In vivo Evaluation of Ixabepilone (BMS247550), A Novel Epothilone B Derivative, against Pediatric Cancer Models

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Abstract

Purpose: *Vinca* alkaloids, agents that cause depolymerization of microtubules, are highly active in treatment of many pediatric cancers. In contrast, taxanes, agents that stabilize microtubules, are far less effective against the same cancer types. The purpose of the current study was to evaluate the antitumor activity of ixabepilone, an epothilone B derivative representing a new class of microtubule-stabilizing antimitotic agent in a wide variety of pediatric solid tumor models.

Experimental Design: Ixabepilone was administered i.v. every 4 days for three doses to *scid* mice bearing s.c. human rhabdomyosarcoma (three lines), neuroblastoma (four), Wilms' tumors (six), osteosarcoma (four), or brain tumors (seven). Tumor diameters were measured weekly, and tumor growth or regressions were determined. Pharmacokinetic studies were done following a single administration of drug at the maximum tolerated dose (MTD) level (10 mg/kg).

Results: At the MTD (10 mg/kg), ixabepilone induced objective responses (all tumors in a group achieved $\geq$50% volume regression) in three of three rhabdomyosarcoma lines, three of five neuroblastomas, six of seven Wilms' tumor models, two of six osteosarcoma, and four of eight brain tumor models. However, the dose-response curve was steep with only 2 of 19 tumors models regressing ($\geq$50%) at 4.4 mg/kg. In comparison, paclitaxel administered at the MTD on the same schedule failed to induce objective regressions of three tumor lines that were highly sensitive to treatment with ixabepilone. Pharmacokinetics following single i.v. administration of ixabepilone at its MTD (10 mg/kg) were biexponential with C_{max} of 12.5 μmol/L, elimination half-life of 19.2 hours, and total area under the curve of 5.8 μmol/L-h. The achieved drug exposure of ixabepilone at this efficacious MTD dose level in mice is similar to those achieved in patients given the recommended phase II dose of 40 mg/m^2 by either 1- or 3-hour infusion every 3 weeks, a regimen that has shown significant anticancer activity in phase II clinical trials in adult patients.

Conclusions: Administered at doses ranging from 66% to 100% of its MTD in mice, the epothilone B derivative ixabepilone shows broad spectrum activity against a panel of pediatric tumor xenograft models. Pharmacokinetic analysis indicates that the systemic ixabepilone exposure achieved in mice at its MTD is similar to that achieved in patients at the recommended phase II dose of 40 mg/m^2 administered every 3 weeks. Importantly, the present results showed a clear distinction in sensitivity of pediatric solid tumors to this epothilone derivative compared with paclitaxel.

The discovery of paclitaxel as a microtubule-stabilizing agent with impressive therapeutic activity against several human cancers has spurred interest in identifying nontaxane entities that have superior activity to the clinically approved agents, taxanes. Epothilones were first isolated as a fermentation product of the myxobacterium *Sorangium cellulosum* (1) and were shown to have taxane-like activity causing microtubule stabilization *in vitro* (2). Ixabepilone (BMS-247550) is a semisynthetic epothilone B derivative (Fig. 1) in which the macrolide ring oxygen atom is replaced with a nitrogen atom to give the corresponding macrolactam. Whereas other natural products, such as discodermalide, elutherobin, and the structurally related sarcodictins, have been shown to exert microtubule-stabilizing properties, ixabepilone was selected for development because of its greater metabolic stability and ease of preparation through fermentation. *In vitro*, ixabepilone is more potent than paclitaxel in stabilizing microtubules, being twice as potent in inducing microtubule polymerization. Of importance is that both *in vitro* and *in vivo*, ixabepilone retains cytotoxic activity in tumor cells that are intrinsically insensitive to paclitaxel and in lines selected for acquired resistance to paclitaxel. Comprehensive *in vivo* evaluation of ixabepilone against tumor lines resistant to paclitaxel showed that this epothilone B derivative

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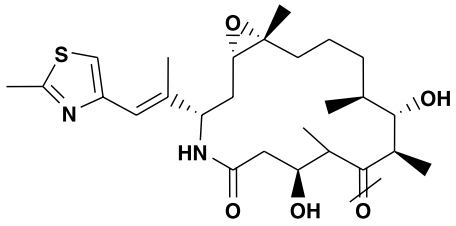

Ixabepilone (BMS-247550-01)

Fig. 1. Chemical structure of ixabepilone [1*S*-[1*R**,3*R**(*E*),7*R**,10S*,12*R**,16*S**]]-7,11-dihydroxy-8, 8,10,12,16-pentam-ethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl) ethenyl]-17-oxa-4-azabicyclo [14.1.0] heptadecane-5,9-dione.

retained activity against paclitaxel-resistant A2780TAX ovarian tumor xenografts and had significant antitumor activity against pancreatic and breast carcinoma xenograft models that are intrinsically insensitive to the taxanes (3). In these studies, ixabepilone showed either equivalent activity or superior antitumor activity compared with paclitaxel administered at optimal doses and schedules. In addition, ixabepilone was shown to be effective in polymerizing tubulin in the freshly biopsied tumor cells of a patient who developed resistance to taxotere and whose tumor exhibited MDR and MRP-1. This patient achieved a partial response in a phase I trial of ixabepilone (4).

