

## Microtubule inhibitors: Differentiating tubulin-inhibiting agents based on mechanisms of action, clinical activity, and resistance

Edith A. Perez

Division of Hematology and Oncology, Mayo Clinic,  
Jacksonville, Florida

### Abstract

Microtubules are important cellular targets for anticancer therapy because of their key role in mitosis. Microtubule inhibitors (MTI) such as taxanes, vinca alkaloids, and epothilones stabilize or destabilize microtubules, thereby suppressing microtubule dynamics required for proper mitotic function, effectively blocking cell cycle progression and resulting in apoptosis. In spite of their antitumor activity, innate or acquired drug resistance to MTIs such as the taxanes is common, limiting their overall clinical efficacy. Further insight into the mechanisms of action of microtubule-targeting drugs has led to the discovery of novel agents that may provide higher efficacy with limited toxicity and help overcome resistance to conventional MTIs. This review will focus on the different mechanisms of action of MTIs, potential factors related to resistance and tolerability, and will discuss the recent approval as well as the development of new antineoplastic agents. [Mol Cancer Ther 2009;8(8):2086–95]

### Introduction

Microtubules are components of the cytoskeleton with important roles in a variety of cellular functions such as intracellular transport, maintenance of cell shape, polarity, cell signaling, and mitosis (1). During mitosis, microtubules form the mitotic spindle that transports daughter chromosomes to separate poles of the dividing cell. The important role of microtubules in cell division makes them a desirable target for the development of chemotherapeutic agents directed against rapidly dividing cancer cells. Microtubule inhibitors (MTI) include agents such as taxanes, vinca alkaloids, and epothilones that are used against many solid and hematologic malignancies. Although MTIs have significant clinical activity in multiple tumor types, their effectiveness

is reduced by drug resistance mechanisms. As a result, there is an ongoing effort to develop new agents within this class to improve efficacy and circumvent drug resistance. The development of new MTIs would benefit from a better understanding of microtubule structure, dynamics, and the different mechanisms of action of currently available agents.

### Microtubule Structure

Microtubules are noncovalent polymers of  $\alpha$ - and  $\beta$ -tubulin heterodimers assembled in a filamentous tube-shaped structure. The two 50-kDa subunits ( $\alpha$ - and  $\beta$ -tubulin) are ~50% identical to one another (2) and polymerize by nucleation-elongation, in which noncovalent tubulin dimers are added at the ends of a short microtubule “nucleus” (3). In the polymerization stage, the tubulin heterodimers lay head to tail, with the  $\alpha$ -subunit of one dimer in contact with the  $\beta$ -subunit of the next. The resulting protofilaments comprise the backbone of the hollow, cylindrical microtubule that is approximately 25 nm in diameter. The microtubule consists of the parallel arrangement of 13 protofilaments in an imperfect helix (3). The head-to-tail order of the  $\alpha/\beta$ -tubulin dimers confers polarity on the microtubule with one end ringed with  $\alpha$ -tubulin, and the other end ringed with  $\beta$ -tubulin. These are considered the (–) and (+) ends, respectively (3). The microtubule-organizing center (MTOC) is a network of microtubule-associated proteins (MAP) to which the microtubules are attached. The (–) ends of microtubules are anchored to the MTOC, whereas the (+) ends are distal (1). Microtubules exist in a dynamic state, growing and shortening by the reversible association and dissociation of  $\alpha/\beta$ -tubulin heterodimers at both ends. The  $\alpha$ -tubulin ringed (–) end is less dynamic, whereas the more dynamic  $\beta$ -tubulin ringed (+) end grows and shortens more rapidly (2).

Each subunit has a guanosine triphosphate (GTP) binding site; termed the nonexchangeable site in  $\alpha$ -tubulin, and the exchangeable site in  $\beta$ -tubulin. Though the GTP bound to  $\alpha$ -tubulin is stable, the GTP in  $\beta$ -tubulin is hydrolyzed to guanosine diphosphate (GDP) after polymerization (1). The rate of tubulin addition is faster than the rate of GTP hydrolysis during polymerization, allowing for the formation of a protofilament. The stability of the microtubule depends on whether the exchangeable site of the  $\beta$ -tubulin is occupied by GDP or GTP. A GTP-capped microtubule is stable and will continue to grow, whereas a microtubule capped with GDP-bound  $\beta$ -tubulin at the (+) end is unstable and will depolymerize rapidly (1, 3). Hence, microtubule growth involves the association of GTP-bound subunits,

Received 4/23/09; accepted 4/27/09; published OnlineFirst 8/11/09.

**Requests for reprints:** Edith A. Perez, Division of Hematology and Oncology, Mayo Clinic, 4500 San Pablo Road Jacksonville, FL32224. Phone: 904-953-7283; Fax: 904-953-1412. E-mail: perez.edith@mayo.edu

Copyright © 2009 American Association for Cancer Research.

doi:10.1158/1535-7163.MCT-09-0366

and shortening involves the dissociation of GDP-bound subunits. Microtubule shortening occurs when the rate of tubulin addition is so slow that the hydrolysis event occurs before additional subunits can be incorporated (3). Consequently, the rate of GTP-bound tubulin addition is another factor that determines microtubule stability. A shrinking microtubule may be “rescued” if GTP-bound subunits are added to the exposed (+) end before the bound GTP is hydrolyzed (3).

## Microtubule Dynamics

The GTP binding and hydrolysis events allow microtubules to exhibit two dynamic behaviors: dynamic instability and treadmilling (3). Dynamic instability is characterized by the stochastic switching of microtubules between periods of slow growth, rapid shortening, and attenuation (a pause in which neither growth nor shortening is detectable; ref. 3). Transition from a growth stage to shortening is referred to as a “catastrophe”; transition from a shortening stage to growth is called a “rescue.” (3) Treadmilling is the net addition of a tubulin subunit to the (+) end, and the corresponding loss at the (–) end (3). Microtubule dynamics are important for successful mitosis, particularly for the proper function of the mitotic spindle (3). To ensure the rapid assembly and disassembly of microtubules during the alignment and separation of chromosomes, spindle microtubules are more dynamic than interphase microtubules (3). Disruption of microtubule dynamics by compounds that inhibit mitosis prevents cell cycle progression with arrest in the G<sub>2</sub>/M phase, eventually resulting in apoptotic cell death (3).

MTIs are classified into two groups: stabilizing and destabilizing agents (Table 1). Microtubule-stabilizing agents, including taxanes and epothilones, operate by promoting polymerization and increasing the microtubule polymer mass in cells. Destabilizing agents, such as the vinca alkaloids, depolymerize microtubules, inhibit polymerization, and decrease polymer mass. At low concentrations, however, both stabilizers and destabilizers suppress microtubule dynamics without changing polymer mass (2, 3). As the understanding of the action of MTIs at the molecular level increases, differences in mechanisms of action of these agents are being elucidated. These differences may be the basis of observed disparities in activity and tolerability of MTIs.

## Microtubule-Destabilizing Agents

### Vincas

Vinca alkaloids were originally isolated from the *Vinca rosea* plant, which is also known as *Catharanthus roseus*. Vinca alkaloids are dimeric molecules, consisting of catharanthine (velbanamine) and vindoline moieties (Fig. 1). The first two vinca alkaloids identified, vinblastine and vincristine, are almost structurally identical, except for the formyl group attached to the dihydroindole nitrogen in vincristine, whereas vinblastine has a methyl group at that position (Fig. 1). Vinorelbine (navelbine, GlaxoSmithKline) is a semisynthetic derivative of vinblastine, which has an eight-membered

ring in the catharanthine moiety instead of the nine-membered ring in the parent vinblastine (Fig. 1).

