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Minireview

Discovery of Ixabepilone

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Abstract

The discovery of the antineoplastic agent paclitaxel and its unique activity as a microtubule-stabilizing agent resulted in dramatic improvements in the treatment of breast, ovarian, and non-small cell lung cancers. Despite the potent antitumor activity of taxanes such as paclitaxel, efficacy of these agents has been limited by development of taxane-resistant tumors in patients. This review describes, with some historical context, our successful efforts to discover a next-generation microtubule-stabilizing agent for the treatment of cancer. In collaboration with the Gesellschaft für Biotechnologische Forschung, we evaluated the epothilones, originally isolated from the myxobacterium *Sorangium cellulosum*, as potential anticancer agents. Experiments performed at Bristol-Myers Squibb confirmed the ability of these agents to induce tubulin polymerization, cell cycle arrest, and apoptosis. Epothilones A and B showed potent cytotoxic activity toward paclitaxel-sensitive and paclitaxel-resistant cells expressing P-glycoprotein or mutant tubulin. Because the parent epothilones were subject to inactivation via esterase cleavage, we used semisynthetic approaches to prepare analogues without this liability. BMS-247550 (ixabepilone), the lactam analogue of epothilone B, showed increased metabolic stability, potent tubulin polymerization activity, and retained activity against paclitaxel-resistant lines. Based on its shown efficacy in clinical trials, ixabepilone was approved by the Food and Drug Administration in 2007 for treatment of drug-resistant/refractory metastatic or locally advanced breast cancer. [Mol Cancer Ther 2009;8(2):275–81]

Introduction

Throughout most of human history, natural products were the nearly universal source of medicinal agents regardless of the nature of the malady. In the beginning of the modern pharmaceutical era, natural products continued to be a mainstay for the derivation of therapeutic agents. As the power of synthetic chemistry continually advanced through the last half of the 20th century, the increased complexity of synthetic organic compounds combined with dramatic increases in high-throughput screening capability and capacity to relegate natural products to a diminished role in drug discovery. Nevertheless, for medicines in selected disease categories such as anti-infective agents and cancer therapeutics, natural products have continued to be an important source of new drugs and new drug lead structures. It is reasonable to postulate that the greater prevalence of natural products found to be effective in such disease states is due to the fact that plant and animal chemical defense mechanisms are highly evolved to combat organisms with survival mechanisms similar to those of human pathogens and human cancer cells. Regardless, even in the current era of sophisticated synthetic chemistry, natural products remain an important source of chemical diversity for drug discovery.

In the early 1990s, the cancer drug discovery group at Bristol-Myers Squibb (BMS) began the search for a successor to our highly successful cancer drug Taxol. Following the original discovery of paclitaxel (1) and the elucidation of its unique mechanism of action as a microtubule-stabilizing agent (2), BMS, working closely with the National Cancer Institute, had developed paclitaxel into a mainstay therapy for breast, non-small cell lung, and other tumors. However, by the mid-1990s, the development of paclitaxel-resistant tumors in patients motivated us to seek a next-generation microtubule-stabilizing agent for cancer therapy.

A Trio of Natural Product Leads

A component of our comprehensive search for a successor to paclitaxel involved the development of next-generation taxanes. These efforts were directed toward identifying novel taxanes that were superior to paclitaxel with respect to its efficacy spectrum and pharmaceutical properties. As a result of an extensive program to discover such compounds, two i.v. delivered taxanes (BMS-184476 and BMS-188797) and one orally delivered taxane (BMS-275183) were advanced into clinical trials in the mid-1990s (3–5). Nevertheless, we have a high level of interest in identifying novel chemical structures that possessed microtubule-stabilizing properties, with the expectation that they might show more profound differentiation from taxanes.

