

## Targeting Microtubules by Natural Agents for Cancer Therapy

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### Abstract

Natural compounds that target microtubules and disrupt the normal function of the mitotic spindle have proven to be one of the best classes of cancer chemotherapeutic drugs available in clinics to date. There is increasing evidence showing that even minor alteration of microtubule dynamics can engage the spindle checkpoint, arresting cell-cycle progression at mitosis and subsequently leading to cell death. Our improved understanding of tumor biology and our continued appreciation for what the microtubule targeting agents (MTAs) can do have helped pave the way for a new era in the treatment of cancer. The effectiveness of these agents for cancer therapy has been impaired, however, by various side effects and drug resistance. Several new MTAs have shown potent activity against the proliferation of various cancer cells, including resistance to the existing MTAs. Sustained investigation of the mechanisms of action of MTAs, development and discovery of new drugs, and exploring new treatment strategies that reduce side effects and circumvent drug resistance could provide more effective therapeutic options for patients with cancer. This review focuses on the successful cancer chemotherapy from natural compounds in clinical settings and the challenges that may abort their usefulness. *Mol Cancer Ther*; 13(2); 275–84. ©2014 AACR.

### Microtubules: Important Target for Cancer Therapy

Microtubules play an essential role in several eukaryotic cellular processes such as cell growth and division, motility, intracellular trafficking, and the ability to adapt to a variety of shapes to interact with the environment (1). As a result of the great success of the mitotic agents in the treatment of cancer thereby, microtubules represent the best cancer target identified thus far. Also, the drugs of this class will continue to be important chemotherapeutic agents in the future as more selective approaches are developed (2).

The biologic functions of microtubules in all cells are regulated in large part by their polymerization dynamics (3). Polymerization of microtubules occurs by a nucleation-elongation mechanism by the reversible, noncovalent addition of  $\alpha$ - and  $\beta$ -tubulin dimers at both ends of microtubules (4).

Microtubules show *in vitro* and *in vivo* two kinds of nonequilibrium dynamics. The dynamic behavior that is highly prominent in cells, called dynamic instability, refers to the individual microtubule ends switching

between phases of growth and shortening (1). The two ends of a microtubule are not equivalent; the plus end grows and shortens more rapidly and extensively than the minus end. When the microtubules neither grow nor shorten detectably, they undergo relatively long periods of slow lengthening, brief periods of rapid shortening, and periods of attenuated dynamics or pause (4). Dynamic instability can be characterized by four main variables: the growth rate of microtubules, the rate of shortening, the frequency of transition from the growth or paused state to shortening, also called a "catastrophe," and the frequency of transition from shortening to growth or pause, also called a "rescue" (4). The second dynamic behavior is called treadmilling, which is the net growth at one microtubule end and balanced net shortening at the opposite end (5). It involves the intrinsic flow of tubulin subunits from the plus end of the microtubule to the minus end and is created by differences in the critical subunit concentrations at the opposite microtubule ends, and might be particularly important in mitosis (6). Both treadmilling and dynamic instability are compatible behaviors; a specific population of microtubules can display one behavior or a mixture of both behaviors. However, the mechanisms that control the degree to which a microtubule population displays one or the other behavior are poorly understood (7).

In interphase, microtubules exchange their tubulin with the soluble tubulin pool relatively slowly, with half-times that range from several minutes to several hours (8). The interphase microtubule network disassembles at the onset of mitosis and is replaced by a new population of spindle microtubules that are four to 100 times more dynamic than

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**doi:** 10.1158/1535-7163.MCT-13-0791

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the microtubules in the interphase cytoskeleton (9). Although there is variation among the various spindle-microtubule subpopulations, the mitotic-spindle microtubules exchange their tubulin with tubulin in the soluble pool rapidly, with half-times on the order of 10 to 30 seconds (8).

Mitosis phase facilitates the equal partitioning of replicated chromosomes into two identical groups. The success of this process requires highly dynamic microtubules in the spindle (1, 8, 9). Microtubule dynamics are critical for the timely and correct attachment of chromosomes at their kinetochores to the spindle during prometaphase after nuclear-envelope breakdown, for the movements of the chromosomes to their properly aligned positions at the metaphase plate, and for the synchronous separation of the chromosomes in anaphase and telophase after the checkpoint of metaphase anaphase is complete (4). During prometaphase, microtubules originating from each of the two spindle poles grow to a maximum of typically 5 to 10  $\mu\text{m}$ , and then shorten nearly completely. Then regrow until they successfully become attached to chromosomes at their kinetochores (10). It is critical that each single chromosome should attach to a bipolar spindle of microtubule; failure to do so is sufficient to stop the cell from transitioning to anaphase; this blocks the cell in a prometaphase/metaphase-like state and it eventually undergoes apoptosis (programmed cell death; refs. 11, 12).