The vinca-binding domain on  $\beta$ -tubulin is located near the exchangeable GTP binding site (Table 2). Vinca alkaloids have two distinct binding sites on microtubules: binding with high affinity to tubulin at the microtubule ends, but with low affinity to tubulin along the sides of microtubule surface (4, 5). They also increase the affinity of tubulin for itself, leading to the formation of spiral aggregates (4). Vincas are classified as destabilizing agents that cause microtubule depolymerization, suppress treadmilling and dynamic instability, inhibit mitotic progression, and ultimately result in cell death by apoptosis.

The vinca alkaloids are part of treatment regimens commonly used to treat patients with solid tumors or hematologic malignancies, and have shown activity as single agents or in combination with other cytotoxic agents.

## Microtubule-Stabilizing Agents

### Taxanes

Paclitaxel (taxol, Bristol-Myers Squibb) is a natural product isolated from *Taxus brevifolia* (6). Docetaxel (taxotere, Sanofi-Aventis) is a water-soluble, semisynthetic analog of the naturally occurring precursor 10-deacetylbaccatin III, which was isolated from *Taxus baccata*. Taxanes are complex diterpenes with a tetracyclic core consisting of two cyclohexanes (rings A and C), a cyclooctane (ring B), and an oxetane (ring D). Paclitaxel and docetaxel have an ester side chain at the 13' position; however, docetaxel is deacetylated at the 10' position, and has a trimethyl group attached to the amide tail, whereas paclitaxel has a phenyl group at that position (Fig. 1).

Both paclitaxel and docetaxel occupy the same binding site in the  $\beta$ -subunit of tubulin with a 1:1 stoichiometry (Table 2; refs. 3, 5, 7, 8). The taxane binding site is on the interior surface of microtubules, resulting in the stabilization of microtubules, increased polymerization, and the suppression of microtubule dynamics, leading to cell cycle arrest in the G<sub>2</sub>/M phase and ultimately, apoptosis (Table 3; refs. 3, 5, 8). At high concentrations, taxanes increase polymer mass by inhibiting the dissociation of tubulin yet allowing addition at both ends of the microtubule (3, 5, 8).

Demonstrating significant activity against solid tumors when used either as single agents or in combination with other chemotherapeutic or targeted agents, paclitaxel and docetaxel have been approved for the treatment of breast and non-small cell lung cancer (NSCLC; refs. 9, 10). Paclitaxel is also indicated as therapy for the treatment of advanced carcinoma of the ovary, whereas docetaxel is also indicated for treatment of androgen-independent (hormone refractory) metastatic prostate cancer, advanced gastric adenocarcinoma, and locally advanced squamous cell carcinoma of the head and neck (SCCHN; refs. 9, 10). Albumin-bound paclitaxel (abraxane, Abraxis Oncology), a cremophor-free formulation of paclitaxel, has also shown activity in phase II-III trials in metastatic breast cancer (MBC) and is indicated for the treatment of breast

Table 1. Classes of MTIs

Compound class	Approved agents	Compounds in development	Effect(s) on microtubules
Taxanes	Paclitaxel Docetaxel Albumin-bound paclitaxel	DJ-927	Polymerization and or stabilization
Epothilones	Ixabepilone	KOS-1584 Epothilone B	
Vinca alkaloids	Vinblastine Vincristine Vinorelbine		Depolymerization and or destabilization
Halichondrin b		Erubilin mesylate	

cancer after failure of combination chemotherapy for metastatic disease or relapse within 6 months of adjuvant chemotherapy (11).

## Resistance

Intrinsic or acquired resistance is a widespread occurrence that limits the efficacy of many chemotherapeutic drugs, including MTIs. One common mechanism of resistance identified in preclinical studies involving the P-glycoprotein (P-gp) efflux pump, which is a product of the multidrug resistance gene (*MDR1*; ref. 12). This membrane-associated ATP-binding cassette transporter (ABC-transporter) is overexpressed in many tumor cell lines, and decreases intracellular drug levels, consequently limiting drug cytotoxicity (12).

The reversal of drug resistance by P-gp inhibitors has been shown *in vitro* (12). However, the role of *MDR1*/P-gp in clinical resistance to therapeutic agents has not been validated because of the paucity of studies in which patient tumor specimens have been tested for P-gp expression. In phase 3 clinical trials, agents such as cyclosporine A, verapamil, and valsopodar did not improve clinical outcome. In patients with chemoresistant disease, the addition of the P-gp inhibitor tariquidar (XR9576) had minimal ability to restore sensitivity to chemotherapy (13). A new P-gp inhibitor zosuquidar (LY335979) was evaluated in phase 1 trials and only modestly increased the plasma concentrations of doxorubicin and docetaxel (14). The effects of this P-gp inhibitor on clinical resistance to docetaxel is being investigated in advanced breast cancer (14).

Alterations in tubulin-binding sites or microtubule dynamics may play a role in the mechanism of resistance to taxanes and vinca alkaloids. Analyses of paclitaxel-resistant ovarian cancer cells showed mutations in the  $\beta$ I isotype of tubulin encoded by the M40 gene (F270V and A364T; ref. 15). Altered expression of tubulin isotypes also have an impact on microtubule dynamics. Microtubules consisting of the  $\beta$ III-tubulin isotype have altered assembly properties, requiring a larger critical mass of tubulin for assembly, and polymerizing at a slower rate than other isotypes (16). *In vitro*, the overexpression of  $\beta$ III tubulin results in increased resistance to taxane and vinorelbine (17, 18). Elevated expression of  $\beta$ III tubulin

has also been correlated with clinical resistance to taxanes in a number of human cancers (19). For example, in a study examining predictors of response to paclitaxel, disease progression was observed in a minority (2%) of breast cancer patients whose tumors had low expression of  $\beta$ III tubulin. By contrast, 38% of patients with elevated expression of  $\beta$ III tubulin progressed during treatment with paclitaxel (20). Similar results were observed in a second study evaluating paclitaxel as first-line containing chemotherapy in patients with advanced breast cancer (21). An association between elevated expression of  $\beta$ III and  $\beta$ I tubulin isotypes in breast tumors and poor response to docetaxel-based chemotherapy has also been reported (22). Patients whose tumors had elevated expression of both  $\beta$ III and  $\beta$ I tubulin had the poorest response rate (15%), compared with patients with elevated expression of only one of the isotypes (50%), or patients with low expression of both isotypes (75%; ref. 22). High expression of  $\beta$ III-tubulin has also been associated with poor response to taxane-based chemotherapy in NSCLC and ovarian cancer (23, 24).

Other proteins such as stathmin, MAP4,  $\gamma$ -actin, and tau may influence resistance to MTIs. In breast cancer cells, the overexpression of stathmin, a regulatory protein that destabilizes microtubules, decreased sensitivity to paclitaxel and vinblastine (25). Higher levels of MAP4 in multiple cancer cell lines increased sensitivity to paclitaxel, but decreased sensitivity to vinca alkaloids (26, 27). MAP4 promotes the polymerization of microtubules, which could explain the disparate response to taxanes (stabilizing agents) and vinca alkaloids (destabilizing agents). Another potential mechanism of resistance involves the cytoskeletal protein  $\gamma$ -actin. A basic isoform ( $\gamma$ -actin 2) is expressed in vinblastine-resistant leukemia cells, and single-point mutations in the  $\gamma$ -actin of NIH 3T3 cells resulted in resistance to MTIs (28). Analysis of the status of  $\gamma$ -actin in relapsed patients with acute lymphoblastic leukemia seemed to suggest a correlation between the reduced expression of  $\gamma$ -actin and the risk of relapse (28). A growing body of evidence indicates that elevated expression of the MAP tau is associated with resistance to paclitaxel (19). However, an analysis of predictive and or prognostic factors in a large phase III study (NSABP-B, ref. 29) in patients with node-positive breast cancer did not show an association between

tau expression levels and benefit from paclitaxel-based chemotherapy (29).