BMS has had a long history in the exploration of natural products as drugs and drug leads both through dedicated internal efforts and through collaborations. In 1994, our

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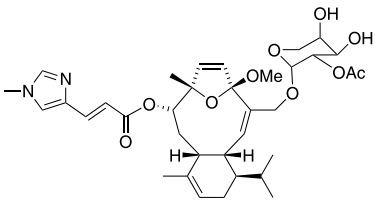
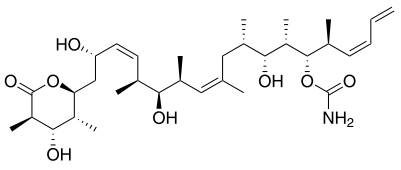
ongoing collaboration with Prof. William Fenical (Scripps Research Institute) led to the isolation of eleutherobin (Fig. 1) from a soft coral and the determination that this compound possessed microtubule-stabilizing properties (6, 7). The scarcity of the soft coral *Eleutherobia* sp. and the low yield of natural product from this source organism suggested that a dependable supply of eleutherobin would require a total synthesis approach, which we embarked on in collaboration with an academic group. In the midst of this collaboration, a group from Merck isolated the known natural products epothilone A and B and showed that they polymerized tubulin with potency and kinetics similar to paclitaxel (8). The epothilones were originally isolated from the myxobacterium *Sorangium cellulosum* by Profs. Hans Reichenbach and Gerhard Höfle [Gesellschaft für Biotechnologische Forschung (GBF)] in the early 1990s. In the original German patent application from GBF, the epothilones were noted to have antifungal activity and cytotoxic activity (9), but it was not until the 1995 article from Bollag et al. (8) that the mechanism of the cytotoxicity was revealed. Not long after the discovery of these two novel classes of tubulin stabilizers, a third natural product class with this property became known, the discodermolides. Discodermolide, isolated from the Caribbean sponge *Discodermia dissoluta* was originally reported in 1991 as an immunosuppressive agent (10, 11). In early 1996, two groups independently reported that the G_2-M block produced by discodermolide is a result of its ability to polymerize microtubules (12, 13).

Faced with three potential starting points for the discovery of a next-generation tubulin polymerizing agent, we attempted to generate fledgling research programs around each. From a chemistry perspective, this involved obtaining initial quantities of each natural product as well as generating a sustainable source of those that would prove to be most promising. We obtained small quantities of eleutherobin via our collaboration with the Fenical group, discodermolide from Harbor Branch Oceanographic Institute via a material transfer agreement, and epothilones A and B from GBF through another material transfer agreement. The exploratory biology effort involved *in vitro* and preliminary *in vivo* studies using the initial quantities of each material that we were able to obtain. As a contingency for anticipated future sourcing needs, we initiated collaborations with two separate academic groups on total synthesis efforts directed toward eleutherobin and discodermolide, respectively. These internal and external total synthesis efforts were driven by the difficulty in sourcing the multigram quantities of each of these complex natural products that we expected would be needed to drive a successful drug discovery program.

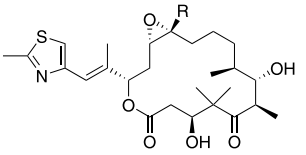
With the publication in 1995 describing the epothilones as having a relevant anticancer mechanism, we immediately recognized that, compared with eleutherobin and discodermolide, the ability to source the epothilones from fermentation provided an enormous advantage to this chemical class in our search for a novel anticancer agent. In addition to obtaining small quantities of the epothilones from GBF through a material transfer agreement,



Eleutherobin



(+)-Discodermolide



Epothilone A, R = H
Epothilone B, R = Me

	Tubulin $EC_{0.01}$	Cytotoxicity IC_{50}	Activity vs Resistant Cell Lines (MDR/Tubulin)	In Vivo Activity	NP Supply/ Synthetic Steps
Eleutherobin	3.1 μM	15 nM	Resistant / Resistant	Not active	Marine coral / 35
Discodermolide	0.4 μM	13 nM	Not Resistant / Resistant	Not active	Marine sponge / 36
Epothilone B	3.5 μM	2.6 nM	Not Resistant / Not Resistant	Borderline active	Myxobacterium fermentation / ~25