Drugs that block mitosis seem to work by a common mechanism, which suppresses the dynamic of microtubules and kills tumor cells. Paclitaxel (taxol) and Vinca alkaloids are the first class of antimetabolic agents to be discovered and inhibit cancer cell proliferation. One possible explanation is that cancer cells are relatively sensitive to these drugs compared with normal cells because they divide more rapidly than normal cells and therefore frequently pass through a stage of vulnerability to mitotic poisons (13).

### Microtubule-Targeting Agents

The microtubule-targeting agents (MTAs) are a very successful class of cancer drugs with therapeutic benefits in both hematopoietic and solid tumors (14). A large number of natural agents and/or their analogs bind to soluble tubulin and/or directly to tubulin in the microtubules. Most of these compounds are antimetabolic agents that inhibit cell proliferation by acting on the polymerization dynamics of spindles, which are essential to proper spindle function of microtubules. The specific effects of MTAs on the polymer mass and the dynamic stability of the microtubules are complex (4).

Microtubule-targeted antimetabolic drugs are classified into two main groups. The first group is microtubule-destabilizing agents, which inhibit microtubule polymerization at high concentrations and include several compounds such as the Vinca alkaloids (vinblastine, vincristine, vinorelbine, vindesine, and vinflunine), cryptophycins, halichondrins, estramustine, colchicines, and combretastatins, that are

used clinically or are under clinical investigation for treatment of cancer (12). The second group is microtubule-stabilizing agents. These agents stimulate microtubule polymerization, and include paclitaxel (the first agent identified in this class), docetaxel (taxotere), the epothilones, and discodermolide (12).

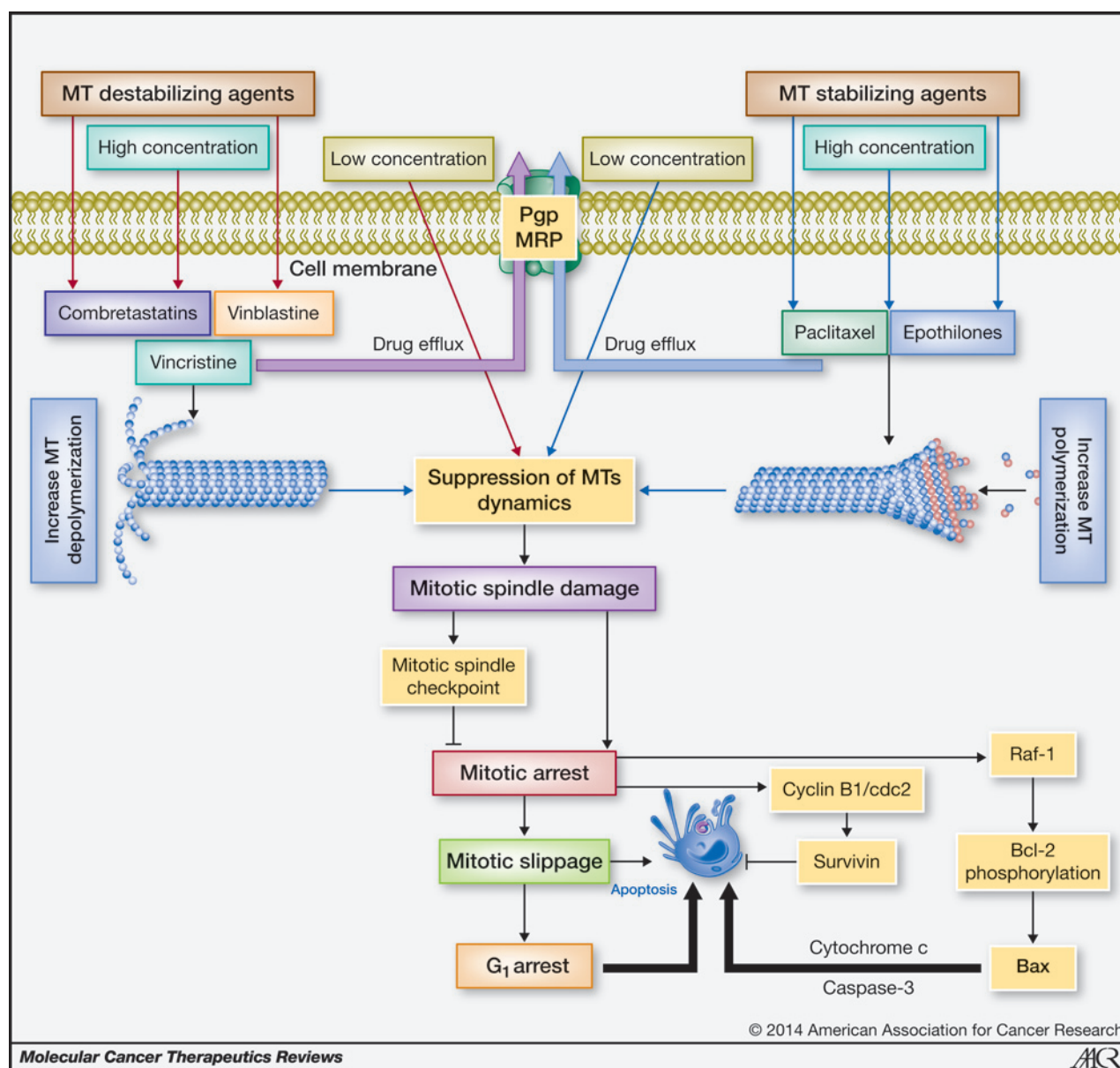
The classification of drugs as "stabilizers" or "destabilizers" of microtubule is that the drugs increase or decrease microtubule polymerization at high concentrations, powerfully suppress microtubule dynamics at 10- to 100-fold lower concentrations, and therefore, kinetically stabilize the microtubules, without changing the microtubule-polymer mass (4). The most important action of the two classes of drugs is the suppression of spindle-microtubule dynamics. This results in the slowing or blocking of mitosis at the metaphase-anaphase transition and induction of apoptotic cell death rather than their effect on microtubule-polymer mass, which requires very high dosage levels to act primarily and continuously on microtubule-polymer mass (refs. 12, 15; Fig. 1).