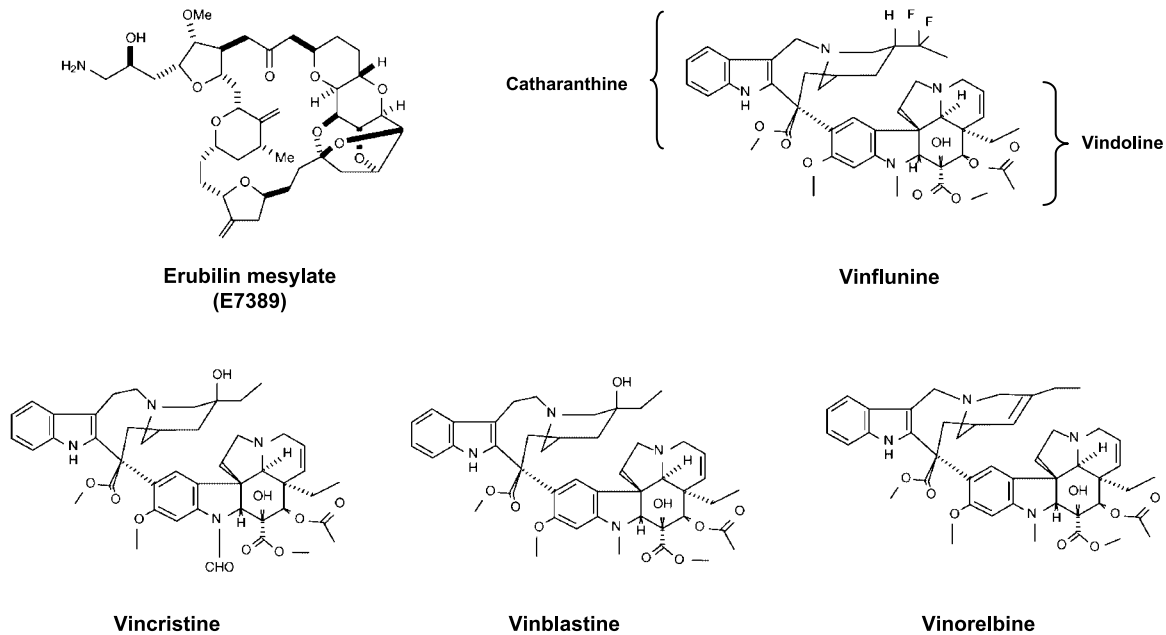
Thus, current evidence indicates that altered drug-binding sites, microtubule-interacting proteins, and impaired assembly properties may be intrinsic or acquired mechanisms that confer resistance to MTIs; however, the clinical

relevance of these resistance mechanisms is not completely understood.

### Novel Microtubule Inhibitors in Development

With the aforementioned issues in mind, novel MTIs are being investigated to offer more effective therapeutic

#### Microtubule-destabilizing Agents



#### Microtubule-stabilizing Agents

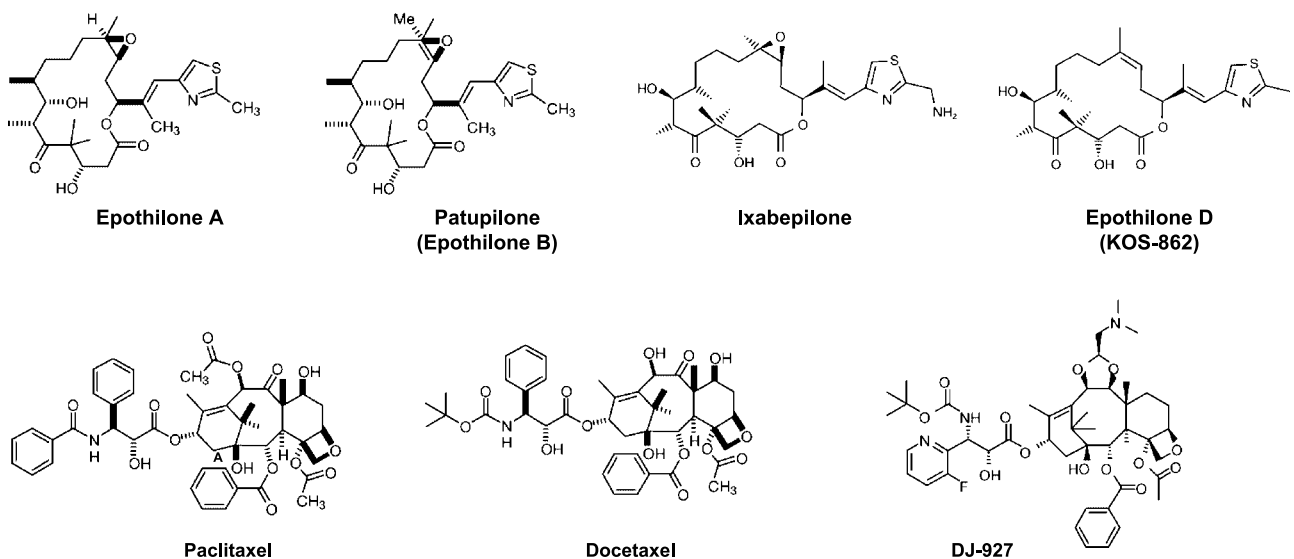


Figure 1. Chemical structures of MTIs.

options. One rationale for the continued development of novel agents is the potential of improving drug activity by exploiting the differences in mechanism of action.

## Epothilones

The epothilones are 16-membered macrolides named for their molecular structure, which includes an *epoxide*, methyl *thiazole*, and *ketone* (Fig. 1; ref. 30). Epothilones A and B were originally isolated from the myxobacterium *Sorangium cellulosum* (30). The structures of the two compounds are nearly identical, with the exception of substitution of a hydrogen for a methyl group at position C-12 in the epothilone B molecule (Fig. 1).

Epothilones are microtubule stabilizing agents with a mechanism of action similar to that of taxanes, including suppression of microtubule dynamics, stabilization of microtubules, promotion of tubulin polymerization, and increased polymer mass at high concentrations (Table 2; refs. 31–33). They induce mitotic arrest in the G<sub>2</sub>-M phase of the cell cycle, resulting in apoptosis (Table 3; refs. 31–33). Similar to paclitaxel, cells exposed to epothilones A and B are characterized by extensive microtubule bundles within the cytoplasm and the formation of aberrant mitotic spindles (31, 32). Both compounds compete with paclitaxel for binding to tubulin and are able to displace [<sup>3</sup>H]-paclitaxel from microtubules, suggesting that they occupy the same binding site as taxanes (31, 32). Despite these similarities, analysis using electron crystallography has shown that epothilones interact with the  $\beta$ -subunit of tubulin through unique and independent molecular interactions (34). The benzoyl phenyl residues of epothilone A were shown to reside in a region of the  $\beta$ -tubulin pocket that is not occupied by paclitaxel. Moreover, four out of the five oxygen-containing polar groups within epothilone A have unique contacts with the  $\beta$ -tubulin protein that are not shared by paclitaxel (34). Three unique molecular interactions were identified that are critical to epothilone A binding with  $\beta$ -tubulin pocket. Threonine 274 and arginine 282 of  $\beta$ -tubulin form cooperative hydrogen bonds with the C3, C5, and C7 oxygens of epothilone A that are necessary for binding (34). In addition, glutamine 292 of  $\beta$ -tubulin forms a hydrogen bond with leucine 275 that is essential to stabilization of the M loop conformation and cooperative hydrogen bonding to epothilone A (34). Alanine 231 also forms a hydrogen bond with histidine 22 that anchors epothilone A within the  $\beta$ -tubulin pocket (34). By contrast, the C3 and C4 substituent groups of paclitaxel form essential contacts with phenylalanine 270 of the  $\beta$ -tubulin protein, a residue that does not play a critical role in epothilone A binding (34).

