Microtubule Active Agents: Beyond the Taxane Frontier

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Abstract
Microtubules are essential to cell transport, signaling, and mitosis. An increasing range of antineoplastic agents interfere with the normal formation and function of microtubules. Vinca alkaloids act as microtubule destabilizers and the taxanes act as microtubule stabilizers. Taxanes are widely used cytotoxic agents that are active in a range of solid tumor malignancies and are routinely used in a variety of settings. Significant limitations with the taxanes exist, including acquired and intrinsic tumor resistance through the expression of multidrug resistance proteins such as P-glycoprotein, risk of hypersensitivity reactions, dose-limiting hematopoietic toxicity, and cumulative neurotoxicity. Hence, there is a need to develop novel agents that act on the microtubules. Epothilones are macrolide antibiotics that bind near the taxane-binding site on microtubules and have been extensively studied in recent and ongoing clinical trials. A variety of other agents that act on the microtubules at different sites with a variety of structures are at varying stages of development.

Background
Microtubules are ubiquitous fibrillar structures that play an important role in a variety of cellular processes including transport, signaling, and mitosis (1). Polymers of α- and β-tubulin combined with microtubular-associated proteins make up microtubules, which are constantly undergoing rearrangement (Fig. 1; ref. 1). The microtubule polymer thus exists in a dynamic equilibrium with the intracellular pool of α- and β-tubulin (1, 2). During mitosis, microtubules and the mitotic spindle are critical to the separation of chromosomes into two daughter cells and so have become a target for cytotoxic agents (Fig. 1). Through a variety of mechanisms, an increasing range of drugs interferes with the normal formation and function of microtubules and the mitotic spindle and cause cells to arrest in metaphase. Cell death then occurs by apoptosis. Until recently, the most important antimicrotubule agents were the taxanes, but many new compounds are at various stages of development.

Classically, drugs that interfered with microtubular structure and function were divided into stabilizers and destabilizers. This division is somewhat simplistic and does not account for all the mechanisms of action such as an effect on tumor vasculature or an effect on microtubule dynamics (2). Microtubule destabilizers consist predominantly of drugs that act at the Vinca alkaloid and colchicine-binding sites (Fig. 2). The oldest class of cytotoxic agents that interfere with microtubules are the Vinca alkaloids, such as vincristine, vinblastine, and vinorelbine. These agents are active in a variety of malignancies including lymphomas, non-small cell lung cancer (NSCLC), and breast cancer. It is thought that the Vinca alkaloids interact with the central portion (or Vinca binding site; Fig. 2) of the β-tubulin subunit and thus prevent polymerization into microtubules (3). Colchicine, which is used for the treatment of gout, acts at a separate site on β-tubulin termed the colchicine-binding site (Fig. 2).

Until recently, the only clinically important microtubule stabilizers were the taxanes, such as paclitaxel and docetaxel. Taxanes are widely used cytotoxic agents that are active in a range of solid tumor malignancies such as breast cancer, NSCLC, ovarian cancer, gastroesophageal cancer, germ cell tumors, as well as cancers of the head and neck. They are routinely used in the neoadjuvant, adjuvant, and metastatic setting alone and in combination with drugs with different mechanisms of action and nonoverlapping toxicity profiles. Paclitaxel was originally derived from the bark of the Pacific yew tree but can now, like docetaxel, be partially synthesized from the precursor 10-deacetylbaccatin III, derived from needles of the European yew (2). The taxanes bind to tubulin, stabilize the microtubule, and inhibit its disassembly leading ultimately to cell death by apoptosis. The clinical use of the taxanes is limited by (a) tumor resistance, (b) risk of hypersensitivity reactions, and (c) toxicity.

Acquired and intrinsic resistance of tumor cells to taxanes remains a significant clinical problem. Resistance occurs through a variety of mechanisms. One important mechanism is the expression of multidrug resistance proteins such as P-glycoprotein, which belongs to a family of ATP-binding cassette transporters and which is the product of the multidrug resistance-1 gene. The expression of these multidrug resistance proteins leads to the production of transporters that act as drug efflux pumps. These pumps cause the efflux of substrate drugs such as taxanes and Vinca alkaloids from tumor cells and prevent the accumulation of therapeutic intracellular concentrations of active drug. P-glycoprotein is expressed on the endothelial cells of the capillaries of the central nervous system and may explain in part why the brain remains a sanctuary site for many chemotherapeutic agents.

Resistance to the taxanes can also occur due to interruption of the interaction between the drug and the target protein, β-tubulin. Tumor cells can overexpress the βIII isoform of β-tubulin. To...
tubulin leading to demonstrable clinical resistance. Intrinsic and acquired mutations in the tubulin protein can interfere with the normal binding of taxanes to the target protein. Altered expression of microtubule-associated proteins can also prevent taxane binding. Elucidating the relative importance of these mechanisms and circumventing them remains a significant challenge.