Phase I clinical trials have evaluated several schedules of administration (5–11). Abraham et al. (5) administered the drug daily as a 1-hour infusion for 5 consecutive days every

Table 1. Responses of pediatric solid tumors to ixabepilone

Tumor	Dose (mg/kg)	Mice/group	Time to 4× volume (wk ± SD)	*P*	PR	CR	MCR
Rhabdomyosarcoma							
Rh18	0	7	1.8 ± 0.4				
	10	7	9.0 ± 2.3	<0.001	3	4	2
Rh30	0	7	2.0 ± 0.0				
	10	7	7.8 ± 2.2	0.002	1	5	1
	6.6	7	7.1 ±1.9	0.002	2	2	0
	4.4	7	3.6 ± 0.5	0.002	0	0	0
Rh36	0	7	2.6 ± 0.5				
	10	7	>12	0.001	0	7	7
Neuroblastoma							
NB1382	0	7	2.0 ± 0.0				
	10	7	>12	0.005	0	7	6
	6.6	7	>12	0.005	0	7	6
	4.4	7	4.8 ± 2.0	0.014	1	2	0
NB1643	0	7	2.0 ± 0.0				
	10	7	11.0 ± 0.0	0.079	5	1	0
	4.4	6	7.8 ± 2.6	0.222	1	2	0
NB1691	0	6	1.8 ± 0.4				
	10	7	5.4 ± 1.7	0.002	0	0	0
	4.4	7	1.9 ± 0.9	0.948	0	1	0
NB7	0	5	1.3 ± 0.5				
	10	5	>12	0.008	0	5	5
	6.6	5	5.0 ± 2.9	0.151	2	0	0
	4.4	5	4.3 ± 0.6	0.032	0	1	0
NB8	0	5	2.0 ± 0.0				
	10	5	2.0 ± 0.0	1.000	0	0	0
	6.6	5	3.0 ± 0.0	0.008	0	0	0
	4.4	5	2.2 ± 0.8	0.444	0	0	0
Osteosarcoma							
OS1	0	5	3.2 ± 0.4				
	10	5	>12	0.008	0	5	5
OS2	0	10	4.2 ± 0.4				
	15	10	8.1 ±1.9	0.001	1	3	0
	10	9	6.9 ± 1.3	0.004	0	0	0
OS17	0	5	2.4 ± 0.5				
	15	5	2.3 ± 0.5	0.432	0	0	0
	10	5	3.4 ± 0.7	0.042	1	0	0
OS21	0	5	2.6 ± 0.5				

(*Continued on the following page*)

21 days. The dose-limiting toxicity was neutropenia, and the recommended phase II dose was 6 mg/m^2. Objective responses were reported in patients who previously received paclitaxel treatment, suggesting a lack of cross-resistance as indicated in the preclinical studies. Responses were observed in patients with breast cancer, cervical cancer, and basal cell carcinoma. Other phase I trials have evaluated ixabepilone administered weekly or every 3 weeks (6, 7). The recommended dose on the weekly schedule was ~30 mg/m^2 and on the every 3-week schedule ~50 mg/m^2. Thus, per cycle of therapy, it seems that weekly dosing allows higher cumulative administration with 80 to 120 mg/m^2 total dose compared with ~30 mg/m^2 on the daily (for 5 days) schedule or 50 mg/m^2 on the 21-day schedule. Responses included patients with ovarian carcinoma, non–small cell carcinoma of the lung, breast carcinoma, and melanoma using the every 3 weeks schedule; and colon carcinoma, ovarian carcinoma, and breast carcinoma on the weekly schedule. The dose-limiting toxicity for weekly ixabepilone was grade 3 fatigue. Additional toxicities included cumulative neuropathy, arthralgia, myalgia, anorexia, nausea, and diarrhea (6, 7).

In the treatment of pediatric solid tumors and hematologic malignancies, *Vinca* alkaloids have an established role (12, 13). Vincristine is typically used in combination therapies for treatment of soft tissue sarcomas, Ewing sarcoma, Wilms' tumor, other extracranial solid tumors as well as medulloblastoma, and other malignant tumors of the central nervous system. Preclinical models of rhabdomyosarcoma at diagnosis and heterografted to immunodeprived mice show marked sensitivity to