*In vitro* studies in tumor cell lines show that epothilone B is more active than epothilone A. Both epothilones have greater potency than paclitaxel or docetaxel *in vitro*, with mean inhibitory concentration (IC<sub>50</sub>) values in the low nanomolar range (31, 32). The distinct mechanism of binding of epothilones to  $\beta$ -tubulin may contribute, at least in part, to their increased potency and to a broader spectrum of

activity compared with taxanes, including their ability to stabilize yeast microtubules (35). The epothilones are also active against cells that overexpress P-gp (32, 36), a mechanism implicated in development of resistance to taxanes (12, 37). In addition, mutations in  $\beta$ -tubulin that confer resistance to taxanes (15) did not significantly alter the cytotoxicity of epothilones A and B. Single point mutations (T274I and R282Q) in  $\beta$ I-tubulin, the major  $\beta$ -tubulin isotype expressed in A2780 ovarian carcinoma cells, are associated with resistance to epothilones A and B *in vitro* (15). However, the prevalence of these  $\beta$ -tubulin mutations and their clinical implications have not been clearly elucidated. The promising anticancer activity of epothilones A and B and their ability to overcome resistance have raised interest in this class of compounds and resulted in the synthesis and evaluation of several epothilone analogs. Ixabepilone is the first of this class of antineoplastic agents to be approved for the treatment of cancer, and other compounds in this class are undergoing clinical evaluation for the treatment of a variety of tumor types (38). A summary of chemical-biological properties and activity of lead compounds in this class is provided below.

## Epothilone B Analog Ixabepilone

A semisynthetic derivative of epothilone B, ixabepilone (aza-epothilone B or BMS247550, Bristol-Myers Squibb) was synthesized by converting the lactone of patupilone to a lactam (36). This structural modification resulted in improved solubility, low plasma protein binding, and high metabolic stability (39, 40). Like the natural epothilones A and B, ixabepilone stabilizes microtubules and induces apoptosis (31, 39–42). In preclinical studies, ixabepilone showed activity against human and murine xenografts *in vivo*, and maintained activity in paclitaxel-resistant cell lines and tumors, including those with elevated expression of P-gp and or  $\beta$ -tubulin III isotype (31, 36, 38–43).

An important feature of ixabepilone is its unique mechanism of action (44, 45). Paclitaxel-induced apoptosis in HL-60 leukemia cells has been linked to a mitochondrial-mediated pathway involving cytochrome C release and activation of Apaf-1 and caspase-9 (46–48). By contrast, in Jurkat human acute leukemia cells, ixabepilone-induced apoptosis is linked to a activation of caspase-3 and caspase-8 (Table 3; ref. 49). Ixabepilone-induced activation of caspase-3 and cytochrome C accumulation has also been observed in human ovarian cancer cells (50). In paclitaxel-refractory ovarian cancer cells, ixabepilone was shown to induce p53-dependent induction of PUMA expression (51). Ixabepilone-induced expression of PUMA led to activation of the death effector Bax and apoptosis (51). Moreover, ixabepilone-induced apoptosis was associated with activation of caspase-2 whereas taxanes were associated with caspase-9 activation (51). Consistent with these results, ixabepilone-induced apoptosis in breast cancer cells was also shown to occur through p53-dependent activation of Bax (52). However, in breast cancer cells, p53-dependent activation of Bax was shown to occur via transcription-dependent (elevated

expression of PUMA) and transcription-independent (direct translocation of p53 to the mitochondria) mechanisms (Table 3; ref. 52). These mechanistic differences may underlie the antitumor activity of ixabepilone observed in taxane-resistant and/or refractory tumors.

Ixabepilone has shown efficacy in chemo-naïve or pretreated patients with breast cancer, and is typically administered once every 3 weeks as a 3-hour IV infusion of a 40 mg/m<sup>2</sup> dose (44, 53). Clinical activity of ixabepilone has also been reported in other tumor types including prostate, renal, NSCLC, and pancreatic cancers (38). A phase 2 trial in patients with MBC resistant to an anthracycline, a taxane, and capecitabine showed a response rate of 12% with stabilization of disease in an additional 50% of patients (54). Ixabepilone treatment led to durable responses in this highly chemo-resistant patient population, even in patients who had not responded to multiple previous therapies (54). Consistent with these results, a 12% response rate was reported in a phase 2 trial in patients with taxane-resistant MBC (55). All patients received prior anthracycline-based therapy and 73% of patients experienced disease progression within 1 month of their last taxane dose. Five of the six patients that responded to ixabepilone did not respond to prior taxane therapy (55). A 42% response rate was reported in a phase 2 trial in MBC patients treated with ixabepilone as first-line chemotherapy for metastatic disease (56). In addition, ixabepilone, monotherapy was active in the neoadjuvant setting in patients with invasive breast cancer not amenable to breast conservation surgery (BCS; ref. 57). Patients received ixabepilone monotherapy for a maximum of four cycles. A 61% best overall response rate was reported, with pathologic complete responses in the breast (pCR<sub>B</sub>) in 18% of patients. Thirty-two percent of patients who had surgery underwent BCS (57). A recent phase 3 trial evaluated the efficacy of ixabepilone in combination with capecitabine in patients with MBC who experienced disease progression after treatment with anthracyclines and taxanes (58). The ixabepilone plus capecitabine regimen was superior to capecitabine monotherapy, resulting in a significant clinical benefit in patients resistant to anthracyclines and taxanes (58).

The main treatment-related adverse events associated with ixabepilone therapy are similar to those reported with other MTIs such as taxanes (44, 53). The treatment-limiting toxicity is grade 3-4 neuropathy. Ixabepilone-induced neuropathy is primarily sensory and reversible after dose reductions. The most common hematologic adverse events include grade 3-4 neutropenia and leucopenia. Other adverse events include fatigue, arthralgia, myalgia, and diarrhea (44, 53, 59).

Based on results of the phase II-III trials, ixabepilone was the first epothilone to be approved by the U.S. Food and Drug Administration (FDA) and is indicated in combination with capecitabine for treatment of MBC or locally advanced breast cancer resistant or refractory to treatment with an anthracycline and a taxane, or as monotherapy for treatment of MBC or locally advanced breast cancer resistant-refractory to anthracyclines, taxanes, and capecitabine.

### Epothilone B (Patupilone, EPO906)

Epothilone B (Novartis) has been evaluated in clinical trials against a variety of solid tumors. Patupilone crosses the blood-brain barrier and has shown activity in patients with recurrent or progressive brain metastases from NSCLC (60). Patupilone is typically administered as a 5- to 20-minute IV infusion of 2 to 10 mg/m<sup>2</sup>. The most commonly reported grade 3-4 adverse events are diarrhea (the dose-limiting toxicity), nausea, and fatigue (60). In an ongoing phase 3 trial, patupilone is being compared with pegylated liposomal doxorubicin in pretreated patients with ovarian cancer (61).