Figure 1. Structures of novel natural product microtubule-stabilizing agents eleutherobin, discodermolide, and epothilone A and B, tubulin-polymerizing activity (defined as an effective concentration to produce an initial slope of 0.01 in absorbance/min; ref. 21), cytotoxic potency (HCT116 human colon carcinoma cell line), cytotoxicity in cell lines resistant to paclitaxel via either MDR expression (HCT116/VM46) or tubulin mutation (A2780Tax), *in vivo* activity (see text for tumor models and route of administration), source organism, and longest linear sequence of total synthesis route shown by the late 1990s.

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we initiated scientific and business development contacts with GBF soon after the mechanistic article was published. Our aim was to establish a collaboration that would afford access to the intellectual property, microbiology expertise, and synthetic expertise that the GBF group had developed around the epothilones and their source organism.

In-house validation of epothilones as potential anticancer agents began in early 1997 with the receipt of small quantities of epothilones A and B from GBF. Our *in vitro* experiments confirmed that both natural products were capable of polymerizing microtubules and possessed potent cytotoxic activity toward not only cell lines that were paclitaxel-sensitive (HCT116 and A2780) but also cell lines that were paclitaxel-resistant by means of either P-glycoprotein expression (HCT116/VM46) or tubulin mutation (A2780Tax22; Fig. 1). Similar experiments with eleutherobin showed that this compound displayed potent cytotoxicity against the HCT116 cell line but was ~50-fold less potent against the HCT116/VM46 cell line, indicating that eleutherobin is a substrate for the P-glycoprotein efflux pump. Somewhat less cross-resistance was observed in the tubulin-mutated cell line. Discodermolide displayed nearly equipotent cytotoxicity in the parental HCT116 and the HCT116/VM46 cell lines. With limited quantities of material in hand, several attempts were made to show *in vivo* efficacy using each of the natural product classes. Using a M109 murine lung tumor model or a P388 murine leukemia tumor implanted i.p., i.p. delivery of eleutherobin failed to yield either an active result or a maximum tolerated dose. Awaiting a possible resupply of the scarce eleutherobin from the Fenical laboratory, we turned our attention to the other natural products. Discodermolide, delivered i.p., was shown to be inactive at its maximum tolerated dose in the i.p. M109 tumor model. In efficacy experiments with epothilones in the i.p. M109 model, i.v. delivery of epothilone A yielded a borderline active result, but epothilone B was inactive at its maximum tolerated dose. When the tumor was implanted s.c., each epothilone failed to show efficacy when given i.v. Understanding this lack of *in vivo* efficacy was identified early on as an important initial goal of the drug discovery program.

Focus Turns to Epothilones

During these initial biology experiments, and while negotiations were ongoing with GBF, the discovery chemistry project group, headed by Dr. Gregory Vite, commenced total synthesis efforts toward the epothilones. Retrosynthetic analysis identified the three key fragments shown in Fig. 2A. The acid fragment 7 and the thiazole fragment 3 were prepared using minor modifications of the methodology reported by Nicolaou et al. (14). The intermediate 6 was prepared by the asymmetric alkylation of the pseudoephedrine amide of propionic acid followed by reductive cleavage to the aldehyde. The total synthesis of epothilone C, and therefore a formal total synthesis of epothilone A, was achieved by a three-step sequence (15). Aldol condensation of the ethyl ketone 7, which carried a protecting group (P) on the alcohol, with the aldehyde 6 afforded 5 along with a diastereomer. Esterification of 5 with 3 to form 1 followed by cyclization via a ruthenium-catalyzed olefin metathesis reaction and subsequent deprotection provided epothilone C, which has been reported to undergo epoxidation to afford epothilone A (16). These internal efforts showed the technical feasibility of driving structure-activity studies of epothilones using total synthesis as was shown previously by successful total synthesis publications from several laboratories (14, 16, 18).

