# The clinical development of new mitotic inhibitors that stabilize the microtubule

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**Microtubule-stabilizing agents are increasingly studied for cancer treatment based largely on the prior success of paclitaxel and docetaxel. In this review, we focus on the clinical development of epothilones and discodermolide, and we discuss salient preclinical and clinical highlights of these two novel natural products. These agents are distinguished by their biochemical features making them poor P-glycoprotein substrates and capable of inducing cytotoxicity in cell lines or *in vivo* tumor models harboring mutations in tubulin. There is now considerable data regarding the efficacy of the epothilones in human beings and discodermolide holds such promise, as well. *Anti-Cancer Drugs* 15:553–558 © 2004 Lippincott Williams & Wilkins.**

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## Introduction

Mitotic inhibitors that are used as anti-cancer drugs fall into two broad categories: (i) those that stabilize microtubule dynamics (microtubule stabilizers) and (ii) those that inhibit tubulin/microtubule assembly (microtubule destabilizers) (Table 1). Typically, drugs that target the paclitaxel-binding site act as microtubule stabilizers [1,2]. Paclitaxel (Fig. 1F) and docetaxel are approved by the US Food and Drug Administration for the treatment of breast cancer and other malignancies; however, a major issue preventing cure is the development of multidrug resistance. The success of paclitaxel in the clinic has stimulated a search for natural products that could preserve this function, yet subvert the typical mechanisms of cellular resistance [e.g. P-glycoprotein (P-gp) export, preserved activity in paclitaxel-resistant cells with mutations in β-tubulin]. These families of extended natural products include the epothilones, discodermolide, laulimalide, the sarcodictyins, eleuthrobin, GS-164 and FR182877. In this review, the focus will be on the clinical development of the epothilones and discodermolide, as both these drugs have entered clinical development and show promise as novel anti-tubulin agents.

The epothilones (a name derived from its molecular features: epoxide, thiazole, ketone) were originally isolated by Gerhard Hofle and Hans Reichenbach from the broth of fermenting soil bacteria, *Sorangium cellulosum*. Bollag *et al.* provided the first evidence that epothilones A and B hyperstabilize microtubules and induce a mitotic arrest similar to that observed with paclitaxel [3]. Recent evidence suggests that epothilones and taxanes bind and interact at a common binding site on β-tubulin of the tubulin heterodimer. However, based on work by Giannakakou *et al.*, the absence of strict taxane/epothilone cross-resistance in their cell lines did not preclude a common binding site, but suggested that detailed binding interactions were weighted differently for the two agents [4]. A crystal structure of epothilone–tubulin interactions has not been conclusively elucidated. Since the original isolation of epothilones, several methods for their preparation have been described, and include fermentation, biosynthesis, biotransformation, total synthesis, semisynthesis and combinatorial synthesis [3,5]. Although the major fermentation products of *S. cellulosum* are epothilones A and B, epothilones C–F were obtained as minor fermentation products, which have since been prepared by chemical synthesis. Chemical synthesis of epothilones has held the most promise as it allows for modifications of the parent molecule. Epothilones A and B are derived from the same polyketide synthase (PKS) gene cluster. Similarly, olefinic epothilones C and D are products of the PKS reactions. Heterologous expression of the epothilone PKS gene cluster in *Streptomyces coelicolor* results in the synthesis of epothilones A and B. Expression of epothilone PKS in myxobacteria, *Myxococcus xanthus*, results in epothilone D. Epothilone analogs (e.g. BMS-247550 and BMS-310705) are produced by partial or total synthetic approaches [6,7].

At least three major regions of the epothilone molecule provide a focus for *in vitro* structure–activity relationship studies (Fig. 1). Although wide variances in results are