### Epothilone D and Analogs (KOS-862 and KOS-1584)

KOS-862, also known as epothilone D and desoxyepothilone B, (Kosan Biosciences) lacks the epoxide moiety in epothilone B (Fig. 1; ref. 36). The compound has a mechanism of action similar to that of taxanes, leading to microtubule stabilization and mitotic arrest. KOS-862 has shown superior *in vivo* anticancer activity relative to patupilone (36). In phase 2 trials, KOS-862 showed activity in chemo-naïve or pretreated patients with breast cancer and NSCLC (62). The most common adverse events noted were grade 1-2 neuropathy, fatigue, nausea, and vomiting (62). Clinical development of KOS-862 has been discontinued.

KOS-1584 (9,10-didehydroepothilone D; Kosan Biosciences) is a novel analog of KOS-862 (Fig. 1). The compound was identified in screens for epothilone analogs with higher potency and improved pharmacologic-pharmacokinetic (PK) properties (63, 64). KOS-1584 stabilizes microtubules, leading to G2-M arrest and apoptosis. KOS-1584 has shown approximately 3- to 12-fold higher potency compared with KOS-862, enhanced tumor tissue penetration, and reduced exposure to selected tissues including CNS (63, 64). An ongoing phase 2 trial is evaluating efficacy of KOS-1584 in patients with advanced or metastatic (stage IIIB-IV) NSCLC (65).

### Vinca Alkaloids, Vinflunine

Vinflunine is a novel MTI of the vinca alkaloid class that was synthesized from a vinca precursor compound by the introduction of two fluorine atoms at the 20' position, and simultaneous reduction of an adjacent 3', 4' double bond in the catharanthine moiety (Fig. 1; refs. 66, 67). Vinflunine destabilizes microtubules, decreases the microtubule growth rate and the time spent in attenuation. Vinflunine presents a differential affinity for tubulin with a higher reversibility of interaction with its target (58, 59). This unique mechanism of action may underlie the superior *in vivo* activity observed with vinflunine compared with other vinca alkaloids (66, 67). In phase 1 pharmacokinetic studies, the active metabolite of vinflunine 4-O-deacetylvinflunine was found to have a half-life of ~5 days (68). Furthermore, as a weak substrate for P-gp, *in vitro* and *in vivo* resistance to vinflunine develops at a slow rate (66, 67).

Vinflunine is freely water soluble, thus eliminating the need for solvent-based formulation and consequently steroid premedication. Administered as a 10- to 20-minute

intravenous (IV) infusion of 320 mg/m<sup>2</sup> once every 3 weeks, vinflunine has anticancer activity against many tumor types (68). Recently completed clinical trials showed the activity of vinflunine in patients with advanced transitional cell carcinoma of the urothelium (TCCU; refs. 69, 70), breast (71), NSCLC (72), and malignant pleural mesothelioma (73). In a recently reported phase 3 trial, the efficacy of vinflunine was equivalent to docetaxel in pretreated patients with NSCLC (74).

Vinflunine-related adverse events were not cumulative and included mostly grade 3-4 neutropenia, fatigue, and constipation (69, 71).

### Halichondrin B Analog Erubilin Mesylate

Erubilin mesylate (E7389, Eisai Medical Research) is a truncated analog of the polyether macrolide natural product

halichondrin B, which was isolated from the marine sponge *Halichondria okadai* in 1986 (74). Halichondrins are noncompetitive inhibitors of vinca alkaloids that occupy the vinca-binding domain on tubulin, suppress the growth of microtubules, and inhibit polymerization, thereby inducing cell cycle arrest and apoptosis (75). The halichondrin analog erubilin mesylate (also known as E7389, ER-086526, and NSC 707389) inhibits the polymerization of purified tubulin more potently than the parent compound halichondrin B (76). Erubilin mesylate has a unique interaction with tubulin as it inhibits microtubule growth with no effect on shortening events (74). Referred to as having an "end-poisoning" mechanism of action, erubilin mesylate either binds directly to the microtubule ends or induces tubulin aggregates, which compete with soluble tubulin for addition to the growing ends of the microtubule (74).

**Table 2. Tubulin/microtubule-binding properties of MTIs**

Property	MTI class		
	Vinca alkaloids	Taxanes-paclitaxel	epothilones-ixabepilone
Mechanism of action	• Direct binding to $\beta$ -subunit of $\alpha/\beta$ -tubulin dimers	• Direct binding to $\beta$ -subunit of $\alpha/\beta$ -tubulin dimers	• Direct binding to $\beta$ -subunit of $\alpha/\beta$ -tubulin dimers
Binding site	• Binds to $\beta$ -tubulin at site near exchangeable GTP-binding site  • Two distinct binding sites on microtubules  • High affinity binding at microtubule ends  • Low affinity binding along microtubule surface	• $\beta$ -tubulin binding site located near inter microtubule protofilament contacts  • Does not overlap binding sites for GTP, colchicine, podophyllotoxin, or vincas  • Poor binding to soluble tubulin  • High affinity binding to polymerized tubulin • Phe270 and Ala364 play essential roles in taxane binding	• Overlaps paclitaxel binding site on $\beta$ -tubulin  • Epothilones competitively inhibit binding of paclitaxel to $\beta$ -tubulin  • High affinity binding to polymerized tubulin • Thr274, Arg282, Glu292, and Ala231 play essential roles in epothilone binding
Biochemical effect(s)	• Suppress microtubule dynamics • Suppress microtubule assembly • Induce microtubule depolymerization • Suppress microtubule treadmilling • Suppress microtubule dynamic instability • Induce tubulin association into coiled spiral aggregates • Decrease polymer mass at high concentrations	• Suppress microtubule dynamics • Enhance microtubule assembly • Induce tubulin polymerization  • Suppress microtubule treadmilling • Suppress microtubule dynamic instability • Induce microtubule bundling  • Induce formation of multipolar spindles • Increase polymer mass at high concentrations	• Suppress microtubule dynamics • Enhance microtubule assembly • Induce tubulin polymerization  • Suppress microtubule treadmilling • Suppress microtubule dynamic instability • Induce microtubule bundling  • Induce formation of multipolar spindles • Increase polymer mass at high concentrations
Selectivity	• No difference in $\beta$ -tubulin isotype binding affinity  • Higher affinity for $\alpha/\beta$ II and $\alpha/\beta$ III tubulin dimers in the presence of GTP observed for vincristine	• Selective binding and inactivation of $\beta$ II-tubulin containing microtubules • Not active against $\beta$ III and $\beta$ IV-tubulin containing microtubules • Do not stabilize microtubules in <i>Saccharomyces cerevisiae</i> • Substrates for drug efflux transporters such as P-gp	• Broad selectivity for microtubules containing multiple $\beta$ -tubulin isotypes • Active against $\beta$ III and $\beta$ IV-tubulin containing microtubules  • Stabilize microtubules in <i>S. cerevisiae</i> cells  • Are not substrates for drug efflux transporters such as P-gp

NOTE: See refs. 3, 5, 7, 8, 31, 34, 35, 42, 85, 86.