The ability of a drug to bind to soluble tubulin or directly to the microtubule depends on the location of the specific binding site in tubulin and the microtubule (15). It is important for the efficacy of these drugs in cancer chemotherapy to block the mitotic phase, slow cells, and subsequently induce apoptosis (12). Although there are important differences in the mechanisms of actions of antimetabolic drugs, the underlying mechanisms of the three classes of drugs are similar (Table 1).

### MTAs: Mechanisms of Action

Microtubules serve as an intracellular cytoskeleton framework, and their unique polymerization dynamics are critical for many cellular functions (16). It is possible that the dysfunction of the cytoskeleton is an intracellular stress, which results in either a disrupted microtubule network or a stabilized microtubule cytoskeleton (17).

MTAs, also known as antimetabolic agents, exert their inhibitory effects on cell proliferation primarily by blocking mitosis, which requires an intense control of microtubule dynamics. Therefore, their actions on microtubule stability and dynamic parameters differ from each other. At relatively high concentrations, MTAs either inhibit microtubule polymerization, destabilizing microtubules and decreasing microtubule polymer mass, or promote microtubule polymerization, stabilizing microtubules and increasing the polymer mass (12, 18). At the cellular level, both types of agents may lead to cell-cycle arrest in mitosis and trigger cell death through apoptosis (12, 16). Mitotic arrest is associated with aberrant spindle formation, thus clearly linking interference with microtubule functionality to inhibition of cell proliferation. It is often assumed that apoptosis induced by microtubule-stabilizing agents is a direct consequence of  $G_2$ -M arrest, which in turn would be a prerequisite for growth inhibition and cell death. However, Chen and colleagues documented in a series of experiments that the situation is more complex



**Figure 1.** Schematic diagram of putative events involved in MTAs-induced apoptosis. The interaction of chemotherapeutic agents that stabilize or destabilize microtubules results in suppression of microtubule dynamics that leads to damage to the mitotic spindle or to massive microtubule damage depending on drug concentration and time of exposure. These actions trigger apoptosis by inducing cell-cycle arrest at the G<sub>2</sub>-M phase or a general failure in microtubule-related functions depending on the level of microtubule damage. These effects, together with the abnormal exit of mitosis, leads to multinucleated cells and eventually to cell death, which are the major mechanisms involved in MTAs-induced apoptosis. However, cancer cells may become resistant to these drugs by activating drug efflux pump.

(19). So, the treatment of human cancer cells with low concentrations of microtubule-stabilizing drugs may lead to mitotic slippage, which is multipolar spindles formation, and subsequent cell-cycle arrest in G<sub>1</sub>. Thus, cells arrested in (aneuploidic) G<sub>1</sub> state and subsequent undergo apoptosis. On the other hand, higher drug concentrations lead to a protracted mitotic block from which the cells eventually exit without division, thus forming tetraploid G<sub>1</sub> cells (19), which will then undergo apoptosis (Fig. 1). These results supported that the entry of cells into

mitosis is a fundamental prerequisite for cell killing by microtubule-stabilizing agents, but that apoptosis does not (necessarily) occur from a G<sub>2</sub>-M arrested state. It is important to note that the induction of tubulin polymerization *in vitro* requires  $\mu\text{mol/L}$  or sub- $\mu\text{mol/L}$  concentrations of microtubule-stabilizing agents (20). However, low nmol/L or even sub-nmol/L IC<sub>50</sub> values for cancer cell growth inhibition are required.