In parallel with our synthetic success, by the middle of 1997, BMS was able to reach an agreement with GBF around a collaboration on the epothilones. This important milestone triggered several enabling capabilities. First, BMS immediately gained access to multigram quantities of several epothilones, which were critical for both advanced biology testing and semisynthesis of novel analogues. Second, BMS gained access to strains of *S. cellulosum*, which, under the expert manipulation of Prof. Reichenbach, had already undergone substantial optimization with respect to production of epothilones. For example, the original strain was genetically heterogeneous and produced not only epothilones but also high amounts of other natural products such as spirangienes. Early optimization at GBF produced a clone that produced 60 mg/L epothilone A and 30 mg/L epothilone B, whereas a subsequent clone derived from this original clone produced 120 mg/L epothilone A and 60 mg/L epothilone B. A third advantage was that we gained access to the extensive knowledge base on the chemistry and biology of the epothilones, which had been accumulated by the work of Profs. Höfle and Reichenbach.

Challenge: Epothilone Metabolic Instability

One of the important pieces of information that we gained from GBF was the fact that the lactone ring of the epothilones was sensitive to esterase cleavage. Before the agreement with GBF being finalized, we have made similar inferences based on studies in the laboratories of Dr. Frank Lee and Dr. William (Griff) Humphreys, which showed that incubation of epothilone B in mouse plasma led to loss of its cytotoxic activity (Fig. 3A) and resulted in a metabolite with a mass 18 units higher than the parent drug. The relevance of this instability in mouse tumor models was made clear by Dr. Lee's subsequent demonstration that administration of the esterase inhibitor bis(4-nitrophenyl)-phosphate along with bolus injection of epothilone produced an active result in the A2780 ovarian carcinoma tumor model. In a similar demonstration that the lack of efficacy of epothilone delivered as a bolus was a result of systemic instability, a 10 h i.v. infusion of epothilone B (100 mpk) in the M5076 murine sarcoma model led to an active result (Fig. 3B). Nevertheless, the relevance and importance of esterase cleavage of the lactone was mitigated to some extent by our contemporaneous observation that the *in vitro* cytotoxic activity of epothilone B was reduced substantially less by incubation in human