Table 1. Responses of pediatric solid tumors to ixabepilone

Tumor	Dose (mg/kg)	Mice/group	Time to 4× volume (wk ± SD)	*P*	PR	CR	MCR
	15	10	3.1 ± 0.3	0.238	0	0	0
	10	10	2.9 ± 0.3	0.505	0	0	0
	6.6		2.6 ± 0.5	1.00	0	0	0
OS29	0	5	4.3 ± 2.2				
	10	5	9.0 ± 0.0	0.003	1	1	0
	6.6	5	4.4 ± 0.5	0.564	0	1	0
	4.4	5	8.6 ± 1.8	0.024	2	1	0
OS33	0	10	2.5 ± 0.7				
	15	10	7.4 ± 0.7	<0.001	1	4	0
	10	10	5.6 ±1.2	<0.001	1	0	0
Wilms' tumor							
WT5	0	5	3.0 ± 1.2				
	10	10	9.5 ± 1.0	0.008	1	4	0
	6.6	10	3.8 ± 0.8	0.685	0	0	0
	4.4	10	4.0 ± 1.2	0.907	0	0	0
WT6	0	5	5.6 ± 0.5				
	10	5	>12	0.008	0	5	5
	6.6	5	9.3 ± 2.9	0.008	0	2	0
	4.4	5	9.5 ± 1.3	0.008	0	0	0
WT8	0	5	4.8 ± 0.4				
	10	5	>12	0.008	0	5	5
	6.6	5	4.2±0.8	0.175	0	0	0
WT9	0	5	2.2 ± 0.4				
	10	5	>12	0.008	0	5	5
	6.6	5	4.0 ± 0.8	0.016	0	2	0
	4.4	5	3.5 ± 1.3	0.103	0	0	0
WT10	0	5	2.0 ± 0.0				
	10	5	3.0 ± 1.2	0.074	0	0	0
	6.6	5	6.0±2.5	0.024	0	3	0
	4.4	5	5.8±1.8	0.008	0	3	0
WT11	0	5	2.0 ± 0.0				
	10	5	8.5 ± 2.1	0.008	1	4	3
SKNEP	0	5	1.2 ± 0.4				
	10	5	>12	0.008	0	5	5
	4.4	5	3.0 ± 0.7	0.016	0	0	0

Abbreviations: PR, partial response; CR, complete response; MCR, maintained complete response.

Table 2. Responses of brain tumor xenografts to ixabepilone

Brain tumors	Dose (mg/kg)	Mice/group	Time to 4× volume (wk ± SD)	*P*	PR	CR	MCR
GBM2	0	7	4.9 ± 0.4				
	10	7	7.0 ± 1.0	0.001	1	3	0
GBM2	0	7	4.7 ± 1.0				
	10	7	6.3 ± 2.4	0.245	2	0	0
BT27	0	7	2.0 ± 0.0				
	10	7	>12	0.001	1	6	6
	4.4	7	9.0 ± 0.0	0.008	0	5	2
BT29	0	5	3.0 ± 0.8				
	10	5	5.8 ± 1.5	0.040	0	0	0
BT35	0	5	2.0 ± 0.0				
	15	5	>12	0.008	0	5	4
	10	5	>12	0.008	0	5	4
BT36	0	7	9.5 ± 3.5				
	10	7	>12	0.192	0	7	4
	6.6	7	7.0 ± 0.0	1.000	0	1	0
	4.4	7	6.5 ± 0.7	0.841	0	1	0
BT37	0	7	2.7 ± 0.5				
	15	7	8.0 ± 0.0	0.001	2	5	1
	10	7	>12	0.001	1	6	4
	6.6	7	6.3 ± 1.5	0.001	1	1	0
	4.4	7	5.3 ± 0.6	0.001	0	0	0
BT40	0	7	2.7 ± 0.5				
	10	7	5.8 ± 0.4	0.001	0	0	0
	6.6	7	5.8 ± 2.2	0.001	1	1	0
	4.4	7	5.2 ± 2.6	0.005	0	1	0
BT41	0	5	8.0 ± 1.9				
	10	5	7.5 ± 2.1	0.198	0	5	0
BT48	0	5	2.0 ± 0.7				
	15	5	3.2 ± 0.4	0.040	0	0	0

Abbreviations: PR, partial response; CR, complete response; MCR, maintained complete response.

vincristine, whereas tumors taken at relapse following vincristine, actinomycin D, and cyclophosphamide therapy and heterografted to rodents are significantly less sensitive to this agent (14). Similarly, xenografts developed from diagnosis samples of favorable histology Wilms' tumors (nephroblastoma) are exquisitely sensitive to vincristine, whereas xenografts developed from diagnosis rhabdoid tumors of the kidney are essentially refractory to this microtubule-destabilizing agent.[4] The implication is that early-passage xenografts from several pediatric cancer types accurately recapitulate the known sensitivity to chemotherapeutic agents of their respective cancer types.

The role of taxanes in pediatric oncology is less well established. Paclitaxel and docetaxel, agents that stabilizes microtubules, showed only sporadic activity in pediatric malignancies in phase I trials (15, 16) and no phase II trials against solid tumors have been reported. In a phase II clinical trial against brain tumors at relapse, paclitaxel showed poor activity, inducing a response rate of only 5.7% (17). This is in contrast to its clinical activity against carcinomas of adults (18–20). Our evaluation of paclitaxel and docetaxel predominantly against rhabdomyosarcoma xenografts showed marginal activity with regression of only occasional tumors (21).[5] Phase I trials of ixabepilone in children, where the drug was administered daily for 5 days, has shown this agent to be well tolerated at dose levels exceeding the maximum tolerated dose (MTD) in adults, with disease stabilization in several patients (22). Thus, it was of interest to evaluate the antitumor activity of ixabepilone and the antimitotic agent with a taxane-like mechanism of action in panels of pediatric cancers.