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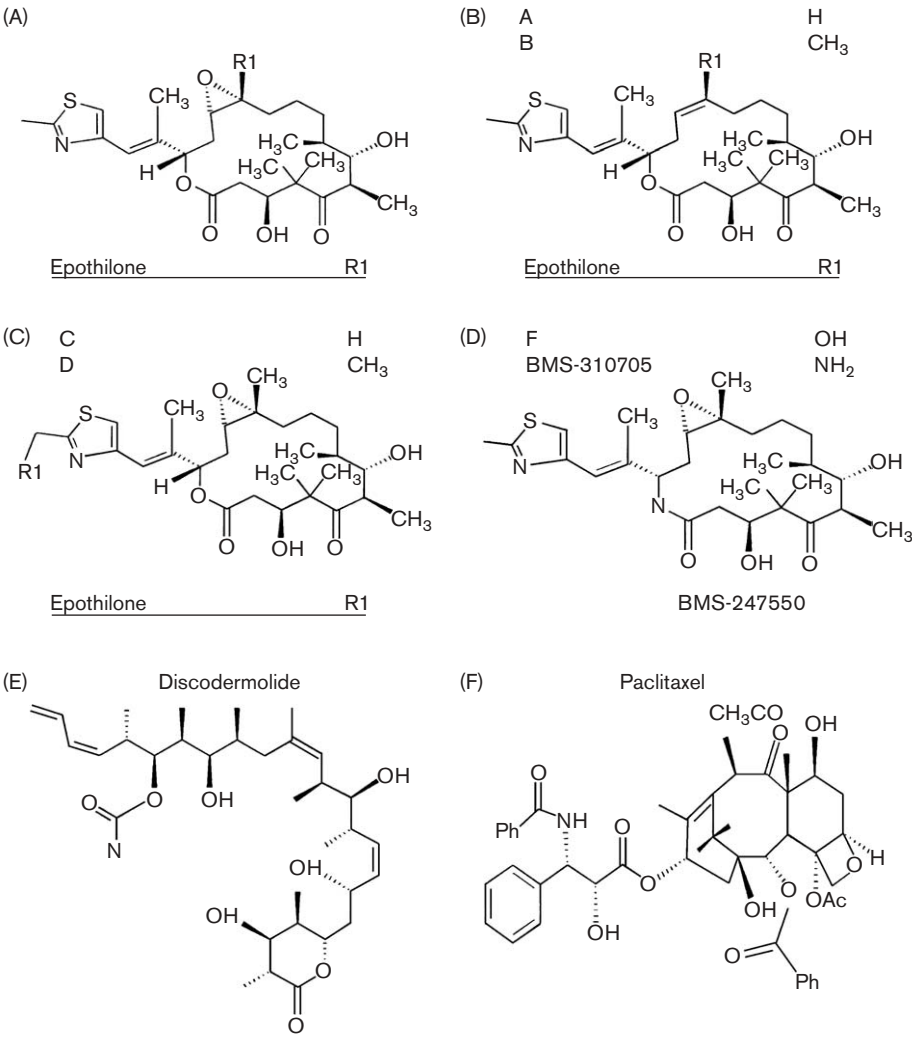
**Table 1**

| Drug/type | Pharmaceutical company | Study phase |
|---|---|---|
| Microtubule stabilizer | | |
| BMS-247550, epothilone analog | Bristol-Myers Squibb | II/III (planned) |
| BMS-310705, epothilone analog | Bristol-Myers Squibb | I |
| BMS-184476, taxane | Bristol-Myers Squibb | II |
| BMS-188791, taxane | Bristol-Myers Squibb | II |
| LEP, liposomal paclitaxel | Pharmacia | II |
| RPR 109881A, non-MDR substrate | Aventis | I/II |
| EPO 906, epothilone B* | Novartis | II |
| TXD 258, taxane | Aventis | II |
| XAA 296, discodermolide* | Novartis | I |
| Microtubule destabilizer | | |
| combrestatin A-4 phosphate | Bristol-Myers Squibb | II |
| ZD 6126 | AstraZeneca | II |
| vinflunine | Pierre Fabre | I/II |
| LU 103793 | BASF | I |
| dolastatin 10 | NCI | II |
| E7010 | Abbott | II |
| T138067 and T900607 | Tularik | II |

**Fig. 1**




Chemical structures of the microtubule-stabilizing drugs.

