mangia A, Lacalamita R, et al. Cytoskeleton and paclitaxel sensitivity in breast cancer: the role of β-tubulins. Int J Cancer 2007;120:2078 – 85.

50. Dumontet C, Isaac S, Souquet PJ, et al. Expression of class III β tubulin in non-small cell lung cancer is correlated with resistance to taxane chemotherapy. Bull Cancer 2005;92:E25 – 30.

51. Seve P, Isaac S, Tredon O, et al. Expression of class III βIII-tubulin is predictive of patient outcome in patients with non-small cell lung cancer receiving vinorelbine-based chemotherapy. Clin Cancer Res 2005;11: 5481 – 6.

52. Okuda K, Sasaki H, Dumontet C, et al. Expression of excision repair cross-complementation group 1 and class III β-tubulin predict survival after chemotherapy for completely resected non-small cell lung cancer. Lung Cancer 2008;62:105 – 12.

53. Umezu T, Shibata K, Kajiyama H, et al. Taxol resistance among the different histological subtypes of ovarian cancer may be associated with the expression of class III β-tubulin. Int J Gynecol Pathol 2008;27: 207 – 12.

54. Raspaglio G, Filippetti F, Prislei S, et al. Hypoxia induces class III β-tubulin gene expression by HIF-1α binding to its 3′ flanking region. Gene 2008;409:100 – 8.

55. Mhaidat NM, Thorneb RF, de Bock CE, Zhang XD, Hersey P. Melanoma cell sensitivity to docetaxel-induced apoptosis is determined by class III β-tubulin levels. FEBS Lett 2008;582:267 – 72.

56. Giannakakou P, Gussio R, Nogales E, et al. A common pharmacophore for epothilone and taxanes: molecular basis for drug resistance conferred by tubulin mutations in human cancer cells. Proc Natl Acad Sci U S A 2000;97:2904 – 9.

57. Kowalski RJ, Giannakakou P, Hamel E. Activities of the microtubule-stabilizing agents epothilones A and B with purified tubulin and in cells resistant to paclitaxel (Taxol®). J Biol Chem 1997;272: 2534 – 41.

58. Yamaguchi H, Chen J, Bhalla K, Wang HG. Regulation of Bax activation and apoptotic response to microtubule-damaging agents by p53 transcription-dependent and -independent pathways. J Biol Chem 2004; 279:39431 – 7.

59. Milross CG, Mason KA, Hunter NR, Chung WK, Peters LJ, Milas L. Relationship of mitotic arrest and apoptosis to antitumor effect of paclitaxel. J Natl Cancer Inst 1996;88:1308 – 14.

60. Kamath K, Jordan MA. Suppression of microtubule dynamics by epothilone B is associated with mitotic arrest. Cancer Res 2003;63: 6026 – 31.

61. Lee FYF, Wen M-L, Shen H, et al. Ixabepilone overcomes multiple mechanisms of drug resistance including overexpression of class III β-tubulin and breast cancer resistance protein. Presented at the 6th European Breast Cancer Conference, April 2008, Berlin, Germany.

62. Roché H, Yelle L, Cognetti F, et al. Phase II clinical trial of ixabepilone (BMS-247550), an epothilone B analog, as first-line therapy in patients with metastatic breast cancer previously treated with anthracycline chemotherapy. J Clin Oncol 2007;25:3415 – 20.

63. Low JA, Wedam SB, Lee JJ, et al. Phase II clinical trial of ixabepilone (BMS-247550), an epothilone B analog, in metastatic and locally advanced breast cancer. J Clin Oncol 2005;23:2726 – 34.

64. Denduluri N, Low JA, Lee JJ, et al. Phase II trial of ixabepilone, an epothilone B analog, in patients with metastatic breast cancer previously untreated with taxanes. J Clin Oncol 2007;25:3421 – 7.

65. Bunnell C, Klimovsky J, Thomas E. Final efficacy results of a phase 1/2 trial of ixabepilone in combination with capecitabine in patients with metastatic breast cancer (MBC) previously treated with a taxane and an anthracycline [abstract 10511]. J Clin Oncol 2006;24.