Materials and Methods

Growth inhibition studies. CB17/Icr female *scid*$^{-/-}$ mice (Charles Rivers, Wilmington, MA) were implanted s.c. with a single tumor fragment as described previously (23, 24). Mice bearing s.c. tumors each received ixabepilone when tumors were ~0.20 to 1 cm diameter. The procedures have been reported previously (24). Tumor-bearing mice were randomized into groups of 5 to 10 before therapy. All mice

[4] J. Dome and P. Houghton, unpublished results.

[5] Unpublished data.

were maintained under barrier conditions. All experiments were conducted using protocols and conditions approved by the St. Jude Children's Research Hospital Institutional Animal Care and Use Committee.

Tumor response and tumor failure time. A partial response was defined as a ≥50% decrease in tumor volume but with measurable tumor (≥0.10 cm^3) remaining at all times. Complete response was defined as a disappearance of measurable tumor mass (<0.10 cm^3) at some point within 12 weeks after initiation of therapy. Maintained complete response was complete response without tumor regrowth within the 12-week study time frame. To meet the definition of partial response or complete response for a tumor line, all tumors within a group had to show that response. Tumor failure time was defined as the time (in weeks) required by individual tumors to quadruple their volume from the initiation of therapy.

Statistical methods. For comparisons of time to tumor failure for different treatment regimens, survival distributions of each treatment group were compared with the survival distribution of the control group using the exact log rank test. Adjustments were made for multiple comparisons using a Bonferroni correction. SAS version 8.2 (with Proc StatXact) was used for statistical analysis.

Drugs and formulation. Ixabepilone and paclitaxel were provided by Bristol Myers Squibb, Co. (Princeton, NJ), formulated in cremophor/ethanol/water (1:1:8), diluted in sterile saline, and administered i.v. (0.1 mL/10 g body weight) at dosages ranging from 4.4 to 15 mg/kg as a short injection (duration of administration was <1 minute) into lateral tail vein. Mice received three administrations of each agent at 4-day intervals (q4d × 3). Vincristine was purchased from the hospital pharmacy and diluted in sterile water. Vincristine was administered weekly for 8 consecutive weeks by i.v. injection.

Plasma pharmacokinetics. Plasma samples (30 μL) were deproteinized with three volumes of acetonitrile containing 5 μg/mL of epothilone A as internal standard. After centrifugation to remove precipitated proteins, a 10 μL portion of clear supernatant was analyzed by high-performance liquid chromatography/tandem mass spectrometry. The high-performance liquid chromatography system consisted of a Hewlett Packard model 1100 HPLC/Autosampler. The column used was a Phenomenex Luna C18-ODS2, 2 × 50 mm, 3 μmol/L particles, maintained at 60°C and a flow rate of 0.5 mL/min. The mobile phase consisted of 5 mmol/L ammonium acetate in water (A) and acetonitrile (B). The initial mobile phase composition was 70% A/30% B. After sample injection, the mobile phase was changed to 50% A/50% B over 4 minutes, and held at that composition for an additional 2 minutes. The mobile phase was then returned to initial conditions and the column was reequilibrated. High-performance liquid chromatography was interfaced to a Finnigan LCQ-Advantage ion-trap mass spectrometer operated in the positive electrospray, full tandem mass spectrometry mode. For ixabepilone, fragmentation of m/z 507 [M + H]+ yielded daughter ions for quantitation at m/z values of 420 and 489. For the internal standard, m/z 494 [M + H]+ was fragmented to yield daughters for quantitation at m/z 476. Helium was the collision gas. The retention times for ixabepilone and the internal standard were 3.2 and 4.1, respectively. The standard curve ranged from 10 nmol/L to 100 μmol/L and was fitted with a quadratic regression weighted by reciprocal concentration ($1 / x$), yielding a fitted line with regression value >0.99. The limit of quantitation for the purposes of this assay was 10 nmol/L. Quality control samples at two levels in the range of the standard curve were used to accept individual analytic sets.

Pharmacokinetics data were analyzed by standard noncompartmental methodology using the software program Kinetica (http://www.innaphase.com). The C_{max} value was obtained directly from experimental observations. The total area under the curve (AUC_{tot}) was calculated using a combination of linear and log trapezoidal summations. The slopes of the initial (α) and terminal (β) phases of the plasma pharmacokinetics profile were determined by unweighted log-linear regression analyses. The apparent elimination half-life is calculated by the equation $T_{1/2\beta} = \ln(2) / \beta$. Total body clearance is obtained by dividing dose with $AUC_{tot.}$ The apparent steady-state volume of distribution is calculated from the total body clearance and $T_{1/2\alpha}$ by standard methods.

