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 antican-
cer drugs interferes 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 neurotox-
hicity. 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 chromo-
somes 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 micro-
tubules 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-deactylbaccatin 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 concen-
trations 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
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. The future development of this compound is therefore unclear, as the hypersensitivity reactions occurred after cycle 4, in contrast to the hypersensitivity reactions seen with standard paclitaxel, which tend to occur after cycle 4 (15). 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). 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 profiles are 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.

IXABEPILON (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/m^2 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 to receive ixabepilone with capicitabine or capicitabine 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.

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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.

Pateplone 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 patients not suitable for treatment with other epothilones (39). ABJ-879 is another semisynthetic derivative of epothilone B, which has showed superior activity to paclitaxel in various premedications (39). 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 disoluta*, has shown promising activity in vitro and possible synergy with paclitaxel, suggesting that the binding sites are not identical (Fig. 2; ref. 49). 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 pelorusside.** 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 hentschelii* 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 antimitotobule activity by binding to the taxane-binding site (55). Further chemical developments are awaited.

**Microtubule-destabilizing 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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References

31. Abrey LE, Wen PN, Govindan R, et al. Activity of 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). LY355703 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. TTT-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 nucelation 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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