Preclinical Pharmacologic Evaluation of MST-997, an Orally Active Taxane with Superior *In vitro* and *In vivo* Efficacy in Paclitaxel- and Docetaxel-Resistant Tumor Models

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Abstract

Purpose: Because resistance to paclitaxel and docetaxel is frequently observed in the clinic, new anti-microtubule agents have been sought. The aim of this study was to evaluate the efficacy and oral activity of a novel taxane (MST-997) in paclitaxel- and docetaxel-resistant tumor models *in vitro* and *in vivo*.

Experimental Design: Tubulin polymerization assays, immunohistochemistry, and cell cycle analysis was used to evaluate mechanism of action of MST-997. The effect of MST-997 on growth inhibition in a panel of paclitaxel- and docetaxel-resistant cell lines that overexpressed P-glycoprotein (MDR1) or harbored β-tubulin mutations were assayed *in vitro* and in murine xenografts.

Results: MST-997 induced microtubule polymerization (EC_{50} = 0.9 μmol/L) and bundling, resulting in G_2-M arrest and apoptosis. In addition, MST-997 was a potent inhibitor of paclitaxel- and docetaxel-sensitive tumor cell lines that did not have detectable P-glycoprotein (IC_{50} = 1.8 ± 1.5 nmol/L). Minimal resistance (1- to 8-fold) to MST-997 was found in cell lines that either overexpressed MDR1 or harbored point mutations in β-tubulin. Most notable, MST-997 displayed superior *in vivo* efficacy as a single i.v. or p.o. dose either partially or completely inhibited tumor growth in paclitaxel- and docetaxel-resistant xenografts.

Conclusions: MST-997 represents a potent and orally active microtubule-stabilizing agent that has greater pharmacologic efficacy *in vitro* and *in vivo* than the currently approved taxanes. Our findings suggest that MST-997, which has entered phase I clinical trials, may have broad therapeutic value.

Based on current estimates, >10 million cases of cancer are documented worldwide, resulting in >6 million deaths annually (1). In most patients with solid tumors, some of the most effective anticancer therapies are palliative rather than curative and extend life only on the order of months rather than years (2). Thus, there remains a significant unmet medical need to develop agents that improve quality of life and prolong survival.

Agents that bind to tubulin and inhibit microtubule function are widely used in the treatment of cancer (2). Such drugs inhibit several processes during cell division, most notably chromatid separation, leading to inhibition of growth and ultimately cell death. Although the exact mechanism of action is not completely understood, all anti-microtubule agents alter the dynamic equilibrium of microtubules such that they either perturb the net addition of tubulin dimers to one end (polymerization) or the net removal of tubulin dimers from the opposite end (depolymerization; ref. 2).

Paclitaxel, originally derived from the inner bark of the pacific yew tree *Taxus brevifolia*, and docetaxel, derived semi-synthetically by esterification of a side chain to 10-deacetyl baccatin III, stabilize microtubules and at stochiometric concentrations enhance microtubule polymerization (3–8). Based on photoaffinity labeling and crystallographic analyses, both paclitaxel and docetaxel inhibit the function of tubulin by binding to a similar, highly defined region within β-tubulin (9). However, recent studies indicate that the antineoplastic activity of taxanes may originate, in part, from induction of genes encoding transcription factors with tumor suppressor effects as well as enzymes governing proliferation, apoptosis, and inflammation (10–12).

The currently approved taxanes have numerous limitations. First, certain tumor types are either completely refractory to these agents (i.e., colon carcinomas) or develop resistance during multiple cycles of therapy (i.e., breast, ovarian, or lung carcinomas; refs. 1, 13). Second, all anti-microtubule drugs

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induce serious side effects, most notably bone marrow suppression and/or peripheral neuropathy. Third, both paclitaxel and docetaxel are prepared in a vehicles that induce hypersensitivity reactions and require patients to be premedicated with corticosteroids.

Tumor cell resistance to paclitaxel or docetaxel is also observed *in vitro* and can be attributed to (*a*) overexpression of drug efflux pumps, such as P-glycoprotein; (*b*) acquired mutations at the drug binding site of tubulin; (*c*) differential expression of tubulin isoforms; (*d*) alteration in apoptotic mechanisms; (*e*) activation of growth factor pathways; or (*f*) other biochemical changes (14–16). The contribution of each of these mechanisms to clinical resistance remains uncertain, although correlations have been made with P-glycoprotein expression levels in some tumor types.

In a continued effort to identify taxanes that are more potent, orally bioavailable, and efficacious in drug-resistant tumors, we evaluated several taxane analogues provided by Taxolog, Inc. (Fairfield, NJ), which are generated by an optimized semisynthetic chemical process. We report the identification of a novel structurally distinct docetaxel analogue, microtubule-stabilizing taxane-997 (MST-997), that has superior *in vitro* and *in vivo* activity in paclitaxel- and docetaxel-resistant models, is orally active, and causes complete tumor regression with a single dose. In addition, the superior *in vivo* efficacy of MST-997 can be obtained in non-Cremophor EL vehicles, potentially providing alternative formulation options and circumventing the need for premedication that is required for paclitaxel administration.

Materials and Methods

Materials. Paclitaxel, vinblastine, Cremophor EL (polyoxyl 35 castor oil), and Tween 80 (polysorbate 80) were purchased from Sigma, Inc. (St. Louis, MO). Intralipid (20% soybean oil, 1.2% egg phospholipids, 2.2% glycerin) was purchased from Baxter Healthcare (Deerfield, IL). Docetaxel was purchased from LKT Laboratories (St. Paul, MN). MST-997 and MAC-321 were obtained from and designated as TL-909 and TL-139, respectively, by Taxolog.

Cell lines. The following human cell lines were purchased from the American Type Culture Collection (Rockville, MD): HCT-116, DLD-1, HCT-15 representing colorectal tumors; NCI H838 derived from non–small cell lung carcinomas; and Lox originating from melanoma tumors. The A549 human lung adenocarcinoma parental cell lines and its counterpart selected for resistance to epothilone B (A549.EpoB40) were kindly obtained from Dr. Susan Band Horwitz (Albert Einstein College of Medicine, Bronx, NY) and have been described previously (17). The human KB series of epidermoid tumors (KB-3-1, KB-8-5, and KB-V1) and MX-1W breast carcinoma have been described and maintained as previously reported (18, 19). The KB-D-15, KB-P-15, and KB-PTX/099 lines were derived from the parental KB-3-1 cells by selecting and clonally expanding in the presence of 15 nmol/L docetaxel (KB-D-15), 15 nmol/L paclitaxel (KB-P-15), or a combination of 15 nmol/L paclitaxel and 5 μmol/L CL-347099 (a P-glycoprotein reversal agent) as described previously (20).

In vitro *tubulin polymerization assays.* For *in vitro* tubulin polymerization assays, lyophilized bovine microtubule-associated protein–free tubulin and PEM buffer [80 mmol/L Na-PIPES (pH 6.9), 1 mmol/L $MgCl_2$, 1 mmol/L EGTA] were purchased from Cytoskeleton (Denver, CO). Microtubule-associated protein–free tubulin (1.5 mg/mL) was incubated with test compounds in PEM-0.3% DMSO at the following concentrations: 0.1, 0.3, 0.9, 2.7, 8.1, and 24.3 μmol/L. Absorbance at 340 nm was measured every minute for 60 minutes at 24°C using a SpectraMax Plus spectrophotomer (Molecular Devices, Sunnyvale, CA).

Immunofluorescence microscopy. The effect of test agents on tubulin morphology in cells was visualized by immunofluorescence microscopy. KB-3-1 epidermoid carcinoma cells were plated at 5,000 per chamber on poly-D-lysine–coated eight-chamber microscope slides (Becton Dickinson Labware, Bedford, MA) and cultured overnight. Compounds, diluted in media, were added to each chamber to achieve the desired final concentrations. Details on the detection of tubulin using the anti-α-tubulin antibody (clone DM 1A, Sigma) followed by FITC-conjugated $F(ab')_2$ fragment of goat anti-mouse IgG (Jackson Immunoresearch, West Grove, PA) and the detection of DNA using 4′,6-diamidino-2-phenylindole have been previously described (19).