**Table 3. Cellular effects of epothilones and taxanes**

Property	Epothilones-Ixabepilone	Taxanes-Paclitaxel
Resistance		
β-tubulin overexpression	<ul style="list-style-type: none"> <li>Retains cytotoxic activity in cells expressing βIII-tubulin</li> </ul>	<ul style="list-style-type: none"> <li>Loss of activity in cells expressing βIII-tubulin</li> </ul>
β-tubulin mutation	<ul style="list-style-type: none"> <li>Active in cells expressing β-tubulin with point mutations (Phe<sup>270</sup> → Val and Ala<sup>364</sup> → Thr) that confer resistance to paclitaxel</li> </ul>	<ul style="list-style-type: none"> <li>Loss of activity in cells expressing β-tubulin with point mutations (Phe<sup>270</sup> → Val and Ala<sup>364</sup> → Thr) that affect binding to βIII-tubulin subunit</li> </ul>
MAP-tau overexpression	<ul style="list-style-type: none"> <li>Reduced activity in cells expressing high levels of MAP-tau</li> </ul>	<ul style="list-style-type: none"> <li>MAP-tau competes with paclitaxel for binding to β-tubulin</li> <li>Reduced activity in cells expressing high levels of MAP-tau</li> </ul>
Cell Damage		
Cell cycle arrest	<ul style="list-style-type: none"> <li>Selective blocking of mitotic spindle microtubule assembly and function</li> <li>Override centrosomal dependent nucleation of microtubules</li> <li>Induces G2-M cell cycle arrest</li> <li>Block mitosis at metaphase-anaphase boundary</li> </ul>	<ul style="list-style-type: none"> <li>Selective blocking of mitotic spindle microtubule assembly and function</li> <li>Override centrosomal dependent nucleation of microtubules</li> <li>Induces G2/M cell cycle arrest</li> <li>Block mitosis at metaphase-anaphase boundary</li> </ul>
Apoptosis	<ul style="list-style-type: none"> <li>Induce phosphorylation of Bcl-2</li> <li>p53-dependent activation of pro-apoptotic effector Bax via transcription-dependent and -independent pathways</li> <li>Cytochrome C and Smac/DIABLO accumulation in paclitaxel-resistant cells</li> <li>Activation of caspase-2, caspase-3, and caspase-8</li> </ul>	<ul style="list-style-type: none"> <li>Induce phosphorylation of Bcl-2</li> <li>Activation of pro-apoptotic effectors Bax, Bad, and Apaf-1</li> <li>Inactivation of the anti-apoptotic effectors Bcl-2 and Bcl<sub>L</sub></li> <li>Cytochrome C accumulation</li> <li>Activation of caspase-2 and caspase-9</li> </ul>

NOTE: See refs. 8, 12, 16, 31, 32, 34, 41, 42, 49–52.

In phase 2 trials, erubilin mesylate has been evaluated in previously treated or treatment-resistant patients with NSCLC, breast, and prostate cancer (77–79). The recommended dosing schedule of erubilin mesylate is 1.4 mg/m<sup>2</sup> administered as a 2- to 5-minute IV bolus on days 1 and 8 of a 21-day cycle. The toxicities associated with erubilin mesylate include grade 3-4 neutropenia, leukopenia, and peripheral neuropathy (77–79). On the basis of the shown activity in treatment-refractory patients, two phase 3 trials have been initiated to evaluate the efficacy and safety of erubilin mesylate in patients with metastatic and or treatment-refractory breast cancer (80, 81).

### Taxane Analog DJ-927

DJ-927 (Daiichi Sankyo Inc.) is a novel docetaxel analog with improved solubility and oral bioavailability (82). With a similar mechanism of action to paclitaxel and docetaxel, DJ-927 is a microtubule-stabilizing agent that has greater potency than paclitaxel or docetaxel against a variety of tumor cell lines *in vitro* and *in vivo* (82). DJ-927 retains activity against P-gp-expressing cells, which could potentially translate to reduced clinical resistance. Intracellular amounts of DJ-927 were higher than paclitaxel and docetaxel in P-gp-positive and -negative cells, and the addition of the P-gp inhibitor verapamil did not affect the *in vitro* activity of DJ-927 (82).

The orally bioavailable DJ-927 has shown promising activity in clinical trials either as a single agent or in combination with other cytotoxic agents. DJ-927 seems active after prior chemotherapy and has a manageable toxicity profile in pretreated patients with gastric and colorectal cancers (Table 2; refs. 83, 84). Administered orally at a dose of 27 mg/m<sup>2</sup> or 35 mg/m<sup>2</sup> once every 3 weeks, the main grade 3-4 adverse events reported were neutropenia, anemia, and diarrhea (83, 84). Further investigations are warranted, because no trials have directly compared the efficacy of DJ-927 with that of docetaxel or paclitaxel.

### Conclusions

MTIs are an important class of compounds in the chemotherapeutic armamentarium. Disparities in their microtubule-binding properties lead to differences in their mechanisms of action with a significant impact on the efficacy or toxicity profile of each agent. Newer agents have shown promising results in clinical settings and may offer improvements in efficacy, tolerability, and the ability to at least partially overcome resistance. Evaluation of combinations of these agents with other targeted and biological therapies for malignancies is an important strategy to follow.

### Disclosure of Potential Conflicts of Interest

Dr. Edith A. Perez received research funding from GlaxoSmith, Bristol-Myers Squibb, and Sanofi-Aventis.