The risk of hypersensitivity reactions with the taxanes, particularly paclitaxel, results from their poor solubility and the need to dissolve in solvents such as polyoxyethylated castor oil (Cremophor EL; BASF) or polysorbate. This risk has been substantially reduced by the use of premedications but remains a clinical problem. It has been suggested that polyoxyethylated castor oil may trap paclitaxel (and other cytotoxic agents administered concurrently) in micelles in plasma and may inhibit the endothelial transcytosis resulting in lower drug delivery to target cells (4). Other limitations to the use of taxanes include dose-limiting hematopoietic toxicity and cumulative neurotoxicity from long-term use. There is therefore a need to develop novel taxane delivery systems, taxane derivatives, and newer agents to target microtubules to overcome these problems.

Clinical-Translational Advances

Microtubule-stabilizing agents

Novel taxane formulations. Several advances in the formulation of paclitaxel have occurred, which avoid the need for polyoxyethylated castor oil and hence lower the risk of hypersensitivity reactions.

ABI-007 (Abraxane; Abraxis BioScience) is a novel albumin-bound, 130 nm particle form of paclitaxel that is solvent-free...
Sorangium cellulosum epothilones A and B, produced by the myxobacterium represent a novel class of antimicrotubule agent. The natural especially given the myriad of other options. paclitaxel, which tend to occur after the first dose (15). The contrast to the hypersensitivity reactions seen with standard these hypersensitivity reactions occurred after cycle 4, in ABI-007 was stopped due to a high rate of neurotoxicity and hypersensitivity reactions in patients with metastatic breast cancer is ongoing (8). ABI-007 is formulated from human albumin, so a theoretical risk of transmission of viruses and prions such as Creutzfeldt-Jakob disease exists. No cases of viral transmission from human-derived albumin have ever been identified, but this theoretical risk may limit the broader use of ABI-007 especially in the adjuvant setting. An adjuvant phase II trial of doxorubicin and cyclophosphamide followed by ABI-007, all in combination with bevacizumab, for breast cancer patients has recently completed accrual (9). The incorporation of ABI-007 into standard taxane-based regimens could potentially remove the need for premedication with steroids and could allow more rapid infusion times.

CT-2103 (Xyotax; Novartis) is a conjugate of α-poly-1-glutamic acid and paclitaxel. From preclinical data, this drug was expected to improve drug delivery to the target tumor while decreasing toxicity. CT-2103 initially showed promising activity in a variety of solid organ tumors including prostate, NSCLC, gastroesophageal, and ovarian cancer (10–13). Neurotoxicity has, however, been a substantial problem (13–15). Recently, a phase II trial in HER-2-negative metastatic breast cancer was stopped due to a high rate of neurotoxicity and hypersensitivity reactions, which occurred in 4 of 18 patients (15). Interestingly, these hypersensitivity reactions occurred after cycle 4, in contrast to the hypersensitivity reactions seen with standard paclitaxel, which tend to occur after the first dose (15). The future development of this compound is therefore unclear, especially given the myriad of other options.

Epothilones. Epothilones are macrolide antibiotics and represent a novel class of antimicrotubule agent. The natural epothilones A and B, produced by the myxobacterium Sorangium cellulosum, are cytotoxic in vitro (16). By binding near the taxane-binding site (Fig. 2), epothilones cause microtubular stabilization and cellular arrest in a similar way to the taxanes. However, their chemical structure is unrelated; moreover, epothilones bind to the tubulin-binding pocket in a specific and independent manner, suggesting that rather than a common pharmacophore for taxanes and epothilones, tubulin has a promiscuous binding pocket, allowing different molecules to interact according to their unique structures. Early clinical use of epothilones was limited by pharmacokinetic difficulties and metabolic instability; therefore, synthetic and semisynthetic derivatives have been developed to overcome these problems. Ixabepilone (BMS-247550). The epothilone most widely clinically investigated is ixabepilone (BMS-247550, Ixempra; Bristol Myers Squibb), which is a semisynthetic derivative of natural epothilone B. The key modification of a lactone to a lactam protects ixabepilone from hepatic degradation by esterases. Ixabepilone does, however, need to be dissolved in polyoxyethylated castor oil. In vitro studies have shown that the cytotoxicity of ixabepilone is 2.5 times that of paclitaxel and that activity is maintained in taxane-resistant cell lines (17). As noted above, tumor resistance to taxanes and other drugs is often mediated by expression of P-glycoprotein or multidrug resistance-associated protein or by mutations within tubulin. The epothilones do not appear to be susceptible to these resistance mechanisms. Phase I studies in a range of malignancies including patients with tumors refractory to conventional therapy investigated two dosing schedules (once every 21 days or daily for 5 consecutive days out of 21 days) and showed promising activity (18–20). The recommended dose for phase II studies was 40 mg/m² every 21 days, which has become the standard (18, 19). Numerous phase II studies have now been reported, which show the activity of ixabepilone in a variety of malignancies and settings, particularly in metastatic prostate and breast cancer (21–32). The combination of paclitaxel and estramustine may have a synergistic cytotoxic effect in vitro (24). Estramustine causes microtubule disassembly by binding to microtubule-associated proteins rather than the taxane-binding site on β-tubulin (24). The combination of estramustine and ixabepilone is thus rational and has shown promising results in a phase II study in metastatic prostate cancer (25). In metastatic breast cancer, several phase II studies have examined ixabepilone in a variety of settings, including the first-line metastatic setting, for patients that are taxane-naïve and in heavily pretreated patients with taxane-resistant disease (28–32).

