

# A Phase I Trial of Doxorubicin, Paclitaxel, and Valspodar (PSC 833), a Modulator of Multidrug Resistance<sup>1</sup>

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## ABSTRACT

**Purpose:** P-glycoprotein is an efflux pump for many drugs including doxorubicin and paclitaxel. This study evaluated the coadministration of these drugs with the P-glycoprotein inhibitor valspodar (PSC 833) with the aim of determining: (a) maximum tolerated doses (MTDs) of doxorubicin followed by paclitaxel (DP); (b) the MTD of DP combined with PSC 833 (DPV), without and with filgrastim (G-CSF); and (c) the pharmacokinetic interactions of PSC 833 with doxorubicin and paclitaxel.

**Experimental Design:** For the first cycle, patients received doxorubicin as a 15-min infusion followed by paclitaxel as a 1-h infusion. For the second cycle, patients received reduced doses of DP with PSC 833 at 5 mg/kg p.o., four times a day for 12 doses.

**Results:** Thirty-three patients with various refractory malignancies were enrolled and assessable. The MTD of DP without PSC 833 was 35 mg/m<sup>2</sup> doxorubicin and 150 mg/m<sup>2</sup> paclitaxel. The MTD of DPV without G-CSF was 12.5 mg/m<sup>2</sup> doxorubicin and 70 mg/m<sup>2</sup> paclitaxel. The dose-limiting toxicity for both DP and DPV was neutropenia without thrombocytopenia. With G-CSF, the MTD for DPV was 20 mg/m<sup>2</sup> doxorubicin and 90 mg/m<sup>2</sup> paclitaxel. No grade 4 nonhematological toxicities were observed. Five partial and two minor tumor remissions were observed. Paired pharmacokinetics with and without PSC 833 revealed substantial drug interactions with both doxorubicin and paclitaxel.

**Conclusions:** PSC 833 can be administered safely with doxorubicin and paclitaxel. The pharmacokinetic profiles of these drugs are significantly affected by PSC 833, requiring ~60% dose reductions for equivalent degrees of myelosuppression.

## INTRODUCTION

P-gp<sup>3</sup> is a transmembrane efflux pump for many drugs including anticancer agents such as *Vinca* alkaloids, anthracyclines, taxanes, and epipodophyllotoxins (1). Encoded by the *MDR1* gene (2), this multidrug transporter is highly expressed in many clinically resistant tumors at diagnosis and/or relapse. Expression of *MDR1* is an adverse prognostic factor in several cancers (3-10), which suggests that P-gp-mediated drug resistance may contribute to a significant portion of treatment failures (11-13). Several noncytotoxic drugs such as verapamil, phenothiazines, and cyclosporines have been shown to modulate this mechanism of MDR by inhibition of P-gp function (14-22).

PSC 833 is a nonimmunosuppressive, nonnephrotoxic analogue of cyclosporine and is ~2- to 10-fold more potent than cyclosporine in its ability to inhibit P-gp (23-24). PSC 833 alters the pharmacokinetics of etoposide causing a decrease in CL, doubling of plasma  $t_{1/2}$ , and an increase in the dose-normalized plasma AUC (25-27). Significant pharmacokinetic effects are also observed when PSC 833 is given in combination with paclitaxel (28) or doxorubicin (29-30). These effects are in part a consequence of inhibition by PSC 833 of endogenous P-gp, which is expressed at high levels in the biliary canaliculi, renal tubules, and intestinal lumen. The drug interactions necessitate dose reductions of the chemotherapeutic agents to avoid excessive toxicity. The P-gp substrates doxorubicin and paclitaxel have recently been used in combination, particularly in breast cancer (31-35). We report here results of a Phase I study, in which we assessed the effect of PSC 833 on the MTD, pharmacokinetics, and pharmacodynamics of doxorubicin in combination with paclitaxel.

## PATIENTS AND METHODS

### Patient Selection

Patients were eligible if they had a pathological confirmation of incurable malignancy and had measurable disease. No chemotherapy for 3 weeks prior to study entry (6 weeks for nitrosoureas) or no radiation therapy for 4 weeks prior to study entry was permitted. Patients were required to have an Eastern

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<sup>3</sup> The abbreviations used are: P-gp, P-glycoprotein; MDR, multidrug resistance; PSC 833, valspodar; MTD, maximum tolerated dose; DP, doxorubicin followed by paclitaxel; DPV, DP combined with PSC 833; G-CSF, filgrastim; AUC, area under the curve; STD, stable disease; LVEF, left ventricular ejection fraction; HPLC, high-performance liquid chromatography; MRT, mean residence time.

Cooperative Oncology Group performance status 0–2 and an anticipated survival of >3 months. Adequate renal function (creatinine < 1.5 mg/dL) and hepatic function (serum bilirubin, <1.0 mg/dL, serum aspartate amino transferase <3 × upper limit of normal) was required. Normal bone marrow function was required as indicated by white blood count greater than 3,500 per mm<sup>3</sup> and platelet count greater than 100,000 per mm<sup>3</sup>. Prior doxorubicin treatment was allowed but could not exceed a cumulative dose of 300 mg/m<sup>2</sup>. LVEF was required to be ≥45% at rest by radionuclide ventriculogram. Patients with active central nervous system metastases were excluded. The protocol was approved by the Panel on Medical Human Subjects of Stanford University.

### Chemotherapy Administration

To obtain paired pharmacokinetic sampling data, patients were hospitalized in the Clinical Research Center of Stanford University Medical Center. Approximately 30 min prior to chemotherapy, all of the patients received i.v. premedication with dexamethasone 20 mg, famotidine 20 mg, and diphenhydramine 25 mg to prevent hypersensitivity reactions to paclitaxel, as well as an antiemetic (ondansetron 10 mg i.v.) prior to the administration of doxorubicin.