observed with *in vitro* tubulin polymerization assays, it is clear that the natural epothilones A and B are at least 2-fold more potent than paclitaxel in tubulin polymerization assays. In cell culture, epothilone B is generally 5–25 times more potent ($IC_{50}$ values about 0.1–0.6 nM) at inhibiting cell growth of human lung (A549, NCI-H460), colon (HCT-116, SW620), prostate (DU145, PC-3), breast (MDA-MB-231, MCF-7, BT-20, ZR-75-1), bladder (T-24), epidermoid (A431, KB-3.1) and ovarian (1A9) carcinoma cells when compared with either epothilone A or paclitaxel ($IC_{50}$ values about 1.3–5.7 nM). In MDR-expressing cancer cell lines, KB-8511, SW620AD-300 and 1A9PTX22 (also has a mutation in β-tubulin), epothilones maintained their activity; however, paclitaxel was at least 2-fold *less* potent compared to activity in parental cells. Modifications to region A, e.g. with epothilone C (Fig. 1B) and D (Fig. 1B), which lack an epoxide group result in potent tubulin polymerization agents [3,8]. Epothilone D is highly potent in inducing polymerization and is 2–10 times more cytotoxic than epothilone C in HCT-116 ($IC_{50}$ = 6.0 nM), A549 ($IC_{50}$ = 20 nM) and KB-3.1 ($IC_{50}$ = 24 nM) carcinoma cells [6,7,9]. BMS-247550, a synthetic analog of epothilone B, is also a potent inducer of tubulin polymerization, as is the case with the natural epothilones. The cytotoxic effects of BMS-247550 are preserved in cell lines that express MDR or harbor tubulin mutations and demonstrate resistance to paclitaxel effect. Despite the vast array of *in vitro* data regarding the cytotoxic effect of these compounds, there is a paucity of *in vivo* pharmacology [10]. Epothilone B is not well tolerated in mice and has a very narrow therapeutic index in this model system [11]. On the contrary, epothilone D is well tolerated and these model systems provide insightful data regarding efficacy of these agents when compared with paclitaxel [6,7,12]. Four epothilones have been studied in the clinic: epothilone B (EPO 906), epothilone D (KOS-862), epothilone B lactam (BMS-247550) and 21-aminoepothilone B (BMS-310705).

EPO 906 was one of the earliest epothilones entering clinical development [13]. It has been evaluated using a 5-min bolus administration every 21 days (42 patients) and a 5-min weekly infusion, administered 6 of every 9 weeks or 3 of every 4 weeks. The maximum dose tolerated was 2.5 and 8.0 mg/m$^2$, respectively, for the weekly and every-21-day schedule. Gastrointestinal toxicity (diarrhea) was the most common dose-limiting toxicity. The 21-day schedule resulted in a greater incidence of fatigue, nausea and peripheral neuropathy. On the weekly schedule, partial responses have been observed in breast, ovarian, carcinoid and endometrial carcinoma. On the every-21-day schedule, partial responses have been observed in breast, colon and ovarian cancer. Phase II studies of EPO 906 are in progress.

Epothilone D (KOS-862) has demonstrated similar potency to that of paclitaxel; however, it remains more potent than paclitaxel in cell lines that demonstrate multidrug resistance, largely through overexpression of P-gp. The $IC_{50}$ values for epothilone D when compared with paclitaxel are approximately 2.9 (MCF-7) to 88 (MX-1) nM and 3.3 (MCF-7) to 177 (MX-1) nM, respectively. *In vivo*, epothilone D has demonstrated superior potency as compared with paclitaxel in a variety of human tumor xenograft models that include, but are not limited to, HCT-116, K562 and SK-OV-3. Chemically synthesized and biologically derived epothilone D has equivalent activity and toxicity in an *in vivo* MX-1 mammary carcinoma model in nude mice. Both i.p. and i.v. methods of administration consistently provided enhanced tumor regressions in these models. In certain models, i.v. dosing appeared superior to i.p. dosing since complete tumor regressions were observed with the former mode of administration. Preclinical studies show that epothilone D is metabolized by CYP3A4 and the liver is a likely route of drug elimination; however, these observations have yet to be proven. Non-clinical toxicology has shown that in two species (rat and dog), the bone marrow compartment is likely to be most affected followed by other organ compartments such as the gonads, peripheral nerves and gastrointestinal tract, and loss of nutritional weight is also observed [6,7,12].

Epothilone D has entered clinical trials but is still in late phase I development. There are two ongoing studies, KOS-101 ($n$ = 27 receiving one dose every 21 days; $n$ = 6 receiving three consecutive daily doses every 21 days) and KOS-102 ($n$ = 8 receiving three consecutive daily doses every 28 days). The dose range studied in KOS-101 was 9–185 mg/m$^2$ for the single dose schedule and 20–40 mg/m$^2$ for the multiple dose schedules. In KOS-102, the dose level of 64 mg/m$^2$ has been exceeded. Overall, the most common drug-related toxicities reported thus far include fatigue (around 44%), anemia, nausea, transaminase elevations and peripheral neuropathy (incidence around 22%), and possibly transient cognitive changes. It is interesting to note that extensive prior chemotherapy use with neurotoxic agents may result in greater neuropathy from epothilone D. Epothilone D plasma distribution shows a rapid distribution phase with slow elimination and linear kinetics across dose levels studied. There is substantial binding to plasma proteins (around 99% bound) and an equally high degree of variation in plasma kinetics between individuals. Kinetic summaries for KOS-101 show a mean elimination $t_{1/2}$ about 9.9 ± 2.7 h, mean clearance approximately 14 l and $V_{dss}$ approximately 187 ± 83 l. These data suggest extensive tissue distribution, but a relatively short plasma half-life, in comparison with other drugs of this class. Finally, a comment worthy of note is that epothilone D is Cremophor based in its formulation, and administration and pre-medications typical of those used for taxanes will likely be used in its clinical development. There is limited myelosuppression, but greater degree of