## References

- Nogales E. Structural insight into microtubule function. *Annu Rev Biophys Biomol Struct* 2001;30:397–420.
- Zhou J, Giannakakou P. Targeting microtubules for cancer chemotherapy. *Curr Med Chem Anticancer Agents* 2005;5:65–71.
- Jordan MA, Wilson L. Microtubules as a target for anticancer drugs. *Nat Rev Cancer* 2004;4:253–65.
- Lobert S, Vulevic B, Correia JJ. Interaction of vinca alkaloids with tubulin: a comparison of vinblastine, vincristine, and vinorelbine. *Biochemistry* 1996;35:6806–14.
- Jordan MA. Mechanism of action of antitumor drugs that interact with microtubules and tubulin. *Curr Med Chem Anticancer Agents* 2002;2:1–17.
- Wani MC, Taylor HL, Wall ME, et al. Plant antitumor agents. VI. The isolation and structure of taxol, a novel antileukemic and antitumor agent from *Taxus brevifolia*. *J Am Chem Soc* 1971;93:2325–7.
- Nogales E, Wolf SG, Khan IA, Ludueña RF, Downing KH. Structure of tubulin at 6.5 Å and location of the taxol-binding site. *Nature* 1995;375:424–7.
- Rowinsky EK. Antimicrotubule agents. In: Chabner B, Longo DL, editors. *Cancer chemotherapy and biotherapy: Principles and practice*. Philadelphia: Lippincott Williams & Wilkins; 2005, p. 237–82.
- Bristol-Myers Squibb Company; Princeton (NJ). Prescribing information taxol (paclitaxel) injection. July 2007.
- Sanofi-Aventis U.S. LLC; Bridgewater (NJ). Prescribing information taxotere (docetaxel) injection concentrate. 2007.
- Abraxis Oncology; Bridgewater (NJ). Prescribing information for abraxane for injectable suspension (paclitaxel protein-bound particles for injectable suspension)(albumin-bound). Version 2005 Jan 7.
- Gottesman MM. Mechanisms of cancer drug resistance. *Annu Rev Med* 2002;53:615–27.
- Pusztai L, Wagner P, Ibrahim N, et al. Phase II study of tariquidar, a selective P-glycoprotein inhibitor, in patients with chemotherapy-resistant, advanced breast carcinoma. *Cancer* 2005;104:682–91.
- Fracasso PM, Goldstein LJ, De Alwis DP, et al. Phase I study of docetaxel in combination with the P-glycoprotein inhibitor, zosuquidar, in resistant malignancies. *Clin Cancer Res* 2004;10:7220–8.
- Giannakakou P, Sackett DL, Kang YK, et al. Paclitaxel-resistant human ovarian cancer cells have mutant  $\beta$ -tubulins that exhibit impaired paclitaxel-driven polymerization. *J Biol Chem* 1997;272:17118–25.
- Lu Q, Ludueña RF. *In vitro* analysis of microtubule assembly of isotypically pure tubulin dimers. Intrinsic differences in the assembly properties of  $\alpha$   $\beta$  II,  $\alpha$   $\beta$  III, and  $\alpha$   $\beta$  IV tubulin dimers in the absence of microtubule-associated proteins. *J Biol Chem* 1994;269:2041–7.
- Kamath K, Wilson L, Cabral F, Jordan MS.  $\beta$ III-tubulin induces paclitaxel resistance in association with reduced effects on microtubule dynamic instability. *J Biol Chem* 2005;280:12902–7.
- Seve P, Isaac S, Tredan O, et al. Expression of class III  $\beta$ -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.
- Pusztai L. Markers predicting clinical benefit in breast cancer from microtubule-targeting agents. *Ann Oncol* 2007;18:xii15–20.
- Paradiso A, Mangia A, Chiratti A, et al. Biomarkers predictive for clinical efficacy of taxol-based chemotherapy in advanced breast cancer. *Ann Oncol* 2005;16:iv14–9.
- Tommasi S, Mangia A, Lacalamita R, et al. Cytoskeleton and paclitaxel sensitivity in breast cancer: the role of  $\beta$ -tubulins. *Int J Cancer* 2007;120:2078–85.
- Noguchi S. Predictive factors for response to docetaxel in human breast cancers. *Cancer Sci* 2006;97:813–20.
- Dumontet C, Isaac S, Souquet PJ, et al. Expression of class III  $\beta$  tubulin in non-small cell lung cancer is correlated with resistance to taxane chemotherapy. *Bull Cancer* 2005;92:E25–30.
- Mozzetti S, Ferlini C, Concolino P, et al. Class III  $\beta$ -tubulin overexpression is a prominent mechanism of paclitaxel resistance in ovarian cancer patients. *Clin Cancer Res* 2005;11:298–305.
- Alli E, Bash-Babula J, Yang JM, Hait WN. Effect of stathmin on the sensitivity to antimicrotubule drugs in human breast cancer. *Cancer Res* 2002;62:6864–9.
- Zhang CC, Yang JM, White E, Murphy M, Levine A, Hait WN. The role of MAP4 expression in the sensitivity to paclitaxel and resistance to vinca alkaloids in p53 mutant cells. *Oncogene* 1998;16:1617–24.
- Zhang CC, Yang JM, Bash-Babula J, et al. DNA damage increases sensitivity to vinca alkaloids and decreases sensitivity to taxanes through p53-dependent repression of microtubule-associated protein 4. *Cancer Res* 1999;59:3663–70.
- Verrills NM, Po'uha ST, Liu ML, et al. Alterations in  $\gamma$ -actin and tubulin-targeted drug resistance in childhood leukemia. *J Natl Cancer Inst* 2006;98:1363–74.
- Pusztai L, Jeong J, Gong Y, et al. Evaluation of microtubule associated protein 1 expression as prognostic and predictive marker in the NSABP-B 28 randomized clinical trial [abstract 54]. Presented at the 31st Annual SABCS Meeting; 2008.
- Reichenbach H, Höfle G. Discovery and development of the epothilones: a novel class of antineoplastic drugs. *Drugs R D* 2008;9:1–10.
- 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.
- Altmann KH, Wartmann M, O'Reilly T. Epothilones and related structures - a new class of microtubule inhibitors with potent *in vivo* antitumor activity. *Biochim Biophys Acta* 2000;1470:M79–91.
- Kamath K, Jordan MA. Suppression of microtubule dynamics by epothilone B is associated with mitotic arrest. *Cancer Res* 2003;63:6026–31.
- Nettles JH, Li H, Cornett B, Krahn JM, Snyder JP, Downing KH. The binding mode of epothilone A on  $\alpha$ , $\beta$ -tubulin by electron crystallography. *Science* 2004;305:866–9.
- Bode CJ, Gupta ML, Jr., Reiff EA, Suprenant KA, Georg GI, Himes RH. Epothilone and paclitaxel: unexpected differences in promoting the assembly and stabilization of yeast microtubules. *Biochemistry* 2002;41:3870–4.
- Fumoleau P, Coudert B, Isambert N, Ferrant E. Novel tubulin-targeting agents: anticancer activity and pharmacologic profile of epothilones and related analogues. *Ann Oncol* 2007;18:v9–15.
- Fojo T, Menefee M. Mechanisms of multidrug resistance: the potential role of microtubule-stabilizing agents. *Ann Oncol* 2007;18:v3–8.
- Lee JJ, Swain SM. The epothilones: translating from the laboratory to the clinic. *Clin Cancer Res* 2008;14:1618–24.
- 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.
- Kolman A. BMS-310705 Bristol-Myers Squibb/GBF. *Curr Opin Investig Drugs* 2004;5:1292–7.
- Lee FY, Smykla R, Johnston K, et al. Preclinical efficacy spectrum and pharmacokinetics of ixabepilone. *Cancer Chemother Pharmacol* 2009;63:201–12.
- Lee FY, Borzilleri R, Fairchild CR, et al. Preclinical discovery of ixabepilone, a highly active antineoplastic agent. *Cancer Chemother Pharmacol* 2008;63:157–66.
- Fornier MN. Ixabepilone, first in a new class of antineoplastic agents: the natural epothilones and their analogues. *Clin Breast Cancer* 2007;7:757–63.
- Vahdat L. Ixabepilone: a novel antineoplastic agent with low susceptibility to multiple tumor resistance mechanisms. *Oncologist* 2008;13:214–21.
- Altmann KH. The chemistry and biology of epothilones - lead structures for the discovery of improved microtubule inhibitors. In: Liang X-T, Fang W-S, editors. *Medicinal chemistry of bioactive natural products*. Hoboken (NJ): John Wiley & Sons, Inc.; 2006, p. 1–34.
- Schimmer AD, Hedley DW, Penn LZ, Minden MD. Receptor- and mitochondrial-mediated apoptosis in acute leukemia: a translational view. *Blood* 2001;98:3541–53.
- Perkins CL, Fang G, Kim CN, Bhalla KN. The role of Apaf-1, caspase-9, and bid proteins in etoposide- or paclitaxel-induced mitochondrial events during apoptosis. *Cancer Res* 2000;60:1645–53.
- Janssen K, Pohlmann S, Jänicke RU, Schulze-Osthoff K, Fischer U. Apaf-1 and caspase-9 deficiency prevents apoptosis in a Bax-controlled pathway and promotes clonogenic survival during paclitaxel treatment. *Blood* 2007;110:3662–72.
- Guo F, Nimmanapalli R, Paranawithana S, et al. Ectopic overexpression of second mitochondria-derived activator of caspases (Smac/DIABLO) or