Cell proliferation assays. Cytotoxicity was assessed by growing cells in the presence or absence of drug agents for 72 hours. Cell survival was measured by the ATP-binding assay using the CellTiter-Glo Luminescent Reporter System (Promega, Inc., Madison, WI). Briefly, cells were plated with a BioMek FX robotic platform (Beckman Instruments, Fullerton, CA) at ~50% confluency in a 384-well plate and allowed to attach for 12 hours at 37°C/5% CO_2. Test agents, diluted in growth media with a BioMek 2000 robotic system (Beckman Instruments), were added to each well and incubated for 72 hours. ATP binding and stabilization of the luminescent signal were done according to manufacturer's protocol (Promega) following cell lysis. Absorbance was read on a Victor V multi-label plate reader (Perkin-Elmer, Gaithersburg, MD) at a wavelength of A_{595} and data collected using Wallac 1420 Workstation software. The drug concentration that reduced the viability of cells by 50% was determined by plotting duplicate data points over a concentration range and using regression analysis (Data Analysis Toolbox, MDL Information Systems, San Leandro, CA) to calculate IC_{50} values.

Cell cycle analysis. KB-3-1 human epidermoid cells were incubated with 0.0, 0.2, 0.4, 0.8, 1.6, 3.1, 6.3, 12.5, 25.0, and 50.0 nmol/L MST-997 or paclitaxel for 16 hours. Cells were harvested, fixed in ethanol, and stained with 0.5 mg/mL of propidium iodide along with 0.1 mg/mL of RNase A (200 KU, Calbiochem, San Diego, CA) and analyzed on a PCA 96 cell sorter (Guava Technologies, Hayward, CA). The resulting DNA histograms were collected from at least 10,000 propidium iodide–stained cells at an emission wavelength of 690 nm. The number of cells in each phase of the cell cycle [G_0-G_1 (gap-zero/gap-one; interphase), S (DNA synthesis), G_2-M (gap-two/mitosis)] was determined, and those in the apoptotic phase were measured by determining the percentage of cells in sub-G_1 peak.

In vivo *tumor xenografts.* All *in vivo* animal studies described here were carried out in compliance with the standards for use of laboratory animals. Athymic *nu/nu* female mice were implanted s.c. with either 2×10^6 Lox cells, 5×10^6 KB-3-1 cells, 2.5×10^6 KB-8-5 cells, 7×10^6 HCT-15 cells, 7×10^6 HT-29 cells, 5×10^6 DLD-1 cells, 5×10^6 Panc 1 cells, or one 3 mm × 3 mm MX-1W tumor fragment. When tumors attained an average mass between 80 and 200 mg (defined as day 0 of staging), 5 or 10 mice were randomized into treatment groups depending upon the experiment.

MST-997 was initially solubilized in 100% ethanol followed by mixing with vehicles used for i.v. or p.o. administration. Mice were treated i.v. with a single dose of MST-997 prepared in 5% ethanol and 95% Intralipid or vehicle alone. Additional i.v. formulations for MST-997 included 5% ethanol and 5% Tween 80 in normal saline and 5% ethanol and 5% Cremophor EL in normal saline. Mice were treated orally (p.o.) with a single dose of MST-997 prepared in 5% ethanol and 5% Cremophor EL in normal saline or vehicle alone. Briefly, paclitaxel powder was initially solubilized in 100% ethanol followed by mixing with Cremophor EL to yield a 25 mg/mL stock of 50% ethanol/50% Cremophor EL that was diluted in saline immediately before administration. Docetaxel powder (20 mg) was dissolved in 100% Tween 80 and then further diluted in 13% ethanol to yield a 10 mg/mL stock. Paclitaxel and docetaxel were given on days 1, 5, and 9 (q4d × 3)

post-staging in 6% ethanol and 6% Cremophor EL in normal saline or 2.5% ethanol and 6% Tween 80 in normal saline, respectively.

Tumor mass ([length × width^2] / 2) was determined once a week for up to 56 days, depending upon the experiment. The percent tumor/control (%T/C) was then calculated for each treatment group for the duration of the experiment. The %T/C is defined as the mean tumor mass of the treated group divided by the mean tumor mass of the vehicle control group multiplied by 100. A drug dose is considered toxic if there is >20% lethality, or if animals have lost ≥20% of their initial body weight. Injection or gavage volumes for all test agents did not exceed 0.5 mL.

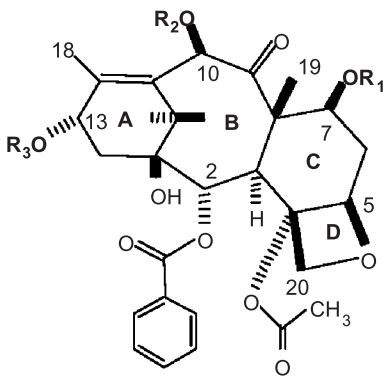
Statistical analysis. Cell proliferation data were imported into Microsoft Excel for analyses and IC_{50} determinations were obtained using Data Analysis Toolbox (MDL Information Systems, v.1.0.1), licensed by Wyeth. Average and SD values were calculated using Microsoft Excel. *In vivo* data were analyzed for significance by a two-tailed Student's *t* test. $P \leq 0.05$ indicates a statistically significant reduction in relative tumor growth of the treated group compared with that of the vehicle control group. A drug is considered active if the %T/C is ≤42, and $P \leq 0.05$ is calculated.

Results

Chemical structure of MST-997. The structure of MST-997 is defined as 5β,20-epoxy-1,2α,4,7β,10β,13α-hexahydroxytax-11-en-9-one 4-acetate-2-benzoate-10-cyclopentane-carboxylate-13-ester with (2*R*,3*S*)-*N*-isopropoxycarbonyl-3-(2-thienyl) isoserine. MST-997 is an analogue of docetaxel with two major substitutions at carbon 10 and the 13 side chain of the baccatin core (Fig. 1). These modifications highlight the structural diversity of MST-997.

MST-997 induces microtubule polymerization and stabilization. The effect of MST-997 on microtubule polymerization was studied *in vitro*. In cell-free assays with purified tubulin, MST-997 was a potent agent that stabilized microtubules in the absence of GTP. Comparable with docetaxel, MST-997 caused an increase in turbidity when incubated with purified tubulin (indicative of microtubule formation) with EC_{50} value of 0.9 μmol/L. At the maximum concentration of 24 μmol/L of MST-997, the overall rate of tubulin polymerization was similar to docetaxel; however, the net amount of polymerized tubulin was slightly enhanced in the presence of MST-997 (Fig. 2A). In the absence of GTP, paclitaxel was a weak inducer of tubulin polymerization. Thus, MST-997 is a more potent tubulin polymerizing agent than paclitaxel.

Fluorescent staining of microtubules in KB-3-1 cells. Another hallmark of taxanes is their ability to induce tubulin bundling in tumor cells. Therefore, MST-997 was examined by immunofluorescence for its ability to disrupt cell division and induce the bundling of microtubules in KB-3-1 epidermoid cells. As a control, paclitaxel was used at concentrations that were 10-fold higher than the concentration needed to inhibit the growth of KB-3-1 human epidermoid carcinoma cells (Table 1). In untreated cells, extensive microtubule networks in the cytoplasm (Fig. 2B) and defined spindle poles surrounding the metaphase plate in dividing cells were observed (Fig. 2B, *inset*).



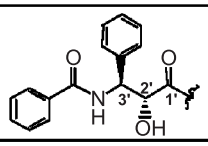
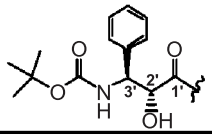
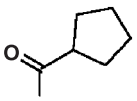
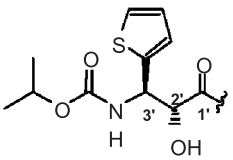
Compound	R_1=	R_2=	R_3=
PTX	H	$CH_3C(=O)O-$	
DTX	H	H	
MST-997	**H**		

Fig. 1. Chemical structures of paclitaxel (*PTX*), docetaxel (*DTX*), and MST-997. Modifications of the baccatin III core structure (*A, B, C*, and *D*) are indicated as R_1, R_2, and R_3.