gastrointestinal toxicity, than epothilone B. The neurotoxicity may be dose or schedule related; however, transient changes in mental status that occur perinfusional are dose dependent [14–18].

BMS-247550 possesses superior anti-tumor potency in comparison with paclitaxel, has entered clinical trials and has undergone significant clinical development [10,19,20]. The National Cancer Institute (NCI) in the US and Bristol-Myers Squibb have sponsored multiple trials, and are generating robust data on the safety and efficacy of this drug [20]. Several schedules in phase I drug testing have been evaluated: once every 21 days, daily × 5, weekly, and combination studies with a variety of other agents including, but not limited to trastuzumab, carboplatin and estramustine [20–22]. In its early phase of development, the three separate 21-day phase I studies indicated that the MTD was 40–50 mg/m$^2$ as a 1-h infusion q 21 days [19,20,23]. At 40 mg/m$^2$, significant neutropenia was still observed in one study, suggesting a very steep dose–toxicity relationship [22]. Overall, neutropenia, peripheral neuropathy and fatigue were dose limiting in all the studies. Since BMS-247550 is formulated in Cremophor EL, recent studies now include $H_1$ and $H_2$ histamine blockers as standard premedications. A remarkable finding in heavily pretreated patients was demonstration of responses in patients with lung, taxane-refractory breast, melanoma, ovarian and other malignancies [19,20,23,24]. Both taxane-refractory and taxane-insensitive tumors (e.g. colorectal cancer) have responded to this drug. A broad phase II development plan in virtually all solid tumor malignancies has been launched by the NCI. *In vivo* pharmacodynamic proof of mechanism of action has been demonstrated by investigators at the Albert Einstein College of Medicine, Bronx, NY, and further explorations of pharmacokinetic/pharmacodynamic relationships are ongoing there and elsewhere [21]. The summary of schedule-dependent toxicities in phase I suggests that the incidence of neuropathy is associated with high peak plasma levels of BMS-247550. Therefore, clinical trials are now focusing on the following alternate schedules: 3-h infusion q 21 days, 1-h infusion q week and 1-h infusion days 1–5 or days 1–3 q 21 days. It remains impressive that despite multiple schedules that have yet to be formally tested in phase II studies, impressive response rate in gastric (9%), prior treated non-small cell lung cancer (14%), and taxane-refractory and taxane-naive metastatic breast cancer (25 and 63%, respectively) have been observed. It is notable that the oral bioavailability of BMS-247550 is about 54% at a dose of 25 mg/m$^2$ [19,20,23,24].

BMS-310705 is the newest drug of this class to enter clinical trials. A critical difference between this drug and other epothilones is that BMS-310705 does not require Cremophor EL for formulation and has improved water solubility in comparison with BMS-247550. Presently, two phase I studies are ongoing and the drug appears to be tolerated at doses exceeding 50 mg/m$^2$ [25,26]. Anti-tumor responses have been observed in lung and ovarian cancer.

Discodermolide (Novartis; XAA 296) is a polyketide natural product originally isolated from extracts of the marine sponge *Discodermia dissolute* [27] and now may be completely synthesized [28–30]. The $IC_{50}$s for discodermolide in breast, prostate, colon, lung and ovarian cell lines vary between below 2.5 and 50 nM [31–34]. In virtually every cell line tested, discodermolide is more potent than paclitaxel and induces microtubule bundle formation at very low concentrations that are well below 10 nM [35]. In some cell lines resistant to paclitaxel due to overexpression of P-gp, discodermolide induces cell death at 5- to 6-fold less drug concentration than paclitaxel [32]. In other words, in comparing MDR phenotype cell lines, XAA296 is 35-fold more potent than paclitaxel. In cell lines resistant to paclitaxel, which are associated with mutations in β-tubulin (e.g. PTX10 and PTX22), discodermolide was about 4-fold more potent than paclitaxel [32]. The potency of effect is best observed with A549.EpoB40 cell lines that are resistant to epothilone B, but require epothilone B for growth [36]. These cell lines harbor a mutation in β-tubulin and the MDR1 gene is not expressed. In comparison with the parental cell line, paclitaxel was 22-fold less active and discodermolide was only 2-fold less active in the mutant cell line [36]. While the binding site of discodermolide is possibly the same as for paclitaxel, this has not been established as a fact. Since there is data suggesting synergy between paclitaxel and discodermolide in a variety of cell lines and *in vivo* animal model systems, it is conceivable that the binding sites of the two drugs are different [37,38].