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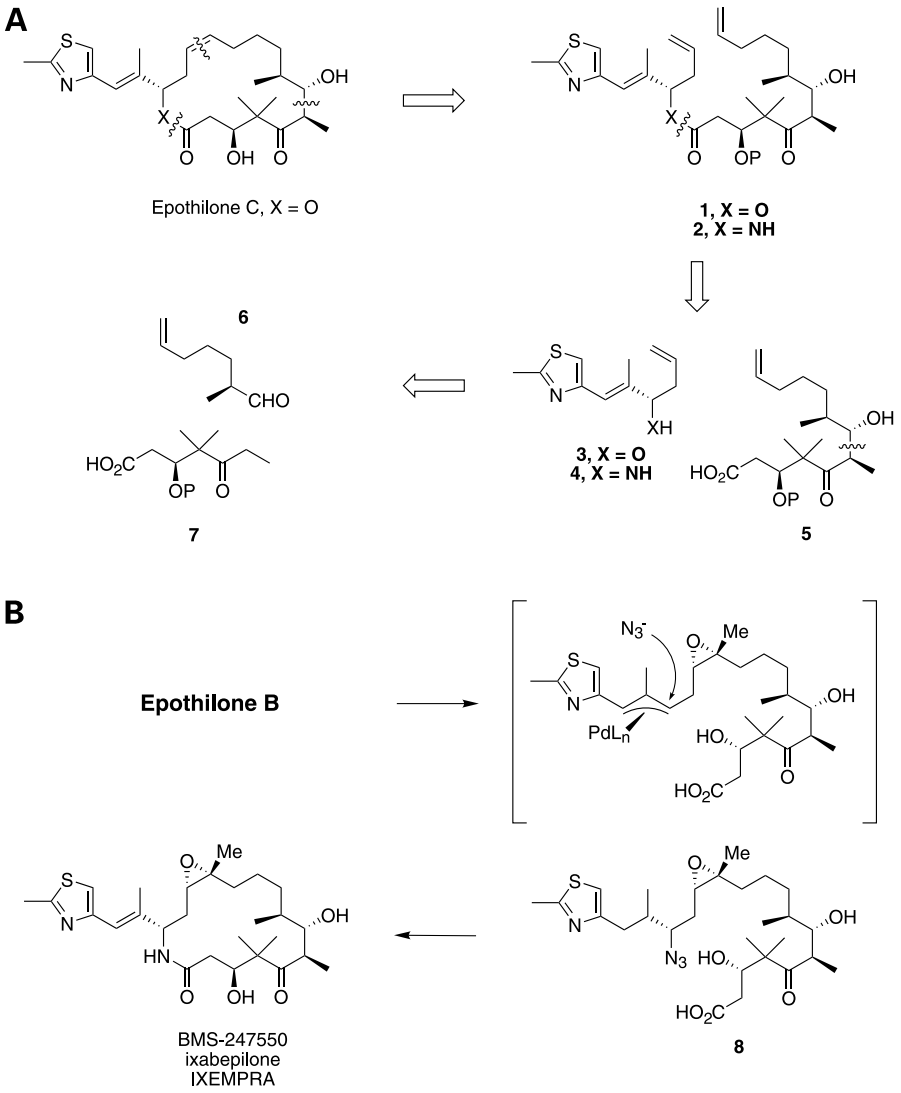

Figure 2. **A,** total synthesis scheme for the preparation of 12,13-olefinic epothilone analogues. **B,** semisynthesis scheme for the preparation of the epothilone β-lactam analogue BMS-247550 (ixabepilone) directly from epothilone B.

plasma, which is known to have much less esterase activity than mouse serum.

Therefore, the issues we faced included the expected difficulty in showing robust antitumor efficacy in mouse models with an epothilone lactone and the uncertainty around whether a lactone drug candidate would be stable in circulation in patients. These uncertainties led us to begin total synthesis efforts toward the lactam analogues of epothilones A and B with the goal of improving metabolic stability, using the key intermediate 4, the amine analogue of fragment 3 (Fig. 2A). Interestingly, incorporating 4 into the synthetic scheme for epothilone C allowed the preparation of the key olefin metathesis precursor, amide 2, but initial attempts at ruthenium-catalyzed metathesis led to the predominant formation of the 12,13-*trans*-olefinic macrocycle rather than the lactam of epothilone C, which has a *cis*-olefin (15). However, with multigram quantities of epothilones A and B now in hand from GBF, we diverted our total synthesis efforts and turned our attention toward semisynthetic approaches to the epothilone lactams and other analogues.

Our structure-activity studies of epothilones, driven by semisynthesis, involved modifications at many positions of the natural product. These included modifications of both the side chain and the macrocycle. The chemical handle of the 12,13-epoxide, the full utility of which was realized by our transformation directly to the 12,13-olefin, led us to focus a substantial amount of our effort in this region of the macrocycle, resulting in interesting new analogues that contained an amide, a cyclopropane, or an aziridine in place of the epoxide (19, 20). Nevertheless, the lactam analogues of epothilones A and B remained prioritized targets. The key insight into the synthesis of the lactams came with the realization by Dr. Bob Borzilleri that the epothilone lactone is allylic and therefore possibly susceptible to a palladium-catalyzed ring opening to form a π-allylpalladium complex, which could be trapped by a nitrogen nucleophile. Indeed, reaction of unprotected

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epothilone B with azide and a palladium(0) catalyst provided a reasonable yield of the ring-opened azide 8 as a single diastereomer (Fig. 2B; ref. 15). The azide was formed with net retention of configuration likely through anti-attack by palladium and then reaction with azide from the face opposite the palladium. Reduction of the azide and cyclization completed the synthesis of the amide analogue of epothilone B, which was given the internal compound number BMS-247550, later to be named ixabepilone and ultimately IXEMPRA. The lactam analogue of epothilone A was produced using a similar route.