Although at low but clinically relevant concentrations of microtubule-stabilizing and -destabilizing drugs

**Table 1.** Natural MTAs: source, binding site, side effects, and clinical use

Binding domain	Drug	Origin	Binding site on tubulin	Side effects	Clinical uses	References
Vinca domain	Vinblastine (Velban)	Plant, <i>Catharanthus roseus</i>	$\beta$ -Tubulin subunit	Neutropenia (dose-limiting toxicity); MDR sensitive; sensitive to $\beta$ III-tubulin	Hodgkin disease, testicular germ-cell cancer, breast cancer, head and neck cancer	(12, 33, 41)
	Vincristine (Oncovin)	Plant, <i>Catharanthus roseus</i>	$\beta$ -Tubulin subunit	Peripheral neuropathy, hyponatremia, constipation, and hair loss	Leukemia, lymphomas	(12, 33, 41)
Taxanes site	Paclitaxel	Plant, <i>Taxus brevifolia</i>	$\beta$ -Tubulin subunit	Neutropenia (dose-limiting toxicity); MDR sensitive; sensitive to $\beta$ III-tubulin	Head and neck, lung, breast, ovarian cancer, and advanced Kaposi sarcoma	(46, 48, 51)
	Epothilones	Bacteria, <i>Sporangium cellulosum</i>	$\beta$ -Tubulin subunit	MDR sensitive; not sensitive to $\beta$ -tubulin content	Ovarian, prostate, lung, breast cancers, refractory solid tumors, glioblastoma; Paclitaxel-resistant tumors	(57, 59, 60, 61)
Colchicine domain	Colchicine	Plant, <i>Colchicum autumnale</i>	$\beta$ -Tubulin subunit	Potent toxicity to normal cells	Nonneoplastic diseases (gout, familial Mediterranean fever)	(36, 37)
	Combretastatins	Plant, <i>Combretum caffrum</i>	$\beta$ -Tubulin subunit	MDR insensitive; sensitive to $\beta$ -tubulin content	Phase III trial of CA-4-P in combination with carboplatin for anaplastic thyroid cancer; potential vascular-targeting agent	(41, 42)

potently suppress microtubule dynamics without affecting microtubule polymer mass, they have the potential to block mitotic progression and induce apoptosis (refs. 11, 12; Fig. 1). Thus, as our understanding of MTAs increases, we realize that the mechanism underlying the antimitotic and anticancer activities of MTAs may be due to their inhibitory effects on spindle microtubule dynamics, rather than their effects on microtubule polymer mass, as proposed previously.

Recent studies have shown that mTOR activation is dependent on dynein-mediated transport that functions as the predominant minus end-directed microtubules motor in eukaryotic cells. It has been shown that molecular dynein is required for perinuclear localization of mTOR into the cells (21). The inhibition of microtubule function by MTAs causes inhibition of the AKT/mTOR

signaling pathway and thus inhibits cancer cell proliferation. This mechanism is independent of MTAs-induced mitotic arrest and could provide an alternative mechanism of drug action that can explain its clinical activity.

Currently there are three well-established drug-binding sites on  $\beta$ -tubulin. The Vinca domain is located adjacent to the exchangeable GTP-binding site in  $\beta$ -tubulin at the plus end interface (22). The taxane site resides in a deep hydrophobic pocket at the lateral interface between adjacent protofilaments, within the lumen of the microtubule (23). Finally, the colchicine site is located at the intradimer interface between  $\beta$ -tubulin and  $\alpha$ -tubulin (24). Laulimalide is another drug that binds on  $\beta$ -tubulin site and stabilizes microtubules. This compound was isolated from marine sponge (*Cacospongia mycofijiensis*) and represents a first class of microtubule-stabilizing



drug that binds at a site distinct from the taxane site on tubulin (25).

## Microtubule-Destabilizing Natural Products

### Vinca alkaloids and related drugs

Vinca alkaloids, among the earliest developed for disruption of microtubule, were isolated from periwinkle leaves, *Catharanthus roseus* (L.) G. Don (also known as *Vinca rosea*). These compounds have been successful in the clinic not only for the treatment of diabetes, high blood pressure, and as disinfectants, but also for being cancer fighters (26). Vinca alkaloids were discovered in the 1950s by Canadian scientists, Robert Noble and Charles Beer, for their antimetabolic and, therefore, cancer chemotherapeutic potential. They were first used as single agent in the treatment of childhood hematologic malignancies, then widely spread into use for solid malignancies and, shortly after, for adult hematologic malignancies (Table 1; ref. 27). Because of their great success in the treatment of childhood leukemia, they were considered "wonder drugs." Further development of the Vinca alkaloids has been motivated by the increased understanding of their mechanisms of action, their synergy in several combination therapies, and the desire to develop orally available analogs. This has led to the development of various novel semisynthetic analogs, including vindesine, vinorelbine, and vinflunine to overcome the side effects of peripheral neuropathy and reversible myelosuppression that commonly occur with these drugs (28).