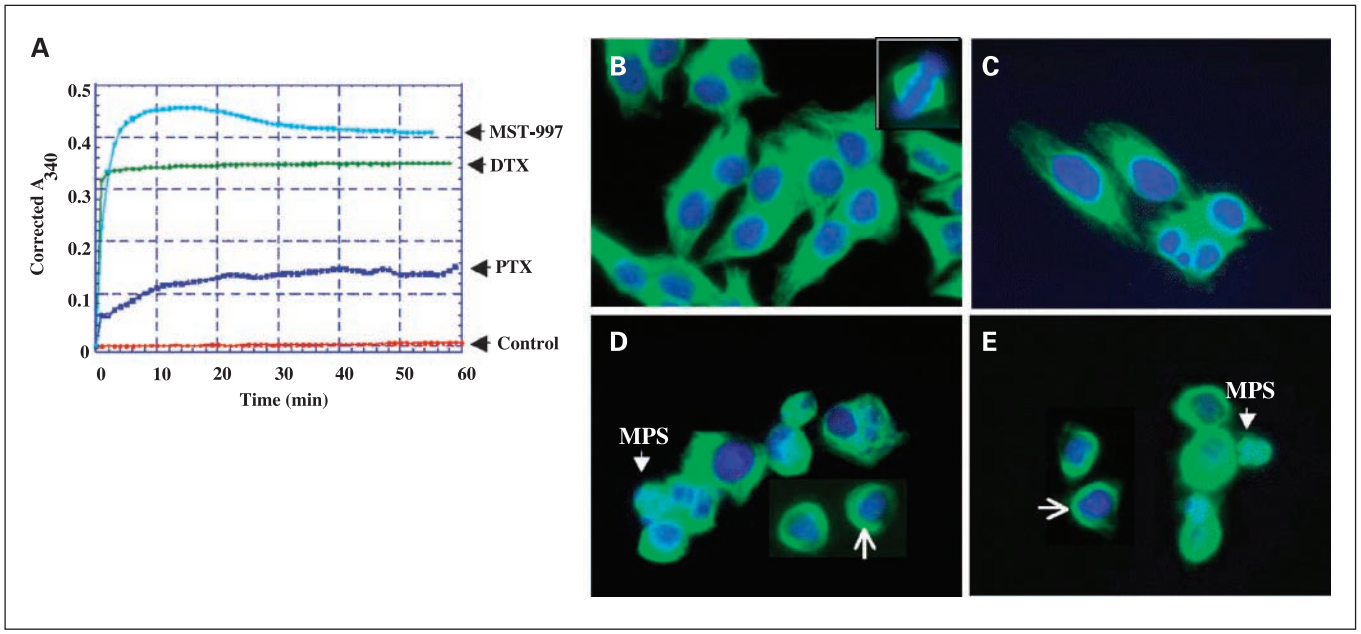


Fig. 2. Effects of MST-997 on tubulin polymerization *in vitro. A,* tubulin polymerization assays were conducted at 24°C using 1.5 mg/mL of purified bovine brain microtubule-associated protein – free tubulin in the presence of vehicle control or 24.3 μmol/L MST-997, docetaxel (*DTX*), or paclitaxel (*PTX*). Turbidity was measured by absorbance (340 nm) for up to 60 minutes. *B* to *E,* structures of microtubules were analyzed in KB-3-1 cells treated with MST-997 by immunofluorescence microscopy. Cells were untreated (*B*) or treated for 16 hours with MST-997 at 1 nmol/L (*C*), 10 nmol/L (*D*), or 40 nmol/L paclitaxel (*E*). After MeOH fixations, cells were stained anti-α-tubulin and 4′,6-diamidino-2-phenylindole to visualize DNA as described in Materials and Methods. Open arrows, bundling of microtubules; closed arrows, multipolar spindle formation (*MPS*) in cells treated with MST-997 (*D*) or paclitaxel (*E*). Magnification, ×600.

At 1 nmol/L MST-997 normal metaphase plates with characteristic spindle poles were rarely observed, and cells were usually rounded (Fig. 2C). However, at the highest concentration tested, both 10 nmol/L MST-997 and 40 nmol/L paclitaxel induced the formation of microtubule bundles in the cytoplasm of numerous cells (Fig. 2D and E). Docetaxel caused similar effects (data not shown). To insure these effects were specific, cells were treated with 8 nmol/L of the depolymerizing agent vinblastine, in which no bundling of tubulin was observed (data not shown).

MST-997 arrests tumor cells at G_2-M phase of cell cycle. To confirm that MST-997 behaved similarly to conventional taxanes, drug-sensitive KB-3-1 epidermoid cells were incubated with varying concentrations of MST-997 and paclitaxel (0.2-50 nmol/L) for 16 hours, and the proportion of cells in each of the different phases of the cell cycle were assessed by fluorescence-activated cell sorting analysis. Although the majority of cells (>75%) were arrested in G_2-M when treated with 25 nmol/L paclitaxel, doses as low as 1.6 nmol/L MST-997 achieved a comparable effect. (Fig. 3A and B). Thus, similar to conventional taxanes, such as paclitaxel and docetaxel, MST-997 arrest cells in G_2-M, however, at much lower doses.

In addition, 0.3 to 30 nmol/L MST-997 caused an accumulation of phosphorylated nucleolin, a selective marker for cells arrested in G_2-M (21, 22) in intact KB-3-1 cells *in vitro*. However, 10-fold greater concentrations of paclitaxel and vinblastine were required to obtain comparable effects (data not shown). Therefore, based on biochemical and cellular data, MST-997 induces G_2-M arrest in tumor cells similar to known agents that disrupt microtubule dynamics but at a 10-fold lower dose than paclitaxel.

MST-997 is a potent inhibitor of tumor cell growth. In addition to KB-3-1 epidermoid cells, the growth inhibitory effects of MST-997 were further evaluated in a panel of paclitaxel- and docetaxel-sensitive cell lines derived from colon and lung tumors. These lines have been shown in a previous study to express little or no P-glycoprotein and thereby render them more sensitive to paclitaxel and docetaxel (19). MST-997 inhibited the growth of all sensitive tumor cell lines tested in tissue culture regardless of tumor origin with an average IC_{50} of 1.8 ± 1.5 nmol/L (Table 1). Overall, MST-997 was equipotent compared with docetaxel and 2.5-fold more potent than paclitaxel in these lines that had no detectable levels of P-glycoprotein (Table 1).

MST-997 overcomes paclitaxel drug resistance due to overexpression of drug efflux pumps. Because MST-997 is an anti-microtubule drug, we reasoned that it would be most useful in those patients where traditional anti-microtubule therapies had failed. Therefore, the activity of MST-997 was compared with other taxanes, with special emphasis on paclitaxel/docetaxel–resistant models, where the basis of resistance was known to be associated with the overexpression of drug efflux pumps, including MDR1 (P-glycoprotein/ABCB1; ref. 23). In a previous study, the increased levels of MDR1 mRNA and protein were confirmed in cell lines selected for resistant to colchcine (KB-8-5), vinblastine (KB-V1), paclitaxel (KB-P-15), docetaxel (KB-D-15), or inherently resistant (HCT-15 and DLD-1) when compared with the drug-sensitive parental lines (19). Indeed, when compared with drug-sensitive P-glycoprotein-negative cell lines, the average IC_{50} for docetaxel and paclitaxel increased to 105.4 ± 178.9 and 737.0 ± 1,226.5 nmol/L in P-glycoprotein-positive tumor lines, respectively (Table 1). This translated into a 87.4- and 178.4-fold increase in the relative drug resistance for docetaxel and paclitaxel, respectively (relative resistance is a ratio of IC_{50} of the drug-resistant cell line versus IC_{50} of the sensitive parental or tumor counterpart

cell line; Table 1). More specifically, cells resistant to paclitaxel, such as KB-8-5, that are 18-fold, were not responsive to the drug *in vivo*, suggesting that this level of resistance *in vitro* translates to resistance in animals (19).