Solution: Lactam BMS-247550

Gratifyingly, the lactam BMS-247550 showed potent tubulin polymerization activity, with an $EC_{0.01}$ value (3.5 μmol/L) similar to that of epothilone A (2.0 μmol/L) or epothilone B (1.8 μmol/L) and ~2-fold lower than paclitaxel (6.5 μmol/L). BMS-247550 showed highly potent cytotoxicity across a broad panel of tumor cells, with a median IC_{50} value of 2.9 nmol/L (21). Potent and expansive activity was also shown across a panel of breast (35 lines), colon (20 lines), and lung (23 lines) cell lines (22). Similar to paclitaxel, BMS-247550 was mechanistically confirmed to block cells in the mitotic phase of the cell division cycle. Moreover, the concentration of BMS-247550 needed to arrest cells in mitosis corresponded well to the concentration required to kill cells over the same treatment duration. Importantly, a comparison of the cytotoxicity of BMS-247550 and paclitaxel in cell lines with different mechanisms of drug resistance indicated that, like the natural product epothilones, the lactam retained sensitivity toward paclitaxel-resistant lines driven by drug efflux pumps as well as tubulin mutations (Table 1).

The success of an anticancer agent is dependent not only on its antitumor activity as a single agent but also on its ability to combine successfully with other antineoplastic drugs. Like paclitaxel, the cytotoxicity of BMS-247550 is strongly dependent on growth phase and cell cycle progression. Colony-forming assays were used to examine the cytotoxicity of BMS-247550 in combination with several selected anticancer agents of diverse mechanisms of action *in vitro.* Isobologram analyses showed that the mode of interaction between BMS-247550 and other cytotoxic agents *in vitro* is highly drug, sequence, and dose dependent. For example, with paclitaxel, combined treatment resulted in additive effects whether BMS-247550 was given first, second, or simultaneously. In the case of cisplatin, additivity was observed when the two agents were used sequentially, but synergism was obtained for simultaneous treatment.

Having established that the lactam BMS-247550 maintained the beneficial *in vitro* properties of the parent epothilones, our attention turned toward establishing that this semisynthetic epothilone analogue had *in vivo* properties suitable for a development compound. These *in vivo* studies, as well as the *in vitro* studies described previously, were being carried out in parallel on many analogues, which were derived from a variety of other semisynthetic epothilone analogues (19, 20). As we have anticipated, the lactam analogue proved to be stable to hydrolysis on incubation in both mouse and human sera (mouse serum; Fig. 3A). Pharmacokinetic analysis showed that BMS-247550, delivered as an i.v. bolus in mice, showed rapid systemic clearance and extensive tissue distribution, with a terminal half-life of ~3 h. The protein binding of BMS-247550 was 87% and 85% in mouse and human plasma, respectively, indicating the presence of substantial free drug in each species.

Our first demonstration of *in vivo* activity occurred in the A2780 human ovarian carcinoma tumor model, where BMS-247550 proved to have superior efficacy compared with paclitaxel in this paclitaxel-sensitive model (Table 1). This activity was shown using our standard q2d × 5 schedule, but even more robust efficacy was observed on a more intermittent schedule (q4d × 3). Therefore, although our additional efficacy experiments used the every other day schedule, we have provided strong evidence that