Although the causes of the neurotoxicity are undefined, they definitely involve the effects of the drugs on microtubules, which are a key component of neurons (29). Neuropathy might result from disruption of axonal flow by bundling of microtubules by paclitaxel (30), from steric hindrance of motor-protein binding to microtubules, or from the effects of altered microtubule dynamics in axonal processes or on transport in growth cones. Another cause of neurotoxicity might result from microtubule destabilization or from suppression of microtubule dynamics due to neuronal retraction and reduced arborization (30); also, from reduced responsiveness of neurons to incoming signals; or from demyelination (29). Myelosuppression, also called bone marrow suppression or myelotoxicity, is a result of blockage of mitosis and proliferation of the rapidly cycling bone marrow cells. This is a common side effect associated with Vinca alkaloids chemotherapy.

Jordan and colleagues reported that Vinca alkaloids destroy mitotic spindles at high concentrations (e.g., 10–100 nmol/L in HeLa cells) and depolymerize microtubules (4), therefore leaving the dividing cancer cells blocked in mitosis with condensed chromosomes. Also, the same authors stated that at low but clinically relevant concentrations, vinblastine does not depolymerize spindle microtubules, yet it powerfully blocks mitosis (e.g.,  $IC_{50}$  0.8 nmol/L in HeLa cells) and cells die by apoptosis (4). Studies from the same laboratory revealed that the

mitotic-blocking action of low concentrations of Vinca alkaloid agent in living cancer cells is due to suppression of microtubule dynamics rather than microtubule depolymerization (12).

Vinblastine binds to the  $\beta$ -subunit of tubulin dimers at a distinct region called the Vinca-binding domain (31). Various other novel chemotherapeutic drugs also bind at this domain. The binding of vinblastine to soluble tubulin is rapid and reversible (12). This induces a conformational change in tubulin in connection with tubulin self-association (32).

*In vitro* studies revealed that vinblastine also binds with high affinity to tubulin at the extreme microtubule ends; however, it binds with low affinity to tubulin that is buried in the tubulin lattice (12, 33). These studies show that binding of one or two molecules of vinblastine per microtubule plus end is sufficient to reduce both treadmilling and dynamic instability by approximately 50%, without significant microtubule depolymerization. This suppression of dynamics prevents the mitotic spindle from assembling normally and it reduces the tension at the kinetochores of the chromosomes. Mitotic progress is delayed with chromosomes often stuck at the spindle poles not capable of assembly at the spindle equator. The cell-cycle signal to the anaphase-promoting complex to pass from metaphase into anaphase is blocked and the cells eventually die by apoptosis (12, 33).

In addition, this group includes a large number of compounds that have not undergone clinical development for cancer therapy, including the antitussive noscapine (15), maytansine, rhizoxin, spongistatins, podophyllotoxin, steganacins, and curacins (34); several herbicides that inhibit microtubule polymerization; antifungal, and antihelminthic agents (35); and some psychoactive drugs (ref. 32; Table 1).

### Colchicine

Colchicine was first isolated in 1820 by French chemists P.S. Pelletier and J.B. Caventou from autumn crocus (*Colchicum autumnale*), and described for treatment of rheumatism and swelling (36). Although colchicine has been used clinically in the treatment of gout, neither colchicine nor compounds that bind to the colchicine site on tubulin have been found to be of significant use in cancer treatment because of their potent toxicity (Table 1; ref. 37).

As with Vinca alkaloids, Colchicine depolymerizes microtubules at high concentrations and stabilizes microtubule dynamics at low concentrations. Colchicine inhibits microtubule polymerization by binding to microtubule ends rather than to the soluble tubulin pool. However, free colchicine itself probably does not bind directly to microtubule ends. Instead, it first binds to soluble tubulin, induces slow conformational changes in the tubulin, and ultimately forms a poorly reversible tubulin–colchicine complex (38), which then copolymerize into the microtubule ends in small numbers along with large numbers of free tubulin molecules (39).

So, despite the differences between the effects at high concentrations of the Vinca/colchicine-like drugs and the taxane-like drugs, almost all of the microtubule-targeted antimetabolic drugs stabilize microtubule dynamics at their lowest effective concentrations (4).

Several natural products that bind in the colchicine domain and disrupt microtubule assembly *in vitro* and *in vivo* are known, such as podophyllotoxin, from the mandrake herb *Podophyllum peltatum*, and combretastatins (40).

### Combretastatins

Combretastatins are a class of natural *cis*-stilbenes (phenols) isolated from the South African tree *Combretum caffrum* (Combretaceae). Combretastatins exhibit cytotoxic properties and tubulin polymerization inhibition in cancer cells *in vitro* (41). The most potent member of this group is combretastatin A-4 with  $IC_{50}$  of 7 nmol/L (42). The combination of this compound with a phosphate prodrug, called combretastatin A4 phosphate, also known as Zybrestat, fosbretabulin (9), progressed into clinical trials for the treatment of solid tumors (Table 1; refs. 41, 42). The advantages of this class of compounds over other antimetabolic agents is that beside their antimetabolic properties, they are also angiogenesis inhibitors and known as vascular disrupting agents (42, 43). In addition, they are being evaluated for their efficacy in the treatment of diabetic retinopathy which is the biggest single cause of blindness (44).