- cotreatment with N-terminus of Smac/DIABLO peptide potentiates epothilone B derivative-(BMS 247550) and Apo-2L/TRAIL-induced apoptosis. *Blood* 2002;99:3419–26.
50. Griffin D, Wittmann S, Guo F, et al. Molecular determinants of epothilone B derivative (BMS 247550) and Apo-2L/TRAIL-induced apoptosis of human ovarian cancer cells. *Gynecol Oncol* 2003;89:37–47.
51. Rojas-Espaillet LA, Uyar D, Grabowski D, et al. Apoptotic pathways induced by ixabepilone in paclitaxel-refractory ovarian carcinoma cells. *Proc Am Assoc Cancer Res* 2005;46:5318.
52. 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.
53. Pivot X, Villanueva C, Chaigneau L, et al. Ixabepilone, a novel epothilone analog in the treatment of breast cancer. *Expert Opin Investig Drugs* 2008;17:593–9.
54. Perez EA, Lerzo G, Pivot X, et al. Efficacy and safety of ixabepilone (BMS-247550), a novel epothilone analog, 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.
55. Thomas E, Taberero 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.
56. 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.
57. Baselga J, Zambetti M, Llombart-Cussac A, et al. Phase II genomics study of ixabepilone as neoadjuvant treatment for breast cancer: efficacy and safety analyses and identification of biomarkers predictive of response. *J Clin Oncol* 2008. Epub 2008 15 Dec.
58. 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.
59. Denduluri N, Swain SM. Ixabepilone for the treatment of solid tumors: a review of clinical data. *Expert Opin Investig Drugs* 2008;17:423–35.
60. Abrey L, Wen PY, Govindan R, et al. Activity of patupilone for the treatment of recurrent or progressive brain metastases in patients (pts) with non-small cell lung cancer (NSCLC): an open-label, multicenter, phase II study. *J Clin Oncol* 2007;25:18058.
61. Patupilone Versus Doxorubicin in Patients With Ovarian, Primary Fallopian, or Peritoneal Cancer [accessed 2009 Jan 19]. Available from: <http://clinicaltrials.gov/ct2/show/NCT00262990>.
62. Overmoyer B, Waintraub S, Kaufman PA, et al. Phase II trial of KOS-862 (epothilone D) in anthracycline and taxane pretreated metastatic breast cancer. *J Clin Oncol* 2005;23:778.
63. Villalona-Calero M, Goel S, Schaaf L, et al. First-in-human phase I trial of a novel epothilone, KOS-1584. *J Clin Oncol* 2006;24:2003.
64. Zhou Y, Zhong Z, Liu F, et al. KOS-1584: a rationally designed epothilone D analog with improved potency and pharmacokinetic (PK) properties. *Proc Am Assoc Cancer Res* 2005;46:2535.
65. Study to evaluate KOS-1584 in patients with advanced or metastatic (stage IIIB/IV) non-small cell lung cancer [accessed 2009 Jan 19]. Available from: <http://clinicaltrials.gov/ct2/show/NCT00651508>.
66. Kruczynski A, Hill BT. Vinflunine, the latest Vinca alkaloid in clinical development. A review of its preclinical anticancer properties. *Crit Rev Oncol Hematol* 2001;40:159–73.
67. Bennouna J, Delord JP, Campone M, Nguyen L. Vinflunine: a new microtubule inhibitor agent. *Clin Cancer Res* 2008;14:1625–32.
68. Bennouna J, Campone M, Delord JP, Pinel MC. Vinflunine: a novel antitubulin agent in solid malignancies. *Expert Opin Investig Drugs* 2005;14:1259–67.
69. Culine S, Theodore C, De Santis M, et al. A phase II study of vinflunine in bladder cancer patients progressing after first-line platinum-containing regimen. *Br J Cancer* 2006;94:1395–401.
70. Petrylak DP, Vaughn DJ, Srinivas S, et al. Phase II study of single-agent vinflunine in platinum-refractory transitional cell carcinoma of the urothelium (TCCU). Barcelona (Spain): Presented at the European Cancer Conference; September 27, 2007.
71. Campone M, Cortes-Funes H, Vorobiof D, et al. Vinflunine: a new active drug for second-line treatment of advanced breast cancer. Results of a phase II and pharmacokinetic study in patients progressing after first-line anthracycline/taxane-based chemotherapy. *Br J Cancer* 2006;95:1161–6.
72. Krzakowski M, Douillard J, Ramlau R, et al. Phase III study of vinflunine versus docetaxel in patients (pts) with advanced non-small cell lung cancer (NSCLC) previously treated with a platinum-containing regimen. *Proc Am Soc Clin Oncol* 2007;25:7511.
73. Talbot DC, Margery J, Dabouis G, et al. Phase II study of vinflunine in malignant pleural mesothelioma. *J Clin Oncol* 2007;25:4751–6.
74. Jordan MA, Kamath K, Manna T, et al. The primary antimetabolic mechanism of action of the synthetic halichondrin E7389 is suppression of microtubule growth. *Mol Cancer Ther* 2005;4:1086–95.
75. Kuznetsov G, Towle MJ, Cheng H, et al. Induction of morphological and biochemical apoptosis following prolonged mitotic blockage by halichondrin B macrocyclic ketone analog E7389. *Cancer Res* 2004;64:5760–6.
76. Dabydeen DA, Burnett JC, Bai R, et al. Comparison of the activities of the truncated halichondrin B analog NSC 707389 (E7389) with those of the parent compound and a proposed binding site on tubulin. *Mol Pharmacol* 2006;70:1866–75.
77. Blum J, Pruitt B, Fabian CJ, et al. Phase II study of eribulin mesylate (E7389) halichondrin b analog in patients with refractory breast cancer. *J Clin Oncol* 2007;25:1034.
78. Spira AI, Iannotti NO, Savin MA, et al. Phase II study of eribulin mesylate (E7389), a mechanistically novel inhibitor of microtubule dynamics, in patients with advanced non-small cell lung cancer (NSCLC). *J Clin Oncol* 2007;25:7546.
79. Molife R, Cartwright TH, Loesch DM, et al. Phase II multicenter, two-stage study of E7389 in patients with hormone refractory prostate cancer with advanced and/or metastatic disease stratified by prior chemotherapy. *J Clin Oncol* 2007;25:15513.
80. E7389 versus capecitabine in patients with locally advanced or metastatic breast cancer previously treated with anthracyclines and taxanes and refractory to the most recent chemotherapy [accessed 2009 Jan 19]. Available from: <http://clinicaltrials.gov/ct2/show/NCT00337103>.
81. E7389 versus treatment of physician's choice in patients with locally recurrent or metastatic breast cancer [accessed 2009 Jan 19]. Available from: <http://clinicaltrials.gov/ct2/show/NCT00388726>.
82. Shionoya M, Jimbo T, Kitagawa M, Soga T, Tohgo A. DJ-927, a novel oral taxane, overcomes P-glycoprotein-mediated multidrug resistance *in vitro* and *in vivo*. *Cancer Sci* 2003;94:459–66.
83. Evans T, Dobrila R, Berardi R, et al. A phase II study of DJ-927 as second-line therapy in patients (pts) with advanced gastric cancer (GC) who have failed a 5-FU non taxane based regimen. *J Clin Oncol* 2006;24:4081.
84. Rhee J, Lee F, Saif MW, et al. Phase II trial of DJ-927 as a second-line treatment for colorectal cancer demonstrates objective responses. *Proc Am Soc Clin Oncol* 2005;23:3654.
85. Lobert S, Frankfurter A, Correia JJ. Energetics of vinca alkaloid interactions with tubulin isotypes: Implications for drug efficacy and toxicity. *Cell Motil Cytoskeleton* 1998;39:107–21.
86. Derry WB, Wilson L, Khan IA, Luduena RF, Jordan MA. Taxol differentially modulates the dynamics of microtubules assembled from unfractionated and purified  $\beta$ -tubulin isotypes. *Biochemistry* 1997;36:3554–62.