In contrast, minimal (1- to 3-fold) resistance to MST-997 was found in cell lines that acquire and are selected for low MDR1 overexpression (KB-8-5, KB-P-15. or DLD-1; Table 1). Overall, the average IC_{50} for MST-997 only increased to 8.5 ± 13.6 nmol/L compared with an average IC_{50} of 1.8 ± 1.5 nmol/L in sensitive tumor lines (Table 1). However, resistance to MST-997 can be mediated by MDR1 in extreme circumstances because 7.9- to 44.5-fold resistance was observed in KB-D-15 and KB-V1 cell lines, respectively, both of which express very high levels of MDR1 (Table 1). Consistent with this observation, only KB-V1 cells had 4-fold lower drug accumulation of ^{14}C-radiolabeled MST-997 compared with the parental KB-3-1 cells and KB-8-5 (data not shown). However, both KB-8-5 and KB-V1 cells had low drug accumulation of radiolabeled paclitaxel (data not shown). The latter is likely due to MDR1 because decreased cellular accumulation of paclitaxel was partially reversed with CL-329,753, an MDR1-specific inhibitor (24). In addition, MST-997 was a potent inhibitor of growth in HCT-15 and DLD-1 that were inherently resistant to paclitaxel in the absence of drug selection (Table 1). For example, the relative level of

Table 1. Cytotoxicity profile of MST-997 in a panel of paclitaxel-sensitive and paclitaxel-resistant tumor cell lines

Cell line (paclitaxel sensitive)	Tumor origin	Resistance phenotype	P-glycoprotein * expression	IC_{50} (nmol/L)		
				MST-997	Docetaxel	Paclitaxel
KB-3-1	Epidermoid	None	0	0.8 ± 0.2	1.1 ± 0.6	3.9 ± 1.3
HCT-116	CRC	None	0	1.5 ± 0.3	5.4 ± 0.8	8.2 ± 1.4
NCI H838	NSCLC	None	0	3.2 ± 0.6	2.1 ± 0.9	6.3 ± 0.7
A549	NSCLC	None	0	1.8 ± 0.9	1.9 ± 0.7	7.5 ± 1.3
Average ± SD				1.8 ± 1.5	2.6 ± 1.9	6.5 ± 1.9

Cell line (paclitaxel resistance)	Tumor origin	Resistance phenotype	P-glycoprotein expression*	IC_{50} (nmol/L)			Fold relative resistance†		
				MST-997	Docetaxel	Paclitaxel	MST-997	DTX	Paclitaxel
KB-8-5	Epidermoid	P-glycoprotein overexpression	++	1.6 ± 0.4	8.8 ± 0.3	70.2 ± 3.7	2.0	8.0	18.0
KB-P-15	Epidermoid	P-glycoprotein overexpression	++	2.4 ± 0.1	17.6 ± 1.0	117.0 ± 15.9	2.6	16.1	30.1
KB-D-15	Epidermoid	P-glycoprotein overexpression	++++	6.4 ± 0.8	68.2 ± 1.5	565.5 ± 29.1	7.9	62.1	144.7
KB-V1	Epidermoid	P-glycoprotein overexpression	+++++	36.0 ± 4.2	467.5 ± 9.2	3201.9 ± 153.0	44.5	425.3	820.5
DLD-1	CRC	P-glycoprotein overexpression	+++	1.5 ± 0.3	16.2 ± 1.4	32.8 ± 7.7	1.5	2.7	4.0
HCT-15	CRC	P-glycoprotein overexpression	++++	3.0 ± 0.9	54.1 ± 2.1	434.6 ± 52.1	1.9	10.1	53.4
Average ± SD				8.5 ± 13.6	105.4 ± 178.9	737.0 ± 1226.5	10.1 ± 17.0	87.4 ± 166.9	178.4 ± 318.5
KB-PTX/099	Epidermoid	β-Tubulin mutation ($26^{Asp \rightarrow Glu}$)	0	0.8 ± 0.3	8.8 ± 2.3	74.1 ± 8.5	0.9	8.2	18.8
A549.EpoB40	NSCLC	β-tubulin mutation ($292^{Gln \rightarrow Glu}$)	0	12.6 ± 0.7	28.5 ± 6.9	127.5 ± 18.3	6.7	15.5	17.1
Average ± SD				6.7 ± 5.9	18.3 ± 9.5	100.8 ± 37.7	3.8 ± 4.12	11.8 ± 5.2	18 ± 1.2

NOTE: Cell toxicity was measured using Cell-Titer Glo as described in Materials and Methods to assess the levels of ATP after a 72-hour incubation with MST-997, docetaxel, or paclitaxel. Values represent average IC_{50} ± SE.

Abbreviations: CRC, colorectal carcinoma; NSCLC, non – small cell lung carcinoma.

*P-glycoprotein expression was determined by Western blotting and quantitative real-time RT-PCR using MDR1-specific antibodies and probes, respectively, as previously described (19). Scoring code based on Western blotting: 0, undetectable; + to ++++, low to high expression.

† Fold relative resistance is defined as a ratio of IC_{50} in the resistant cell line to the IC_{50} of the corresponding sensitive/parental cell counterpart. For example, drug-resistant epidermoid tumors were compared with the KB-3-1 parental line. HCT-116 was used as the respective sensitive colon cell line for DLD-1 and HCT-15. A549 was used as the respective parental line for A549.EpoB40.

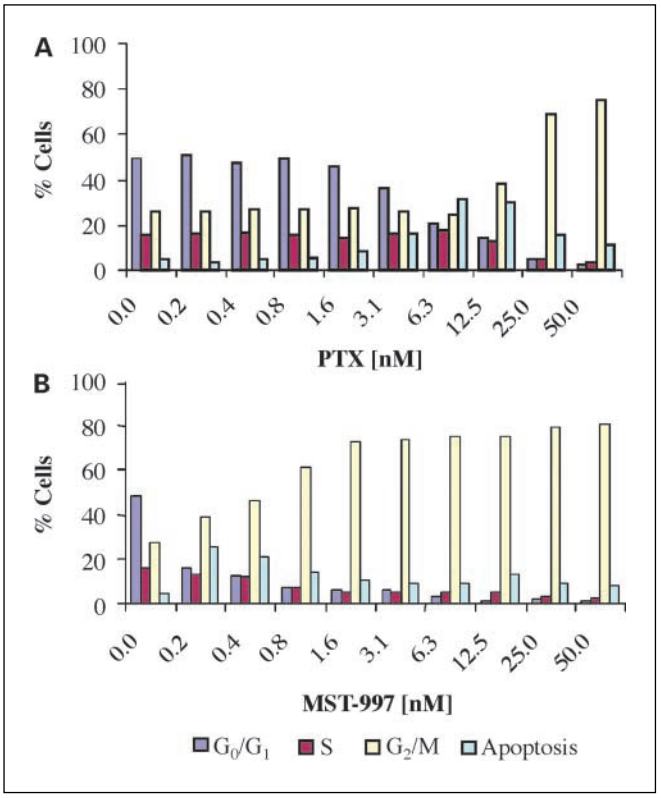


Fig. 3. Cell cycle analysis of MST-997 versus paclitaxel (*PTX*). KB-3-1 epidermoid cells were plated at 10,000 per well and treated in triplicate at the indicated doses for 16 hours. Cells were detached, fixed, and analyzed using a PCA 96 Cell Sorter as described in Materials and Methods. The resulting DNA histograms were collected from at least 2,000 propidium iodide – stained cells at an emission wavelength of 690 nm. Percentage of cells in each phase of the cell cycle and in apoptosis as indicated.

resistance to MST-997 and paclitaxel in the HCT-15 colon tumor cell line, which overexpresses very high levels of MDR1, was 1.9- and 53.4-fold, respectively, compared with HCT-116 colon cells that are sensitive to these agents. MDR1 mediates resistance, at least in part, in the HCT-15 lines as well because the reversal agent CL-329,753 resensitized cells to MDR1 substrates, such as paclitaxel (24).