### Microtubule-Stabilizing Natural Products

#### Paclitaxel and related drugs

The taxanes (paclitaxel, docetaxel), a novel class of antimicrotubule agents, are the most important addition to the chemotherapeutic armamentarium against cancer over the past several decades. Paclitaxel was discovered as part of a National Cancer Institute (Bethesda, MD) screening program which extracted thousands of plants for anticancer activity (45). Paclitaxel, *Taxus brevifolia*, was extracted from the stem bark of the Pacific yew tree in 1966 (46). The structure of paclitaxel was reported in 1971; however, the microtubule-stabilizing properties of this compound were discovered later by Schiff and colleagues in 1979 (ref. 47; Table 1). Although taxanes bind poorly to soluble tubulin, they bind to tubulin along the length of the microtubule with high affinity (48). Paclitaxel binds to the  $\beta$ -tubulin subunit, and promotes microtubule stabilization by inducing conformational changes of the M-loop of  $\beta$ -tubulin that result in more stable lateral interactions between adjacent protofilaments (48). In contrast with a high concentration of taxane required to increase microtubule polymerization, a study found that the binding of a very small number of paclitaxel molecules powerfully stabilizes the dynamics of the microtubules without increasing microtubule polymerization (11). Suppression of microtubule dynamics by paclitaxel leads to mitotic

blocking in the absence of significant microtubule bundling (11). Jordan and colleagues show that half of HeLa cells in mitosis are blocked at 8 nmol/L paclitaxel, whereas there is no increase in microtubule-polymer mass below 10 nmol/L paclitaxel (12). The polymer mass of paclitaxel is half increased at 80 nmol/L (12). The suppression of spindle-microtubule dynamics by taxanes prevents the dividing cancer cells from progressing from metaphase into anaphase and the cells eventually die by apoptosis (11, 12, 49).

Because of the limited quantities of paclitaxel from the bark of *Taxus brevifolia*, alternate sources, particularly an approved semisynthesis of 10-deacetylbaccatin III, were obtained to cover the need (4). The U.S. Food and Drug Administration (FDA) approved taxol for the treatment of metastatic ovarian cancer in 1992. Today, taxol is one of the most important anticancer drugs and is widely used clinically for the treatment of ovarian, breast, and non-small cell lung cancer, either as monotherapy or in combination with *cis*-platin. It is also used for the treatment of acquired immunodeficiency syndrome-related Kaposi sarcoma.

Paclitaxel and its semisynthetic analogue docetaxel are widely prescribed antineoplastic agents for a broad range of malignancies (50). The side effects are neurotoxicity and myelosuppression similar to those associated with Vinca alkaloids (51). Treatment of prostate cancer with taxanes also inhibits androgen receptor signaling by inhibiting the androgen-dependent nuclear translocation of the androgen receptor (AR; ref. 52). In the absence of ligand dihydrotestosterone, AR binds with Hsp90 chaperone in the cytoskeleton. However, in the presence of dihydrotestosterone, the ligand binding results in AR homodimerization and translocation in the nucleus, where it binds with specific androgen-dependent genes, resulting in proliferative and trophic effects (53). This finding is an evidence that in addition to blocking cell division, microtubule-targeting drugs also impair AR signaling.

Paclitaxel was the first microtubule-stabilizing agent that was investigated in an animal model of neurodegenerative tauopathies, the T44 tau Tg mouse. The ability of paclitaxel and docetaxel to cross the blood-brain barrier (BBB) is limited. This is believed to be caused at least in part by the P-glycoprotein (Pgp) efflux pump, which is highly expressed in the BBB (54). Thus, taxane analogs that are capable of overcoming Pgp-mediated transport may result in improving the effectiveness of the drugs. Several compounds of this type have been reported, such as cabazitaxel, an FDA-approved semisynthetic taxane with weak Pgp substrates that can saturate the active transporter (55). Furthermore, pharmacokinetic studies with cabazitaxel show that administering the compound via rapid infusions in the brain significantly enhances the drug concentration, which results in plasma drug levels that are above the threshold needed to saturate Pgp (55). Other examples of taxanes that circumvent Pgp-mediated efflux are orally

active BMS-275183 and milataxel (MAC-321; ref. 56). In addition, other approaches with brain-targeted delivery have been reported with favorable results, such as paclitaxel-peptide conjugate GRN1005, a Pgp-insensitive prodrug that exploits the low density lipoprotein receptor-related protein 1. Albumin nanoparticle carriers proposed for taxanes not only enhance the microtubule inhibition, but also prevent the use of solubilizing cremophors, which sometimes induce severe hypersensitivity (15). Other nanoparticle-based carriers, such as nanoporous silicon particles, for chemotherapeutic shuttling, have been recently proposed.