Results

Activity of ixabepilone against pediatric solid tumor models. Initial studies using daily dosing for 5 days showed that ixabepilone was toxic to all tumor-bearing *scid* mice at dose levels as low as 4.4 mg/kg (data not shown). All studies reported here used the q4d × 3 i.v. schedule for drug administration. At a dose level of 10 mg/kg, there were 12 toxicity-related deaths in the 287 mice treated (4.2% mortality). A limited number of studies were undertaken using 15 mg/kg per administration. Although no toxicity-related deaths were observed (n = 55), occasionally severe weight loss was

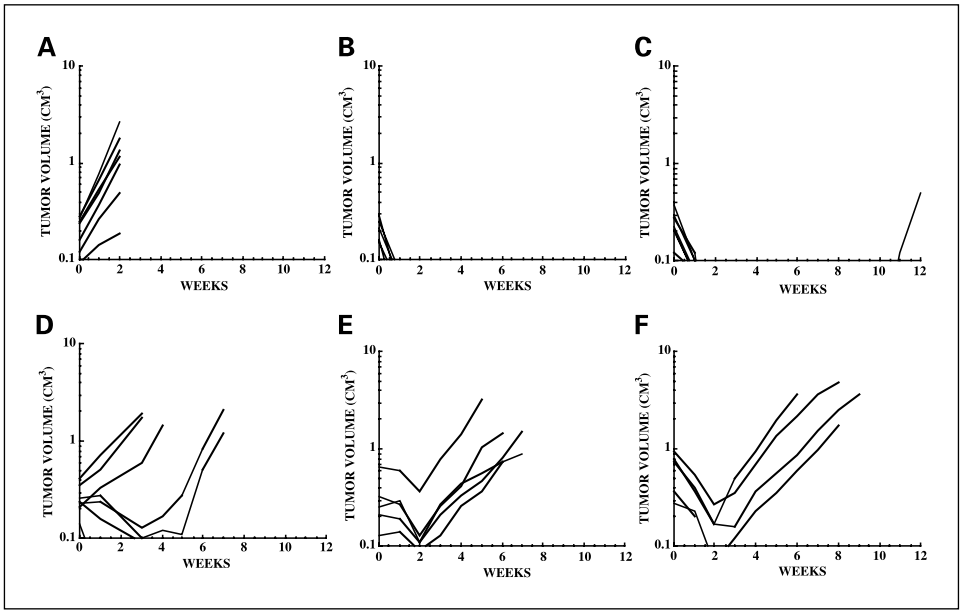

Fig. 2. Responses of NB-1382 neuroblastoma xenografts to treatment with ixabepilone or paclitaxel. *A*, control; *B*, 10 mg/kg ixabepilone; *C*, 6.6 mg/kg ixabepilone; *D*, 4.4 mg/kg ixabepilone; *E*, 15 mg/kg paclitaxel; *F*, 20 mg/kg paclitaxel. Both drugs were administered i.v. every 4 days for three administrations (q4d × 3 i.v.). Each curve shows the growth of a tumor in an individual mouse with time after the start of treatment. Note that one of five mice died in the highest paclitaxel dose group.

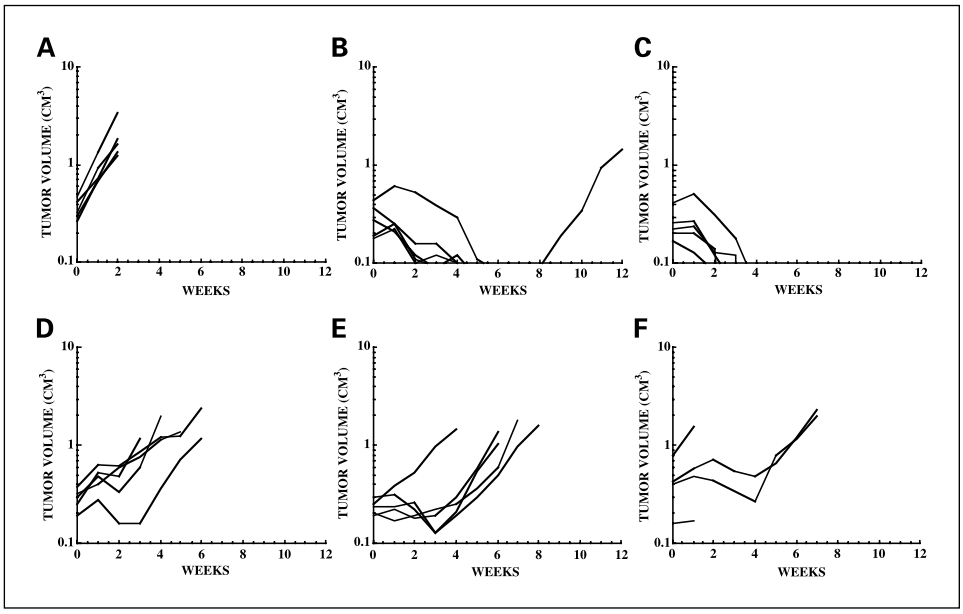

Fig. 3. Responses of SJ-BT35 (anaplastic astrocytoma) xenografts to treatment with ixabepilone or paclitaxel. *A*, control; *B*, 15 mg/kg ixabepilone; *C*, 10 mg/kg ixabepilone; *D*, 10 mg/kg paclitaxel; *E*, 15 mg/kg paclitaxel; *F*, 20 mg/kg (note three of five deaths). Both drugs were administered i.v. every 4 days for three administrations (q4d × 3 i.v.). Each curve shows the growth of a tumor in an individual mouse with time after the start of treatment.