Resistance models with mutations in the taxane binding site of tubulin. In cell culture, resistance to paclitaxel and other tubulin polymerizing agents, such as epothilones, can be attributed to tubulin mutations (17, 20, 25, 26). Epothilone A and B promote microtubule polymerization and bind to a similar site in tubulin compared with paclitaxel and docetaxel (27, 28). Therefore, we determined if MST-997 could overcome this mode of resistance by using the KB-PTX/099 line derived from the human KB-3-1 epidermoid cells selected in the presence of paclitaxel and an MDR1 reversal agent (20). Additional comparisons were also done with an A549 human lung carcinoma selected for resistance to epothilone B (17). These resistant cell lines express β-tubulin containing distinct point mutations in the taxane- or epothilone-binding sites but do not overexpress drug efflux pumps and have been previously reported as amino acid $292^{Gln \rightarrow Glu}$ (A549.EpoB40) and amino acid $26^{Asp \rightarrow Glu}$ (KB/099; refs. 17, 20). Cross-resistance to docetaxel and paclitaxel was observed in both tubulin-mutant lines tested and on average was ~11.8- to 18.0-fold, respectively (Table 1). However, MST-997 displayed a lower level of cross-resistance (3.8-fold) in both lines when compared with paclitaxel and docetaxel (Table 1). In contrast to tubulin-polymerizing agents, no cross-resistance was observed for vinblastine or for dolastatin-10, which presumably bind to the *Vinca* and *Vinca* peptide–binding domains of tubulin, respectively (data not shown). The binding domain for these agents is believed to be distinct from the taxane pharmacophore based on pharmacologic, biochemical, and crystallographic data (9).

***MST-997 is highly efficacious when given as a single i.v. or p.o. dose in paclitaxel-sensitive tumor xenografts* in vivo.** The activity of MST-997 was assessed in several nude mouse xenograft models that are known to be sensitive to treatment with paclitaxel and docetaxel (29–31). The first set of experiments was done using Lox melanoma and KB-3-1 epidermoid xenograft models. We have established that these tumor models are highly responsive to paclitaxel such that the optimal dose of 60 mg/kg paclitaxel when given on days 1, 5, and 9 (q4d × 3) is ~90% of the maximum tolerated dose (MTD; based on the maximal acceptable weight loss of 20% compared with control-treated animals). Animals bearing small-established Lox melanoma xenografts were treated with 10 to 120 mg/kg MST-997 given as a single i.v. dose in Intralipid on day 1 (defined henceforth as the day after tumor weight of ~100 mg was achieved). A clear dose response was observed with a maximum efficacious dose of 100 mg/kg and the minimum efficacious dose of 10 mg/kg (Fig. 4A). No tumors were detected in 9 of 10 animals receiving the 100 and 70 mg/kg doses up to 56 days after drug administration and as such were defined as cured (Fig. 4A). The MTD was 120 mg/kg. Consistent with previously published data (19, 29), paclitaxel was also highly effective when given at its optimal dose of 60 mg/kg q4d × 3 such that tumor growth was inhibited by >95% up to day 35 (Fig. 4A) with no observable weight loss.

In animals bearing staged HT-29 colon tumors, the minimum efficacious dose of MST-997 was 30 mg/kg, and cures were observed at 70 mg/kg (data not shown). Again, with exception of the 120 mg/kg MTD dose, little or no weight loss was observed in the latter experiment either. Similar to LOX and HT-29, single i.v. doses of MST-997 > 60 mg/kg were also highly effective in eliminating tumor growth in MX-1, Panc-1, and KB-3-1 xenografts such that no tumors were detected 30 to 60 days post-administration, and animals were again classified as cured (8 of 10 animals; data not shown). However, single i.v. doses of paclitaxel given at concentrations of >60 mg/kg were completely ineffective in reducing tumor growth in all sensitive models tested (data not shown). Thus, a single i.v. dose of MST-997 is highly effective in a non-Cremophor EL vehicle, such as Intralipid; well tolerated; and has a broad therapeutic window in animals bearing paclitaxel-sensitive tumors.

MST-997 was also benchmarked directly to paclitaxel with regard to a multiple i.v. dose schedule in sensitive models to determine it tolerability. MST-997 was given i.v. on days 1, 5, and 9 to animals bearing Lox melanoma and MX-1 breast tumor xenografts. Doses of MST-997 ranged from 5 to 40 mg/kg/dose, and drug was prepared in Intralipid. All doses of MST-997 significantly inhibited tumor growth of LOX melanoma xenografts with cures observed at 20 to 40 mg/kg in 10 of 10 animals (Fig. 4B). Again, the increased potency of

MST-997 in sensitive xenograft models is underscored by the observation that a 2-fold less dose was more efficacious than paclitaxel (Fig. 5B; 30 mg/kg MST-997 versus 60 mg/kg paclitaxel). Similar results were obtained using the human breast carcinoma MX-1 (data not shown). No significant weight loss was noted at any of the doses in either Lox or MX-1 models, suggesting that MST-997 is well tolerated at multiple low doses.

MST-997 was also highly efficacious in the KB-3-1 paclitaxel-sensitive model when given orally. Animals bearing KB-3-1 xenografts were treated with vehicle or 10 to 300 mg/kg MST-997 given as a single p.o. dose prepared in Cremophor EL in normal saline (Fig. 4C). The MTD of MST-997 when given orally was 300 mg/kg, and the minimum efficacious dose was 30 mg/kg. Cures were observed in all animals receiving as low as 100 mg/kg MST-997 (Fig. 4C). Little or no weight loss was detected at any dose tested. Although paclitaxel is not orally bioavailable, a single p.o. administration of MST-997 was as effective as multiple i.v. doses of paclitaxel given at its optimal concentration and schedule in KB-3-1 xenografts (Fig. 4D). However, >90% tumor growth inhibition was observed when animals were treated with 70 mg/kg MST-997 p.o., where a comparable i.v. dose resulted in cures in 9 of 10 animals (Fig. 4D). Similar p.o. results were obtained in the Lox melanoma xenograft model (data not shown). The data obtained in the above *in vivo* experiments confirm that MST-997 is a potent inhibitor of growth in paclitaxel-sensitive tumors when given either i.v. or p.o. However, cures were observed at lower i.v. doses, suggesting that higher or more frequent dosing may be required when MST-997 is given orally.

MST-997 has superior activity in paclitaxel- and docetaxel-resistant xenograft animal models. Experiments were done in xenograft models that were either inherently resistant (DLD-1 and HCT-15 colorectal carcinoma) or have acquired resistance to paclitaxel and docetaxel (KB-8-5). As described previously, the DLD-1 cell line overexpressed MDR1 to equivalent levels found in KB-8-5 cells (19, 29) and was ~2.7- to 4.0-fold resistant to docetaxel and paclitaxel relative to the HCT-116 P-glycoprotein-negative cell lines described in Table 1. For example, in tumors derived from DLD-1, 20 mg/kg docetaxel or 60 mg/kg given i.v. q4d × 3 did not inhibit the growth of tumors (Fig. 5A). The results were markedly different for MST-997 because tumor growth was inhibited by >90% (10% T/C) with a single i.v. dose of 70 mg/kg in Intralipid (Fig. 5A) in 9 of 10 animals. The minimum efficacious dose of MST-997 was