Search for other drugs that enhance microtubule polymerization has yielded several promising compounds, including the epothilones, discodermolide, the sarcodictyins, eleutherobin, and laulimalide. Some of these compounds compete with paclitaxel for binding to microtubules and bind at or near the taxane site (epothilones, discodermolide, eleutherobins, and sarcodictyins), but others, such as laulimalide, seem to bind to unique sites on microtubules (25).

### Epothilones

Epothilones are a family of novel MTAs, stabilizing microtubules and inhibiting microtubule dynamic behavior at mitotic spindle and, therefore, preventing cancer cells from mitosis. Epothilones A and B were discovered by Gerth and colleagues as antifungal agents produced by the soil bacterium *Sorangium cellulosus* (57). These compounds compete with paclitaxel for the taxane-binding site on  $\beta$ -tubulin, suggesting that this class of compounds may act on microtubules in a paclitaxel-like manner and promote microtubule assembly (58). This observation led to the suggestion that epothilones, taxanes, and other classes of microtubule-stabilizing natural products may share a similar pharmacophore (ref. 59; Table 1). Many studies support this common pharmacophore model (60); however, other studies evaluated the complex of epothilone A with tubulin polymerized in zinc-stabilized sheets by electron crystallography and demonstrated that epothilone A and paclitaxel interact in substantially different ways within the same binding pocket in  $\beta$ -tubulin (61). Such differences in the binding modes of  $\beta$ -tubulin may provide a possible explanation of how the epothilones maintain their high antimitotic activity in cell lines that are resistant to taxanes because of point mutations in the  $\beta$ -tubulin subunit (62). Another unique feature of the epothilones is that unlike docetaxel and paclitaxel, epothilones maintain their cytotoxic performance even in cells overexpressing Pgp.

Currently there are five epothilone or epothilone-derived compounds undergoing clinical evaluation. One of the most advanced of these compounds is epothilone B (Patupilone; EP0-906). This drug is being evaluated by Novartis and is in phase III clinical trials for a number of cancer types (63). Similarly, lactam analog (Ixabepilone; BMS-247550) of epothilone B is in phase III trial targeting

breast cancer being developed by Bristol-Myers Squibb (BMS).

In addition to epothilones A and B, epothilone D has been isolated as a minor component of fermentation of myxobacteria (64). This compound revealed a greater therapeutic index as a chemotherapeutic agent, compared with epothilones B (65); however, severe side effects including central nervous system toxicities were reported from clinical trials (66).

### Limitation of the MTAs in Clinical Setting

#### Toxicity and side effects

Although MTAs have proven to be highly effective for the treatment of cancer, the adverse side effects associated with their long- and short-term use, affect their applicability in the clinical setting. DNA damage and apoptosis are the main causes of drug-induced cytotoxicity (67). Neurologic and hematologic side effects are the main and often dose-limiting toxicities, but several other side effects also occur during the treatment with each individual drug (68).

Neurotoxicity such as peripheral neuropathy, which is characterized by the loss of deep tendon reflex at the ankle, numbness, and motor weakness, is very common. Cranial neuropathy may also occur after treatment with the Vinca alkaloids and taxanes and may cause different symptoms such as jaw pain and vocal cord dysfunction. Another common symptom is autonomic neuropathy causing constipation, abdominal cramping, and urinary retention. In addition, headache, dizziness, and mental depression are other symptom of neurotoxicity. Neurotoxicity typically occurs after prolonged treatment with MTAs. The inhibition of axonal microtubules, which are crucial for axonal transport in neurons, is at least in part the reason for such toxicity (29).

Hematologic toxicity is another major side effect as a result of treatment with MTAs, and is referred to as myelosuppression (68). Severe neutropenia, in particular, occurs early after treatment. Myelosuppression may result from the inhibition of the rapidly dividing hematopoietic cells. Nausea, vomiting, and diarrhea are other common symptoms besides the neurologic and hematologic toxicities (ref. 68; Table 1).