An international phase III study of 752 patients randomized patients to receive ixabepilone with capcitabine or capecitabine alone and showed a statistically significant prolongation in progression-free survival of 4.2 to 5.8 months in favor of the combination (33). The most common treatment-related toxicities were neutropenia, sensory neuropathy, and fatigue (33). Although 65% of patients treated with ixabepilone experienced neuropathy, this was grade 3/4 in 21% (33). In October 2007, ixabepilone was approved by the Food and Drug Administration for metastatic breast cancer.

Patupilone. Naturally occurring epothilone B, patupilone (EPO906; Novartis), is up to 20 times more potent than paclitaxel against a variety of cell lines in vitro and this activity is maintained in taxane-resistant cell lines. It has a different side-effect profile to ixabepilone with minimal neurotoxicity and myelosuppression. The main dose-limiting toxicity is diarrhea. Although these substances are very similar chemically, their differing side-effect profile is puzzling and likely related to tissue distribution and metabolism (34). This may in part be due to the fact that patupilone, unlike ixabepilone, is inactivated by esterases (34). This different side-effect profile of patupilone suggests a possible use in patients with neurotoxicity from prior taxane therapy.

Interestingly, patupilone has been shown to cross the blood-brain barrier and has antitumor effects in the central nervous system in animal models (35). Results from a phase II trial of refractory brain metastases in NSCLC were encouraging (35). A phase II trial of patupilone is ongoing in patients with progressive brain metastases from breast cancer following
whole-brain radiotherapy. These are interesting and important studies because of the dearth of therapeutic options for these patients. The brain remains a sanctuary site for many cytotoxic agents. With improvements in systemic control of many malignancies, brain metastases are an increasing clinical problem and therapeutic advances in this area are welcome.

Patupilone has shown activity in preclinical models of rarer malignancies such as in multiple myeloma cell lines, in hepatocellular carcinoma cell lines, and in a rat glioma model in combination with imatinib (36–38). These data offer possible avenues for further investigation in the future.

Other epothilone B analogues. Other semisynthetic analogues of epothilone B are at various stages of development. BMS-310705 is a semisynthetic analogue of epothilone B, which is more water-soluble than ixabepilone (39). BMS-310705 has been safely administered without the need for premedications (39). In a phase I study, responses were seen in gastric, breast, and ovarian cancer, but difficulties have arisen with diarrhea and neurotoxicity. Its future use may be limited to patients not suitable for treatment with other epothilones (39). ABJ-879 is another semisynthetic derivative of epothilone B, which has shown superior activity to paclitaxel in various cell lines and in xenograft tumor models (40). Although ABJ-879 remains active in vitro against multidrug-resistant cell lines, the absence of clinical studies to date suggest that this compound may not be important in the future (40). ZK-EPO, a rationally designed derivative of epothilone B and the first fully synthetic epothilone, has shown remarkable activity in a variety of cell lines as well as an ability to evade the cellular efflux pumps responsible for multidrug resistance (41). A phase II study in platinum-resistant ovarian cancer has enrolled 63 patients with promising early efficacy results (42). The most notable toxicity appears to be neurotoxicity (42).

Epothilone D derivatives. In vitro studies have suggested that epothilone D and its analogues have substantially less activity that epothilone B (41). KOS-862 is a derivative of epothilone D, which has shown particular activity against taxane-resistant cells in vitro (43). In phase I studies, KOS-862 has been successfully combined with drugs with differing mechanisms of actions such as carboplatin, gemcitabine, and trastuzumab (43–46). Phase II studies in metastatic breast cancer, platinum-refractory NSCLC, and metastatic hormone-refractory prostate cancer have shown disappointing efficacy and substantial neurotoxicity (43, 47, 48). The future development of this compound could lie in taxane-naive patients or at lower doses in combination with other cytotoxic agents. Early clinical experience with KOS-1584, also derived from epothilone D, has been encouraging with responses in NSCLC, ovarian, and head and neck cancer (48). Diarrhea has emerged as a dose-limiting toxicity and antidiarrheal prophylaxis is now routinely given in studies (48).