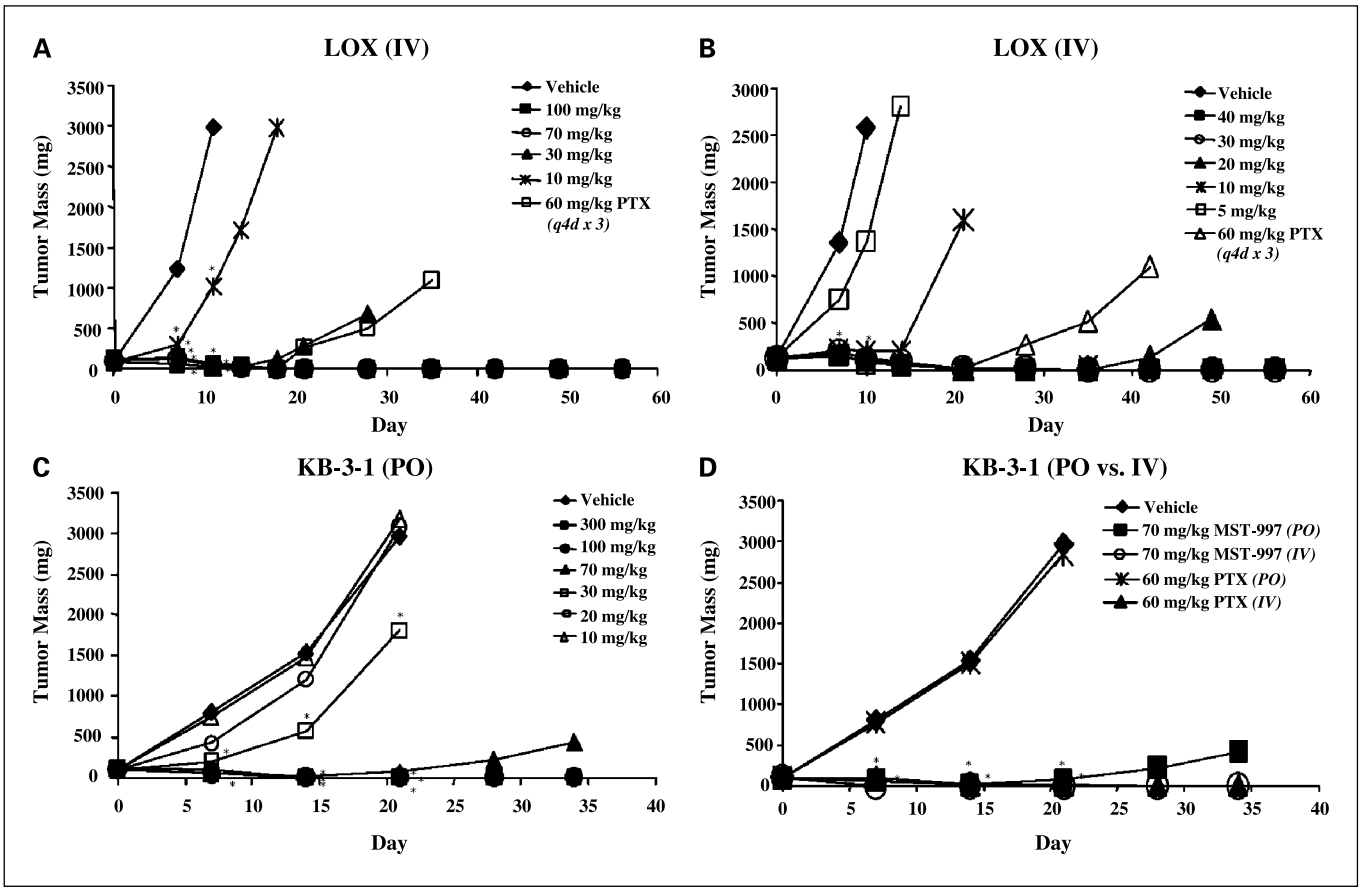


Fig. 4. Efficacy of MST-997 in paclitaxel (*PTX*) – sensitive xenograft tumor models *in vivo* by i.v. and p.o. administration. *A* and *B,* female *nu/nu* mice ($n = 10$) bearing paclitaxel-sensitive Lox melanoma tumors ~100 mg in size were treated i.v. with vehicle (Intralipid), 10 to 100 mg/kg of MST-997 given qd × 1 (*A*) or 5 to 40 mg/kg MST-997 dosed q4d × 3 (*B*). As a control, paclitaxel was administered i.v. at its optimal dose and schedule of 60 mg/kg q4d × 3 (*A* and *B*). *C,* animals bearing KB-3-1 epidermoid tumors ($n = 10$) were treated with vehicle (Cremophor EL) or a single p.o. dose of 10 to 300 mg/kg MST-997. *D,* animals bearing KB-3-1 tumors ($n = 10$) were dosed with vehicle (Intralipid), single i.v. and p.o. doses of 70 mg/kg MST-997 on day 1, or multiple 60 mg/kg doses of paclitaxel given q4d ×3. Tumor growth was determined every 7 days for 28 or 60 days depending on the model tested. *, $P < 0.01$.