There are some notable features of discodermolide that will have an impact on clinical development. In human tumor mouse xenograft models, single i.v. bolus administration appears to be an optimal schedule. In these mouse models, weight loss following administration of discodermolide was significant, which may be due to release of cytokines from tumors, and is currently under further study. Similar schedule-based efficacy has been observed with syngeneic rat tumor models. Single- and multiple-dose toxicology studies have been conducted in mice, rats and marmosets. Clinically relevant toxicities reflect cytotoxic effects in rapidly proliferating tissues including the bone marrow compartment, crypt cells of the intestines, and gonadal tissue and its derivatives. The maximally tolerated dose of discodermolide when administered to marmosets and rats was 0.15 and 1.0 mg/kg, respectively. The drug was administered as an i.v. dose every 3 weeks for 4 cycles. Preliminary ADME studies show that discodermolide is bound to protein and rapidly cleared from plasma. Discodermolide is extensively

distributed to most bodily tissues and has a long total body half-life (over 24 h) with metabolism likely restricted to the liver (predominantly CYP3A4/5 mediated) [39].

Presently, a single phase I study on-going at the Cancer Therapy and Research Center, San Antonio, Texas and Vanderbilt University, in which discodermolide is administered once every 22 days [39]. Dose levels up to 14.4 mg/m$^2$ have been explored thus far. Toxicities have included fatigue and diarrhea, but little neutropenia and neuropathy have been seen. The preliminary data on pharmacokinetics of discodermolide show multiphasic disposition of the drug in humans with blood concentrations rapidly falling to below 10% of $C_{max}$ in the first 24 h post-treatment. Non-linear kinetics is observed with a high variance between patients that is suggestive of significant inter-patient variation in metabolism. Efficacy evaluations are pending as dose escalation continues.

## Summary

Many new agents have been discovered and developed whose target is the polymerized microtubule. While many of these agents appear to be more potent than the first-generation taxanes, the results from preclinical data have been mixed with respect to determining if these new agents will have a greater therapeutic window in humans. A major reason for this uncertainty is that the discovery and development process of anti-microtubule agent is still virtually entirely empirical. Many agents are still discovered through screening of natural compounds. Generation of potentially useful derivatives has not been driven by exploitation of differences between the microtubules of malignant cells and the microtubules of normal cells. In fact, thus far there are no known differences between the microtubules of malignant and normal cells. Instead, development has focused on identification of agents that circumvent known markers of resistance to earlier generations of anti-tubulin agents, such as P-gp expression and tubulin mutation.

For now, the clinical development of this class of agents continues to be based on empirical testing in humans with a broad variety of tumors across a broad variety of schedules, for we cannot predict based on molecular biological principles the spectrum of activity of these new agents. In many respects, the clinical development of these new agents is identical to the development of the first generation taxanes, when it was not known if the spectrum of clinical utility would be similar or different from other tubulin interactive agents such as the vincas. Why is a malignant cell more likely to undergo apoptosis after exposure to a tubulin interactive agent, and what is the difference between tumor cells of different types that yield specific patterns of susceptibility and resistance? Until we have a better understanding of the pharmacodynamics of these agents from a molecular perspective, development will be business as usual.

However, some vexing problems in the clinical use of these drugs persist. Individualized dosing, which is now possible, has to be achieved in order to 'optimize' dosing schedules. More may not be better, as 'other' mechanisms of drug effect may need to be explored as in metronomic dosing (e.g. paclitaxel) or cyclic dosing targeting the various tumor/matrix compartments. The relative merits of dosing normalized to body surface area versus flat milligram or gram dosing strategies need to be explored further. Some of these problems are being addressed at our institution as there are further preclinical and clinical studies being performed that evaluate the effects of metabolism on the disposition of these drugs in the human body. Nerve conduction studies are the first step towards defining the pathophysiology of these drugs, after which preclinical biopharmacology would be required to define the signature molecular lesions in nerves. So while steps are being taken to get better drugs, there are significant strides being made towards defining physiologic based dosing that may be individualized for each person.

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