#### Drug resistance

Drug resistance refers to the status of poor responsiveness of tumor cells to chemotherapeutic drugs. Multiple mechanisms of drug resistance in cancer have been described, but typically involve efflux mechanisms and membrane-associated changes to prevent drug accumulation within tumor cells (4). Acquired drug resistance may be a result of the cellular response to MTAs, which modulate by adaptive changes of the cell. This is a common theme of anticancer drugs. Otherwise, because of the presence of specific resistance proteins, cells may be inherently protected from the antiproliferative effects of growth inhibitors even upon first exposure (4). Drug

efflux by ATP-binding cassette (ABC) transporters such as Pgp, is one of the most frequent mechanisms of drug resistance encountered in cancer cells (4). Although Pgp-mediated drug efflux is a major resistance mechanism for taxol, some of the newer MTAs have been found to be less vulnerable to Pgp action, and thus may offer more benefits over taxol (4). Concrete efforts to reveal the specific resistance mechanisms to MTAs have been investigated over the last several years. Three major mechanisms have been proposed: first, overexpression of specific tubulin isotypes, especially of  $\beta$ III-tubulin; second, inherit tubulin mutations that may affect microtubule stability and dynamics; third, the emergence of tubulin mutations, which lead to impaired ligand binding. Several groups have reported that the overexpression of class III  $\beta$ -tubulin is the cause of taxol resistance in cancer cell lines and human tumors as well (32). In addition, *in vitro* studies reported that microtubules that assemble solely from  $\alpha$  $\beta$ III dimers exhibit different assembly properties and significantly enhanced dynamicity over microtubules that assemble from  $\alpha$  $\beta$ II or  $\alpha$  $\beta$ IV dimers or unfractionated tubulin (63). In paclitaxel resistance studies, taxol-resistant A549 lung carcinoma cells, which overexpress  $\beta$ III-tubulin, exhibited inherently increased microtubule dynamics. This phenomenon does not allow the cells to proceed from metaphase to anaphase (4). However, upon treatment with low doses of taxol, the normal cell-cycle progression is restored. These results support the theory that these cells are dependent on taxol to normalize their microtubule dynamics and to successfully proceed through mitosis. Another study showed the effects of  $\beta$ III-tubulin overexpression on microtubule dynamics and taxol sensitivity by inducing either  $\beta$ I- or  $\beta$ III-tubulin overexpression in CHO cells (51). Cells that harbor specific tubulin mutations may be resistant to MTAs as a result of microtubule stability having been reduced and/or increased microtubule dynamics. This concept is supported by the observation that these cells often become dependent on the selecting agent and are hypersensitive to microtubule-destabilizing agents, thus indicating inherently reduced microtubule stability (69). None of these mechanisms, however, have been proven to be the cause of drug resistance in clinical settings (70). The importance of  $\beta$ -tubulin mutations in the clinical resistance to taxol in patients with non-small cell lung cancer by DNA sequence of the  $\beta$ -tubulin gene was proposed (71). However, these findings were highly controversial and could not be confirmed in a number of subsequent prospective studies. Berrieman and colleagues investigated the possible role of  $\beta$ -tubulin mutations in the resistance to cancer chemotherapy and concluded that  $\beta$ -tubulin mutations in clinical samples are rare and unlikely to contribute to drug resistance (72). It should be noted that  $\beta$ -tubulin pseudogenes belong to a family of homologous DNA sequences that need further investigation (73).

Enhanced DNA repair by O6-alkylguanine DNA alkyltransferase has been known as a common mecha-

nism of resistance to drugs (74). Another mechanism of chemoresistance has been proposed by which the alteration in death-inducing signaling pathway may result in inhibition or prevention of apoptosis (75). Overexpression of antiapoptotic genes such as Bcl-2, besides mutation or reduced expression of proapoptotic genes such as p53, has been shown to induce cancer cells to become resistant to anticancer treatment. The way that Pgp can protect tumor cells from apoptosis has recently been discussed in view of observations that inhibition of Pgp by chemosensitizing agents can restore the normal apoptotic cascade in cells with defective signaling pathways (75).

Overall, there are multiple, complex and, possibly, interrelated mechanisms of drug resistance. These mechanisms are often found in combination, which can further complicate the chemotherapy of tumors (Table 1).

## Conclusions and Future Developments

As our knowledge of MTAs increases, we realize that the mechanism underlying the anticancer activity of these agents may mainly lie in their inhibitory effects on spindle microtubule dynamics, rather than in their effects on microtubule polymer mass. More understanding of mechanisms of action of MTAs will enhance the use and effectiveness of these compounds. This knowledge of the mechanistic differences among this class is vital to understanding their tissue and cell specificity and the development of resistance. Our ability to understand resistance-mediated mechanisms in tumor biology has led to the creation of cabazitaxel as an alternative for those patients who become resistant to docetaxel-based chemotherapy regimens. The identification and further elucidation of microtubule-dependent tumor-specific pathways not only may help us to better understand the molecular basis of clinical taxane resistance, but also help identify individual patients more likely to respond to treatment. Combination therapy at much lower doses than the doses already used are needed that will be nontoxic, yet effective in suppressing microtubule dynamics. Furthermore, the uses of adjuvants in chemotherapy that suppress microtubule dynamics need further investigation.

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

## Grant Support

The work was supported by United States Public Health Service Grant RO1 CA 160867 and RO1 CA 160867 S1 (to H. Mukhtar).

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Received September 25, 2013; revised November 7, 2013; accepted November 13, 2013; published OnlineFirst January 16, 2014.



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