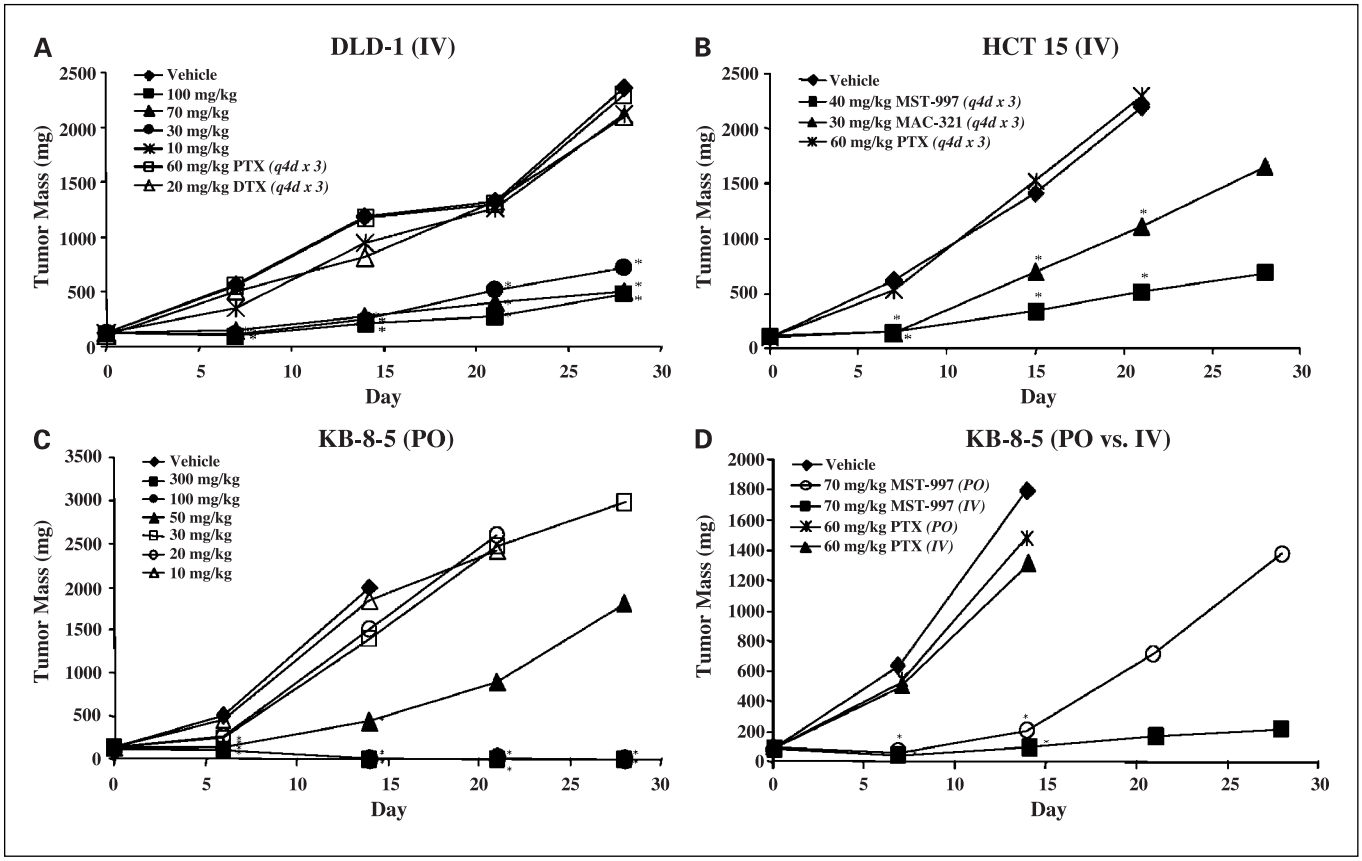


Fig. 5. Efficacy of MST-997 in paclitaxel (*PTX*) – resistant xenograft tumor models *in vivo* by i.v. and p.o. administration. *A,* female *nu/nu* mice ($n = 10$) bearing DLD-1 colon tumors ~100 mg in size were treated with vehicle (Intralipid) or the following: 10 to 100 mg/kg MST-997 qd ×1, 60 mg/kg paclitaxel or 25 mg/kg docetaxel (*DTX*) administered q4d × 3. *B,* animals bearing stage HCT-15 tumors ($n = 10$) were treated i.v. with vehicle (Intralipid), 40 mg/kg of MST-997, 30 mg/kg MAC-321, or 60 mg/kg paclitaxel given q4d × 3 as indicated. *C,* animals bearing KB-8-5 epidermoid tumors ($n = 10$) were treated with vehicle (Cremophor EL) or a single p.o. dose of 10 to 300 mg/kg. *D,* animals bearing KB-8-5 tumors ($n = 10$) were dosed with vehicle (Intralipid), single p.o. or i.v. doses of 70 mg/kg MST-997 qd × 1 or 60 mg/kg doses of paclitaxel (p.o. and i.v.) given q4d x 3. Tumor growth was determined every 7 days for 28 or 60 days depending on the model tested. *, $P < 0.01$.

30 mg/kg in the DLD-1 model versus 10 mg/kg in the Lox melanoma model, and the MTD was 100 mg/kg (Fig. 5A). Little or no weight loss was observed at any of the doses tested.

To further explore the use of MST-997 in paclitaxel-resistant models, the MDR1-positive epidermoid cell line KB-8-5, which is ~19-fold resistant to paclitaxel or docetaxel, was used. This level of resistance *in vitro* translates to resistance in animals as well (19). KB-8-5 xenografts were dosed i.v. from 10 to 100 mg/kg with MST-997 in Intralipid, and >50% inhibition was observed with doses as low as 30 mg/kg (data not shown). Maximum tumor growth inhibition at 90% (10% T/C) was observed in all animals tested at 70 mg/kg MST-997 (data not shown). The growth of KB-8-5 tumors treated with paclitaxel was not inhibited when given as a single i.v. dose of 60 mg/kg or on q4d × 3 schedules, which is efficacious in the paclitaxel-sensitive KB-3-1 model (data not shown).

To explore the efficacy and tolerability of multidose i.v. regimens, 40 mg/kg MST-997 was given q4d × 3 in HCT-15 xenografts, a highly resistant paclitaxel model. Indeed, in tissue culture and *in vivo*, these colon carcinoma cells are inherently resistant to both paclitaxel and docetaxel due to very high levels of MDR1 (refs. 19, 29; Fig. 5B). Given that repeated high doses of 70 mg/kg were toxic, lower doses of MST-997 were required on the multidose schedule. Interestingly, compared with MAC-321, another docetaxel analogue that was identified by Taxolog and characterized in our laboratory (29), which is only partially effective at multiple doses of 30 mg/kg q4d × 3; 40 mg/kg of MST-997 given i.v. on the same schedule in Intralipid resulted in >90% inhibition in 9 of 10 animals (Fig. 5B). Thus, multidose scheduling of MST-997 at low doses is extremely effective in reducing tumor growth in both paclitaxel-sensitive and highly resistant tumor xenografts and, more importantly, are well tolerated.

MST-997 was also tested orally in the KB-8-5 xenograft model. Animals bearing KB-8-5 xenografts were dosed p.o. with vehicle or 10 to 300 mg/kg MST-997 prepared in Cremophor EL. The minimum efficacious dose was 50 mg/kg, and cures were observed in 8 of 10 animals at the 100 and 300 mg/kg dose levels (Fig. 5C). However, MST-997 was partially effective at the 70 mg/kg dose, which typically resulted in >95% inhibition when given i.v. (Fig. 5D). Nevertheless, p.o. administration of MST-997 was more effective than either i.v. or p.o. dosing of paclitaxel in the KB-8-5 model (Fig. 5D). Thus, a single dose of MST-997 significantly inhibited, or in some cases completely repressed, tumor growth in paclitaxel-resistant tumor xenografts when given either i.v. or p.o.

Discussion

The prototypic taxanes paclitaxel and docetaxel are novel anti-microtubule cytotoxic agents that have been widely used to treat ovarian, lung, and breast cancers since their approval in the 1990s (2, 3). However, neither paclitaxel nor docetaxel is an optimal anticancer agent because they only prolong survival in some patients and are rarely used with curative intent. For example, the response rate with paclitaxel as first-line therapy in ovarian cancer and non–small cell lung carcinoma is ~59% and 25%, respectively, and the median survival time is ~36 and 9 months, respectively. The response rate to docetaxel as second-line therapy in breast cancer and non–small cell lung carcinoma is ~28% to 45% and 6%, respectively, and the median survival time is ~13 and 6 months, respectively. In most cases, those patients that initially respond to these taxanes ultimately develop resistance. In addition to these limitations, the compounds are poorly soluble, require the use of toxic vehicles, and induce numerous adverse reactions, including profound neutropenia and leukopenia (both agents) or fluid retention (unique to docetaxel).

Although the baccatin core of both these molecules is a natural product (10-deacetyl baccatin III) and difficult to synthesize, complete synthesis of the molecule is achieved by esterification of 10-deacetyl baccatin III with chemically synthesized side chains to yield the parent drug products (4). This allows for considerable modifications of the side chains and other positions on the baccatin core that can be altered in the laboratory. Based on our evaluation of taxane analogues produced by incorporating a semisynthetic approach and engineered by Taxolog, we identified MAC-321, our first clinical candidate. MAC-321 [5β,20-epoxy-1,2α,4,7β,10β,13α-hexahydroxytax-11-en-9-one 4 acetate-2-benzoate-7-propionate-13-ester with (2*R*,3*S*)-*N*-tertbutoxycarbonyl-3-(2-furyl)isoserine] is a docetaxel analogue that has superior efficacy in suppressing tumor growth *in vitro* and *in vivo* compared with paclitaxel and docetaxel (29). The second clinical candidate identified was MST-997, another analogue of docetaxel that is also has superior pharmacologic activity compared with the marketed taxanes and in some cases to MAC-321 itself in terms of improved efficacy in highly drug resistant tumor models *in vivo*. The synthesis of MST-997 further underscores the ability to derive highly potent and orally active yet structurally distinct taxanes by Taxolog's semisynthetic process.

Qualitatively, MST-997 is similar to paclitaxel and docetaxel, given that it disrupts microtubule function, induces polymerization of tubulin, and blocks cell cycle progression in the G_2-M phase of the cell cycle, resulting in apoptosis. The increased potency of MST-997, compared with paclitaxel, in inducing G_2-M arrest may be indicative of its ability to rapidly polymerize tubulin dimers. Although taxanes alter the polymer mass at high concentrations, alternations in the dynamic instability of microtubules without changes in polymer mass have been observed (5). Therefore, it is likely that the observed enhanced polymerization by MST-997 alters chromatid separation during mitosis, leading to cell death. The primary mechanism of cell death by MST-997 is mediated by apoptosis given that within 24 to 48 hours of treatment; ~40% of the KB-3-1 epidermoid cells displayed increased DNA fragmentation based on fluorescence-activated cell sorting analysis. Comparable with docetaxel, MST-997 is an equipotent cytotoxic agent *in vitro* in sensitive cell lines. However, *in vivo*, a single i.v. dose of MST-997 at ~70% of the MTD had curative effects, whereas multiple doses of either paclitaxel or docetaxel was required to achieve >90% growth inhibition. Most notably, 25 to 30 days after administration of paclitaxel or docetaxel, tumor regrowth occurred, whereas no tumors were present after a single dose of MST-997 treatment. There were no differences with regard to overall toxicity *in vivo* at the MTD between MST-997 and paclitaxel or docetaxel with >20% body weight loss most commonly observed. Therefore, in paclitaxel-sensitive tumor models, MST-997 is more potent *in vivo* and has curative effects after a single i.v. dose.