Discodermolide and dictyostatin. There has been considerable interest in substances derived from marine organisms as anticancer agents. For many sedentary marine organisms, the production of toxic substances that act on microtubules forms an important defense mechanism. These substances tend to be in short supply and considerable time has been needed to identify their chemical structure and formulate them synthetically. For many of these agents, research is therefore at an early stage. Discodermolide, isolated from the marine sponge Discodermola dissoluta, has shown promising activity in vitro and possible synergy with paclitaxel, suggesting that the binding sites are not identical (Fig. 2; ref. 49). Dictyostatin is structurally related to discodermolide and was initially isolated from a marine sponge of the genus Spongia (50). Dictyostatin and discodermolide maintain antiproliferative activity in cells expressing β-tubulin mutation genes (2, 50). Early clinical results with discodermolide were encouraging, but further clinical development has been limited by unforeseen pulmonary toxicity (2) The possibility of developing structural analogues remains.

Laulimalide and peloruside. Laulimalide is a structurally complex substance derived from marine sponges that also maintains antimitotic activity against paclitaxel-resistant cells (51). The interaction of laulimalide and microtubules is complex, but there is evidence for a distinct laulimalide-binding site on α-tubulin (Fig. 2; ref. 52). Xenograft studies in mice have shown that the drug has a narrow therapeutic index and marked toxicity without evidence of efficacy, probably limiting its further development (51). Peloruside A is a metabolite of the New Zealand marine sponge Mycale hentscheli with a similar structure to the epothilones (52). It has the advantage of being less lipophilic than paclitaxel and binds to α-tubulin on the laulimalide-binding site (52). This binding site distinct from the taxanes raises the possibility of combining drugs that act on laulimalide and taxane-binding sites—a synergistic antiproliferative effect has already been seen in vitro (53).

Other agents. Cyclosporin was originally recovered from the fermentation broth of a bacterium from the Streptomyces species (54). Although it is less cytotoxic than paclitaxel in vitro, it is active in taxane-resistant cell lines possibly because of a novel mechanism of action involving covalently cross-linking with β-tubulin (54). Eleutherobin and sarcodictyins A and B are chemically related natural compounds derived from coral with potent antimitotocute activity by binding to the taxane-binding site (55). Further chemical developments are awaited.

Microtubule-stabilizing agents

Colchicine-binding site. Although colchicine itself has no clinical use in malignancy, many orally available compounds that act at the colchicine-binding site of tubulin (Fig. 2) are undergoing investigation for possible cytotoxicity. These agents include 2-methoxyestradiol, sulfonamide derivatives, and synthetic derivatives of Aspergillus species (56–58). As yet, no dominant compound has emerged, but there is substantial potential for development of agents that act as this novel site and could theoretically be combined with other antimicrotubule agents and drugs with other mechanisms of action.

Vinca-binding site. As noted, Vinca alkaloids have a well-established role in a variety of malignancies. Vinflunine is a synthetic Vinca alkaloid with greater in vitro activity than older Vinca alkaloids and has now shown clinical efficacy in NSCLC (59). Synthetic derivatives of other naturally occurring compounds that act at the Vinca site are undergoing investigation (Fig. 2). Halichondrin B is a large polyether macrolide derived from the marine sponge Halichondria okadaic (60) Eribulin mesylate (E7389) is a synthetic derivative of

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halichondrin, which is currently in phase II trials in breast, prostate, and NSCLC (60). Cryptophycins are naturally occurring compounds isolated from blue-green algae (61). LYS5703 is a synthetic cryptophycin derivative, which has shown some activity in platinum-refractory ovarian cancer (61). Although the activity of these compounds in refractory malignancies may be modest, a future role in a variety of settings is possible. Future synthetic derivatives may also show greater activity.

**Dolastatins.** Dolastatins were originally isolated from the Indian Ocean mollusk, *Dolabella auricularia* (also known as the sea hare), and screened for anticancer activity and are thought to bind near the Vinca binding site (62). Although many compounds have been tested, clinical results have been disappointing. TZT-1027, a derivative of dolastatin-10, showed no activity in NSCLC in a phase II trial despite promising results in preclinical studies (62). Tasidotin (ILX651), a synthetic derivative of dolastatin-15, inhibits microtubule nucleation at low concentrations and is potentially a more promising drug—phase II studies in melanoma and NSCLC are ongoing (63).

**Conclusion**

Many promising agents that interfere with microtubules are at varying stages of development. The challenges lie in how these novel agents can be incorporated into standard management for diseases with many different treatment options as well as for diseases with minimal therapeutic options. The use of rational combinations of drugs with nonoverlapping side-effect profiles and differing mechanisms of action is particularly interesting.

**Disclosure of Potential Conflicts of Interest**


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**References**