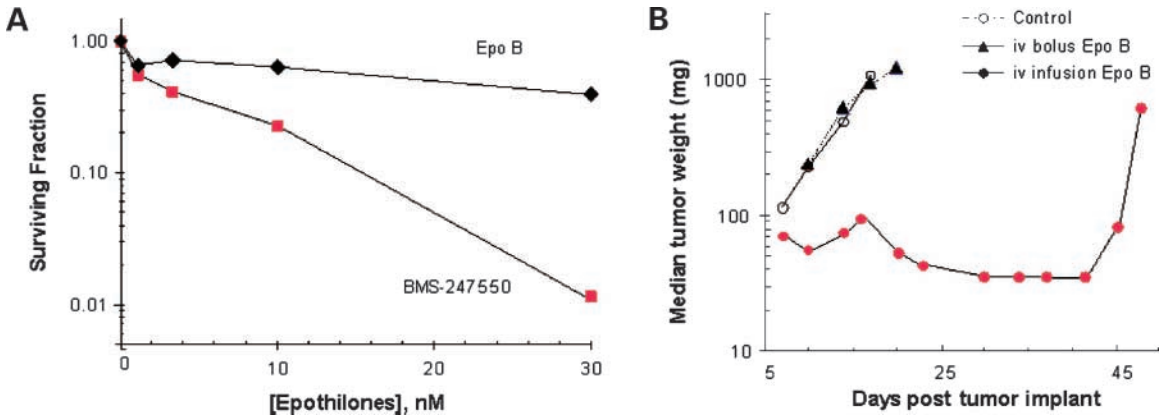

Figure 3. **A,** plasma stability of epothilone B and BMS-247550 in mouse serum measured by residual cytotoxicity against HCT116 human colon carcinoma cells. **B,** antitumor efficacy (M5076 murine sarcoma model) of epothilone B delivered as either a bolus injection or as a 10 h i.v. infusion.

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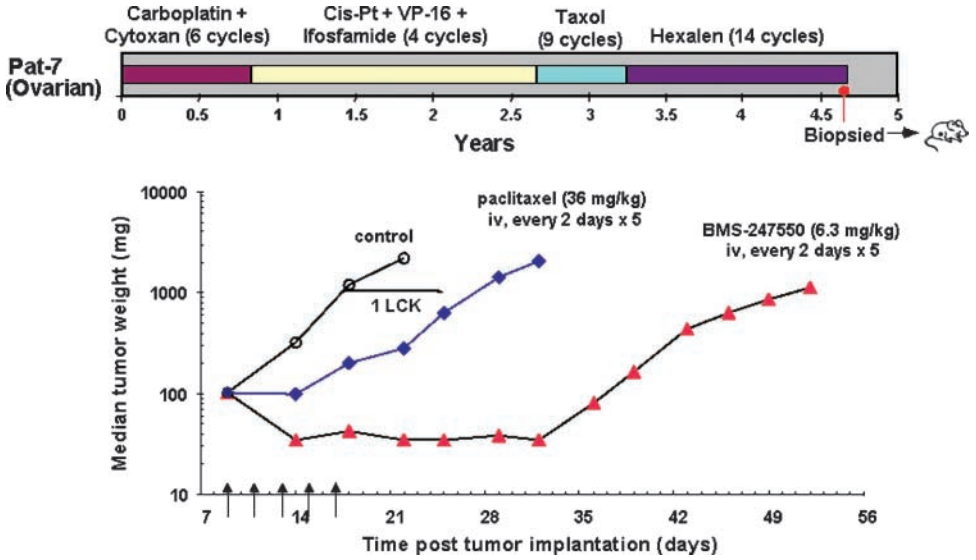

Figure 4. Antitumor efficacy of paclitaxel and BMS-247550 in the Pat-7 human ovarian carcinoma model.