determined (body weight nadir ~72% of day 0), although the median nadir for body weight loss in treated groups was 13%. Thus, the MTD level used here (10 mg/kg) may be conservative. Results evaluating ixabepilone against models of pediatric solid tumors are presented in Table 1. Data show growth delay, statistical significance, and the number of partial and complete regressions and complete regressions maintained at week 12 after initiation of treatment. At 10 mg/kg, ixabepilone induced partial or complete regressions in many tumor models. Criteria for response (i.e., all tumors within a group showed at least partial response) were met in three of three rhabdomyosarcoma lines, three of five neuroblastoma lines, two of six osteosarcoma models, six of seven Wilms' tumor models, including the diffuse anaplastic variant, SKNEP, and four of eight brain tumor lines. At lower dose levels (6.6 and 4.4 mg/kg), the incidence of objective regressions decreased although significant growth inhibition was determined at 6.6 mg/kg but not at the lowest dose evaluated. Higher dose ixabepilone (15 mg/kg) was evaluated in several osteosarcoma models. This resulted in enhanced growth inhibition only in the OS2 and OS33 models with an increase in the frequency of regressions.

Activity of ixabepilone against pediatric brain tumor models. The activity of ixabepilone against pediatric brain tumors is shown in Table 2, with data analysis similar to that presented above. Of interest is the quite broad spectrum activity of ixabepilone against a variety of histologies. Objective regressions

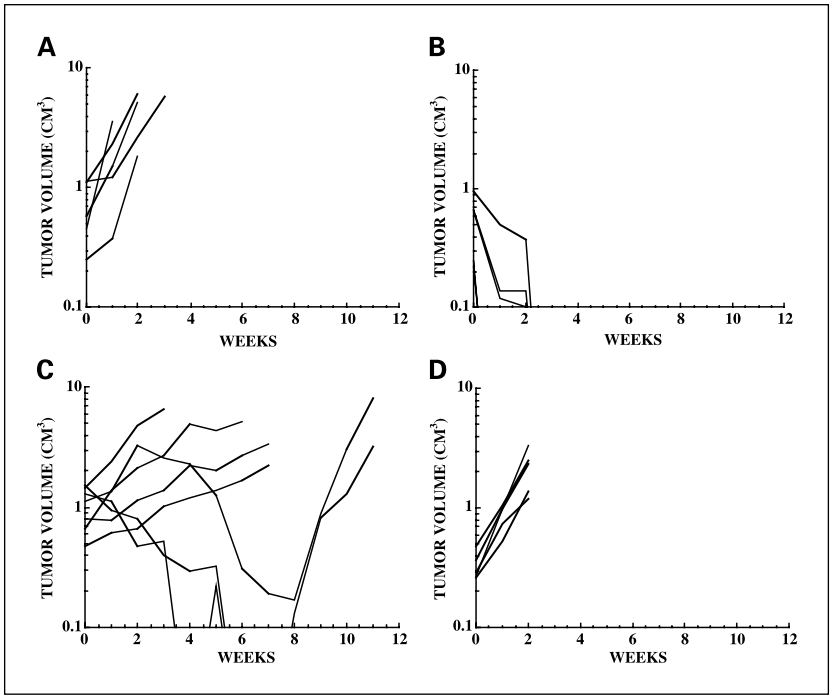

Fig. 4. Responses of SKNEP Wilms' tumor (diffuse anaplastic variant) xenografts to antimitotic agents. *A*, control; *B*, 10 mg/kg ixabepilone (q4d × 3 i.v.); *C*, 1 mg/kg vincristine (q7d × 8 i.v.); *D*, 20 mg/kg paclitaxel (q4d × 3 i.v.). Each curve shows the growth of a tumor in an individual mouse with time after the start of treatment.

Table 3. Pharmacokinetic parameters of ixabepilone in mice

Parameter (unit)	
Dose (mg/kg)	10
C_{max} (μmol/L)	14.7
AUC_{tot} (μmol/L-h)	5.77
$T_{1/2\alpha}$ (h)	0.204
$T_{1/2\beta}$ (h)	9.23
MRT (h)	3.62
Clearance (mL/min/kg)	57.8
Vss (L/kg)	46.2

were induced in both primitive neuroectodermal tumors (SJ-BT27 and SJ-BT41) and complete responses in anaplastic astrocytoma and ependymoma models. Only one of three atypical teratoid rhabdoid tumors of the central nervous system responded to ixabepilone. Ixabepilone showed significant activity only against early stage SJ-GBM2 glioblastoma xenografts. As was observed with the non–central nervous system tumors, activity of ixabepilone decreased very substantially as the dose was reduced from 10 mg/kg.