Drug resistance to paclitaxel and docetaxel is a major therapeutic limitation and is usually inherent (i.e., in colon carcinomas) or acquired (after multiple rounds of therapy). Perhaps, the most widely studied mechanism of paclitaxel and docetaxel resistance is associated with overexpression of the drug efflux pumps, such as MDR1 (14, 15). For example, cells transfected with MDR1 are resistant to paclitaxel and docetaxel, and MDR1 inhibitors resensitize cells to these agents (22, 23). Therefore, both agents seem to be transported by MDR1, indicating that overexpression of MDR1 mediates preferential resistance to paclitaxel and docetaxel compared with other agents. The effects of MST-997 were explored in several cell lines that had acquired resistance to paclitaxel and docetaxel (KB-8-5 and KB-V1) or were inherently resistant (DLD-1 and HCT-15) as a result of MDR1 overexpression. It was found that cells, such as KB-8-5, DLD-1, and HCT-15, were not resistant and retained sensitivity to MST-997 compared with taxane-sensitive counterparts. Once again, the level of potency of MST-997 in these MDR1-positive tumor cells lines translated *in vivo* as single i.v. dose at ~70% of the MTD resulted in >95% inhibition of tumor xenografts that were resistant to paclitaxel and docetaxel even when these agents were given in their optimal schedules of q4d × 3 or q5d × 2, respectively. It is noteworthy, however, that ~44-fold resistance to MST-997 is observed in a cell line that expresses extraordinarily high levels of MDR1 (KB-V1). The reversibility of this resistance by an MDR1-specific inhibitor suggests that MST-997 is a minor, albeit less potent, MDR1 substrate. However, such resistance in KB-V-1 cells is extraordinary because MDR1 expression at this level is rarely observed in the clinic (32). Rather, the level of P-glycoprotein expressed in KB-8-5 cells is more typically found in resistant patients (32). The above data suggest that MST-997 overcomes most cases of clinically relevant MDR1-mediated resistance in *in vitro* and *in vivo* models.

In addition to drug efflux pumps, resistance to paclitaxel, docetaxel, and epothilones in tissue cell culture has been attributed to tubulin point mutations (17, 20, 25, 26). In these lines, there is moderate to high cross-resistance to paclitaxel, docetaxel, and epothilone B, whereas lower cross-resistance was observed for MST-997 in cells. The tubulin mutant data suggest that MST-997 may interact in a similar but distinct binding domain of β-tubulin compared with other agents that bind to the taxane pharmacophore. However, the clinical relevance of these observations in patients remains to be validated. For example, one study reported mutations in class I β-tubulin DNA of serum samples isolated from 33% of patients with non–small cell lung carcinoma that were associated with

resistance to paclitaxel (33). However, the results have not been confirmed by subsequent studies where DNA or cDNA was obtained from tumor or serum samples (34–37). In addition, no mutations in β-tubulin that encode a different protein structure have been found in 62 human breast cancers (38). The discrepancy between the original report and subsequent studies is likely attributed to the use of nonselective primers used during PCR amplification of β-tubulin that would allow hybridization of probes to tubulin pseudogenes present in genomic DNA (35–37). The lack of positive results, however, does not exclude the possibility that clinical resistance to paclitaxel may be correlated with mutations in other isomers of α-tubulin or β-tubulin.

The lack of resistance to MST-997 *in vitro* and *in vivo* in cell lines, such as KB-8-5, DLD-1, or HCT-15, that overexpress P-glycoprotein at clinically relevant levels suggests that MST-997 may have use in patients who have failed previous taxane therapy due to P-glycoprotein overexpression. However, it should be noted that the contribution of each of these mechanism to the clinical response to taxanes is either controversial (i.e., MDR1), has not been substantiated (i.e., tubulin mutations), or is poorly studied (i.e., apoptotic mechanisms; refs. 4, 10, 13). Moreover, because P-glycoprotein is present and functional in normal tissues (15), including progenitors of the hematopoietic system (39) and endothelial cells within the blood brain barrier (15), it remains to be determined if enhanced efficacy of MST-997 in a P-glycoprotein-expressing tumor will also be associated with increased toxicity in humans.

Previously, it has been shown that paclitaxel and docetaxel are ineffective when given orally and both agents have poor bioavailability (40–42). Because paclitaxel and docetaxel are excellent substrates for MDR1, this effect is likely due to high levels of MDR1 that are present in the gastrointestinal tract (43, 44). Consistent with this hypothesis, the oral bioavailability of paclitaxel is improved in $MDR^{-/-}$ mice as well as in patients co-administered a MDR inhibitor (45, 46). The efficacy of orally given MST-997 at a single dose is comparable with i.v. given paclitaxel in sensitive tumor models. More importantly, the superior efficacy of MST-997 achieved with oral administration in paclitaxel-resistant xenografts is comparable with that observed with i.v. administration. The efficacy and tolerability of oral MST-997 makes it feasible to consider daily low dose therapy as a viable alternative to a high-dose infrequent therapy. This metronomic approach, which produces little side effects but is highly efficacious with other agents (47), may be effective with MST-997 as well. In addition, low-dose scheduling of MST-997 would also make alternative regimens amendable to testing in combination with other standard therapies or those based on signal transduction inhibitors (i.e., estrogen or epidermal growth factor receptor inhibitors) where oral dosing is efficacious.

Another advantage of MST-997 may indeed involve the dosing schedule. In the experiments presented, a single dose of MST-997 can be highly effective; therefore, dividing the dose was usually unnecessary. This is similar to docetaxel where an equivalent total dose of the compound has been reported to be equally efficacious when given on an intermittent schedule (days 1 and 6 or days 1, 5, 9, and 13) or thrice a day for 5 days (41). By comparison, paclitaxel often requires repetitive dosing (i.e., daily doses on days 1 to 5 or days 1, 5, and 9) to show efficacy in numerous models (31). Multiple-dose therapy with MST-997 may still be preferred in paclitaxel-resistant models where a single dose of MST-997 is only partially effective. Notably, multiple low doses of MST-997 was more effective than MAC-321, given at a similar schedule and dose, at overcoming drug resistance in the HCT-15 cell lines, which overexpresses high levels of MDR1. The latter observation suggests that MST-997 may be an effective alternative to MAC-321 in treating highly resistant tumors that overexpress comparable levels of MDR1.

Presently, paclitaxel and docetaxel are given in Cremophor EL or Tween 80, respectively. In both cases, patients must be premedicated to avoid a hyperallergenic response that has been attributed to the vehicle rather than the taxane (48, 49). Given that MST-997 was highly efficacious in a non-Cremophor vehicle, such as Intralipid, when given i.v., it may not induce a hypersensitive response in patients. Furthermore, MST-997, unlike paclitaxel is highly soluble in ethanol (~275 versus 39 mg/mL, respectively), thus eliminating the need for Cremophor EL or Tween 80. However, it is important to note that MST-997 is equally efficacious in either Cremophor EL or Tween 80. Therefore, it is likely that the superior activity observed for MST-997 is due to the compound and not the formulation. An alternative formulation to Cremophor EL that does not require premedications, such as Intralipid, however, will be clearly advantageous when MST-997 is given in the clinic.

In conclusion, MST-997 can be distinguished from other anti-microtubule agents in development or marketed because it has a unique chemical structure that is amenable to scale-up and administration in non-Cremophor vehicles. Like other taxanes, however, it induces polymerization of purified tubulin and G_2-M arrest in cells albeit at a more rapid rate and lower doses. Most notably, it is a highly potent orally bioavailable taxane that overcomes paclitaxel and docetaxel resistance *in vitro* and *in vivo* at single doses. The data presented here provide further support for aggressive development of MST-997, which is currently in phase I clinical trial for the treatment of human cancers.

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