trials with ixabepilone could explore intermittent schedules with the likelihood of showing clinical activity. To make a stronger case for BMS-247550 as a development compound, we tested it in more stringent models that were paclitaxel-insensitive or paclitaxel-refractory. In concordance with results from *in vitro* cytotoxicity comparisons, BMS-247550 showed substantial efficacy in two models in which paclitaxel was inactive: the A2780Tax model, which is resistant by means of tubulin mutation, and the HCT/VM46 model, which is resistant by means of MDR expression. Seeking to extend our *in vivo* studies to a more clinically relevant tumor, we established the Pat-7 tumor. This tumor was derived directly, without intermediate passage *in vitro*, from the ovarian carcinoma of a patient who had developed resistance to paclitaxel following nine courses of monotherapy. This patient had also failed repeated cycles of other anticancer agents, including carboplatin/cytoxan, cisplatin/VP-16/ifosfamide, and Hexalen. The Pat-7 tumor showed only a modest response to an optimized treatment with paclitaxel likely as a result of the expression of MDR as well as multidrug resistance protein. In contrast, a dramatic response was observed on treatment with BMS-247550 (Fig. 4).

To Clinical Development and Beyond

The complete preclinical data package, but particularly the robust *in vivo* efficacy profile, convinced us that BMS-247550 had the potential to provide significant clinical benefit to patients. We therefore triggered the considerable investment in resources to deliver BMS-247550 to patients in the clinic. There were still major issues that needed to be addressed going forward. These included further fermentation optimization to produce development quantities of epothilone B, modifying the discovery synthetic route to ensure a scalable process, addressing pharmaceutics and stability issues to allow i.v. delivery of the drug in patients, the need to more completely understand the combinability of BMS-247550 with other anticancer agents, and the desire to understand the mechanisms behind the efficacy of BMS-247550 in paclitaxel-resistant tumors

Table 1. ***In vitro*** **and** ***in vivo*** **activity of ixabepilone in paclitaxel-sensitive and paclitaxel-resistant cells and tumors**

	Ixabepilone	Paclitaxel
In vitro IC_{90} (nmol/L)		
HCT116 (paclitaxel-sensitive)	7.3	18
HCT/VM46 (paclitaxel-resistant, MDR)	16	450
A2780S (paclitaxel-sensitive)	6.9	9.7
A2780Tax (paclitaxel-resistant, tubulin mutation)	12	140
In vivo log cell kill		
HCT116 (paclitaxel-sensitive)	>6.3 (7/8 cures)	>4.8 (3/8 cures)
HCT116/VM46 (paclitaxel-resistant, MDR)	2.4	0.6
A2780S (paclitaxel-sensitive)	≥4.8	2
A2780Tax (paclitaxel-resistant, tubulin mutation)	2.5	0.8

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(The story of how elevated expression of specific tubulin proteins such as βIII tubulin contribute to development of clinical resistance to taxanes is the subject of a separate minireview in this issue of *Molecular Cancer Therapeutics*.). The BMS discovery and development organizations united behind BMS-247550 to solve these and other issues with the compound, enabling the implementation of a robust clinical development plan. Our commitment to BMS-247550 proved to be justified, with the approval by the Food and Drug Administration in 2007 of ixabepilone (IXEMPRA) as monotherapy for the treatment of metastatic or locally advanced breast cancer resistant or refractory to anthracyclines, a taxane, and capecitabine. The Food and Drug Administration also granted approval of IXEMPRA in combination with capecitabine for the treatment of metastatic or locally advanced breast cancer resistant to treatment with an anthracycline, a taxane, or whose cancer is taxane resistant and for whom further anthracycline therapy is contraindicated.

Conclusion

The road from the identification of an obscure secondary metabolite with antifungal properties to an approved drug that is now providing benefit to cancer patients was a long one. Along the way, there were numerous tangents, as we sought to define if natural products other than epothilones were the preferred drug lead. There was an exemplary collaboration between GBF, a private research institution, and BMS, a pharmaceutical company, with a shared goal of delivering an improved medicine to patients. There was an intense and focused effort by a team of dedicated scientists in both discovery and development. But in the end, the approval of ixabepilone for the treatment of metastatic breast cancer validated and rewarded our efforts.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Discovery of Ixabepilone

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