Comparison of ixabepilone with other antimicrotubule targeted agents. *Vinca* alkaloids, vincristine and vinblastine, natural products that destabilize microtubules and cause mitotic arrest, form the backbone of many curative regimens used in pediatric oncology. In contrast, taxanes (paclitaxel and docetaxel) stabilize microtubules, also inducing mitotic arrest. However, neither paclitaxel nor docetaxel have shown significant activity against pediatric malignancies. It was, therefore, of importance to determine the antitumor activity of ixabepilone relative to paclitaxel. Paclitaxel was administered using the same schedule (q4d × 3 i.v.) as used for ixabepilone. The highest dose used (20 mg/kg) caused some deaths (4 of 20 mice); hence, it represents a more toxic regimen than ixabepilone administered at the highest dose level (15 mg/kg). As shown in Fig. 2, ixabepilone administered at 10 and 6.6 mg/kg induced complete regression of NB-1382 neuroblastoma xenografts, and induced some regressions at 4.4 mg/kg. Of note, even at 6.6 mg/kg, only one tumor regrew by week 12, whereas all complete regressions were maintained at the higher dose level. In contrast, paclitaxel at the MTD induced transient regressions only. Responses of an anaplastic astrocytoma (SJ-BT35) in mice receiving ixabepilone or paclitaxel are shown in Fig. 3. In this experiment, paclitaxel (20 mg/kg) was toxic. Although paclitaxel inhibited tumor growth at 10 and 15 mg/kg, this was not significant ($P > 0.079$). Ixabepilone at both dose levels examined induced complete regression of this tumor model. The final model used to compare the activity of antimicrotubule targeting agents was the diffuse anaplastic Wilms' tumor (SKNEP). This model is highly sensitive to ixabepilone (Fig. 4) but poorly sensitive to vincristine, and completely refractory to treatment with paclitaxel.

Pharmacokinetic studies with ixabepilone. The plasma pharmacokinetics of ixabepilone (10 mg/kg, i.v.) are summarized in Table 3. Figure 5 shows the plasma time course for ixabepilone in CB17/Icr female *scid*$^{-/-}$ mice. Plasma pharmacokinetics were biexponential, with a short initial distribution phase ($T_{1/2\alpha}$ = 0.204 hours) and a considerably longer terminal elimination phase ($T_{1/2\beta}$ = 9.23 hours). The systemic plasma clearance of ixabepilone was moderate (57.8 mL/min/kg), being somewhat less than the hepatic blood flow in mice. The steady-state volume of distribution was high (Vss 46.2 L/kg), which is likely indicative of significant extravascular distribution. The mean residence time (MRT) of ixabepilone was estimated to be 3.62 hours.

Discussion

Cancer in children is a relatively rare disease with an incidence of ~12,500 new cases annually in the United States for children and adolescents under 21 years of age (25). Multimodality therapy, including intensive combination chemotherapy, results in cure rates exceeding 70%. However, for many childhood malignancies, prognosis for patients with advanced, metastatic disease remains bleak. Whereas understanding the molecular basis for pediatric malignant diseases remains a primary goal in ultimately developing specific and nontoxic therapies, the past success of cytotoxic drug combinations suggests that new cytotoxic agents may be identified that will contribute to improved survival rates. However, drug development in pediatric oncology is a relatively slow process due to a paucity of eligible patients for experimental drug trials and good initial response rates to standard agents, even among those patients with advance disease at diagnosis. Historically less than one drug in three evaluated against adult cancers is evaluated against pediatric populations. Further, drugs that may be highly active in patients at diagnosis are first tested in a heavily pretreated population at time of relapse that may not be optimal for identifying active agents (26). As one approach to identifying agents that may deserve some priority in their development in pediatric cancer populations, we have developed comprehensive preclinical models of many childhood solid tumors and several histologies of brain tumors. In the study reported here, we have evaluated the epothilone B derivative ixabepilone, and have compared its activity with paclitaxel

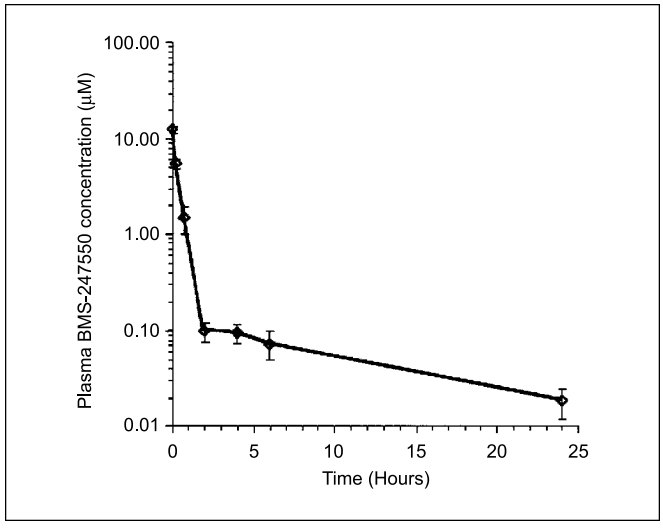

Fig. 5. Plasma pharmacokinetics of ixabepilone in *scid* mice following a single i.v. bolus administration at 10 mg/kg. Points, mean plasma concentration of five mice; bars, SE.

and, in one experiment, vincristine. Our results indicate that ixabepilone has broad-spectrum activity against rhabdomyosarcoma, neuroblastoma, and Wilms' tumor models. In addition, anaplastic astrocytoma and ependymoma xenografts, at least when growing at a s.c. site, showed very significant sensitivity to this agent. In our studies, we administered the agent every 4 days for three administrations only. Tumor volumes were measured every 7 days and animals observed for up to 12 weeks. Our criteria for biologically meaningful activity was objective regressions, as this will be the criteria used clinically to evaluate this agent in phase I/II clinical trials in children. Most of our studies used 10 mg/kg as the highest dose level. This may be conservative, as in a limited set of experiments a dose of 15 mg/kg was well tolerated. Administered as a single cycle of treatment ixabepilone showed very impressive activity given at 10 mg/kg/injection. However, as with most cytotoxic agents, the dose response relationship is steep. There were few objective regressions at 4.4 mg/kg/injection.

We compared the activity of ixabepilone with that of paclitaxel in three tumor models, NB-1382 neuroblastoma, SJ-BT35 anaplastic astrocytoma, and SKNEP diffuse anaplastic Wilms' tumor. The highest dose of paclitaxel used on the q4d $\times$ 3 i.v. schedule was 20 mg/kg/injection. This is significantly less than that used by Lee et al. (3) who administered 36 mg/kg/injection using a q2d $\times$ 5 schedule to tumor-bearing athymic nude mice. In our study, *scid* mice were less tolerant of paclitaxel treatment, with several deaths at the highest dose used. Thus, in this study, we have compared ixabepilone and paclitaxel at dose levels that approximate the MTD. In each model, ixabepilone showed superior activity compared with paclitaxel. The epothilone B derivative caused complete regression of the three tumor lines and, in most instances, complete regression was maintained through week 12. In contrast, paclitaxel caused, at best, partial regressions. We also compared the activity of vincristine with that of ixabepilone in the SKNEP xenograft. SKNEP was developed from a diffuse anaplastic Wilms' tumor (27), a variant that shows poor sensitivity to conventional cytotoxic agents, in contrast to Favorable Histology Wilms' tumor, and has a poor prognosis. Vincristine was administered at the MTD (1 mg/kg q7d $\times$ 8 i.v.) and caused some regressions in the SKNEP model. Lower doses of vincristine (0.66 mg/kg) have no activity against this tumor (data not shown). In contrast, SKNEP tumors are very sensitive to ixabepilone. We plan to evaluate this agent in additional models of diffuse anaplastic nephroblastoma as they are developed.

Clearly, there are examples where agents show quite dramatic activity against preclinical tumor models, but have little subsequent clinical efficacy (28–30). We and others have proposed that a useful index is to relate the antitumor activity of the agent with the systemic exposure in the host rather than merely relating tumor responses to host toxicity (31, 32). For example, mice are highly tolerant of certain camptothecin derivatives that target DNA topoisomerase I. Testing of camptothecin analogues at the mouse MTD gives an artificially high response rate in many tumor models. However, evaluation of topotecan or irinotecan at doses in mice that give representative clinical systemic exposures results in response rates that are similar to those observed in the clinic (33–35). Conversely, it is possible to use the mouse systemic exposure data at dose levels causing objective tumor responses and target that systemic exposure in clinical trials (36).

Such an approach may also have value in the development of ixabepilone for pediatric malignancies. In clinical trials in adult patient populations, ixabepilone has shown robust and broad-spectrum antitumor activity against a variety of solid malignancies (5, 11, 37), including taxol-resistant populations. Ixabepilone has been evaluated for efficacy and safety when administered by a number of widely different dosing schedules, including every 3 weeks, continuous weekly, and qd $\times$ 5 every 3 weeks. For each of these schedules, a reasonable characterization of the effective dose and the associated pharmacokinetics has been achieved. It is clear from these studies that the efficacious dose and, therefore, the corresponding efficacious exposure, can vary depending on the dose density (or frequency) of the regimen. For the every 3 weeks schedule, the efficacious dose ranged from 30 to 40 mg/m^2 (5, 11), with 40 mg/m^2 being the recommended phase II dose. In the case of the daily $\times$ 5 schedule, significant activity had been observed at 6 mg/m^2 (5). The systemic drug exposures (AUC_{tot}) corresponding to these efficacious doses were reported to be 5.1 and 0.54 μmol/L-h for 40 and 6 mg/m^2, respectively (4, 10). The same schedule in children has shown stable disease in several patients with refractory tumors, at dose levels that exceed the MTD in adults. In the present preclinical study, ixabepilone administered on an intermittent every 4 days $\times$ 3 schedule was found to produce significant antitumor activities in the majority of pediatric tumor xenografts at doses between 6.6 and 15 mg/kg. At the conservative MTD of 10 mg/kg, the corresponding ixabepilone exposure was 5.8 μmol/L-h. It seems, therefore, that efficacious exposure of ixabepilone is similar in mice as in humans with solid malignancies. Moreover, the drug exposure necessary for robust antitumor activity against pediatric tumor xenografts in mice can readily be achieved in adult patients. It remains to be determined whether such exposure can be obtained in the pediatric patient populations.

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