Patients received doxorubicin by i.v. 15-min infusion, followed by paclitaxel 1 h i.v. infusion, for the first cycle. Three weeks later, patients received reduced doses of doxorubicin and paclitaxel (as described below) in combination with PSC 833. PSC 833 (provided by Novartis Pharmaceuticals Corporation, East Hanover, New Jersey) was administered at 5 mg/kg p.o. four times daily for a total of 12 doses using the liquid formulation. If significant toxicity particular to PSC 833 occurred (*i.e.*, grade 3 ataxia), the PSC 833 dose was reduced to 4 mg/kg. Chemotherapy was administered on day 2, after the fifth or sixth dose of PSC 833.

Patients continued to receive this combination of doxorubicin, paclitaxel, and PSC 833 every 3 to 4 weeks, with assessment of toxicity and tumor response. In the absence of grade 3 or 4 neutropenia, or other grade 3 toxicity, the dosage of chemotherapy was increased stepwise, to ensure that the patient was receiving a therapeutic dose. If grade 3 or 4 neutropenia occurred the doses remained fixed for that patient. If grade 3 or 4 toxicity occurred (excluding neutropenia, hyperbilirubinemia, and nausea/vomiting), the patient was removed from the study or treated at a lower dose of chemotherapy, as agreed on by patient and investigator. On documentation of disease progression, or StD after six cycles of the combination treatment, the patient was removed from the study.

### Study Design

Separate dose escalation cohorts were planned for cycle 1 (DP) *versus* cycle 2 (DPV), as further described. If one patient among the first three in a cohort developed grade 3 or 4 toxicity (excluding neutropenia, hyperbilirubinemia, and nausea/vomiting), the cohort was expanded to six. If two of these six patients developed grade 3 or 4 toxicity (exclusions as noted above), this represented dose-limiting toxicity, and the maximal tolerated dose was defined as the next lower dose level.

Because grade 4 neutropenia represents a significant risk to

patients, but is no longer usually considered a dose-limiting toxicity, we decided that a >50% incidence of grade 4 neutropenia was unacceptable, even if there were no other dose-limiting toxicities in the cohort. Grade 4 neutropenia was, therefore, handled as follows. If any of the three patients in a cohort of three had grade 4 neutropenia, the cohort was expanded from three to six. If one to three of six patients in a cohort of six had grade 4 neutropenia, without fever, the next cohort was enrolled and treated at the next dose level. If 4 of 6 patients had grade 4 neutropenia, the cohort was expanded to 8 or 10 patients (see cohort I of DP and cohort III of DPV).

Once the maximally tolerated dose of doxorubicin and paclitaxel (DP) with PSC 833 was determined, we proceeded to establish the maximally tolerated dose with G-CSF (Neupogen; Amgen, Thousand Oaks, CA) support. Patients were treated at the maximally tolerated dose of DP for one cycle, and then, on cycle 2, received doxorubicin, paclitaxel, and PSC 833 (DPV) with G-CSF support, at one dose level higher than that previously established as the maximally tolerated dose without G-CSF support. This was based on our finding that the dose-limiting toxicity with DPV is neutropenia. Patients were enrolled in cohorts of three, until the maximally tolerated dose was determined.

Dose levels (all doses are in mg/m<sup>2</sup>) were as follows. The initial starting doses for DP alone were 40 and 175 mg/m<sup>2</sup> and for DPV were 10 and 70 mg/m<sup>2</sup>, respectively. Because of dose-limiting neutropenia observed in the first cohort of patients receiving DP (40/175), the second cohort was treated at a lower dose level of 35/150, respectively. For DPV, dose escalation entailed doses of 12.5/70 and then 15/80.

Dose escalation by cohort and determination of the MTD was based on dose-limiting toxicities observed during the first cycle of DP or DPV, respectively. After cycle 2, a patient whose nadir showed only grade 1 or 2 neutropenia received a higher dose of chemotherapy, with the increase according to the scheme presented above to ensure that patients were not undertreated.

### Assessment of Toxicities and Antitumor Response

Quantitative hematological and nonhematological toxicities were assessed by the common toxicity criteria of the National Cancer Institute (36). Patients were monitored closely and nadir counts checked on days 11 and 15. Imaging studies were performed after every two cycles of treatment.

Although antitumor efficacy was not a primary objective of the study, tumor response rate and time to progression were recorded. Partial remission was defined as a 50% reduction in the sum of products of the two largest perpendicular diameters of all of the measurable lesions for at least 4 weeks and with no new lesion or progression of assessable disease. Minor response was defined as partial regression of disease of <50%. StD was any measurement not fulfilling the criteria for response or progression and lasting at least 4 months. Tumor progression was defined as a 25% or greater increase in tumor size measured as the bidimensional product of perpendicular diameters. The time to progression was measured from the time of first study drug administration to documented progressive disease.

Cardiac function was monitored in all of the patients. All of the patients had a baseline radionuclide ventriculogram

(MUGA) at rest to determine the LVEF. Patients had a repeat study after every two cycles of treatment once the doxorubicin cumulative dose reached 300 mg/m<sup>2</sup>. Patients were taken off study if there was a >10% decrease in the absolute LVEF or a decline in the resting ejection fraction to ≤45%.

Cerebellar ataxia has previously been shown to be a limiting toxicity for PSC 833 (12). This toxicity was graded as follows: grade 1, slight subjective sense of incoordination, without difficulty in walking and a normal exam; grade 2, definite subjective incoordination on walking but able to walk without assistance and, on physical examination, broad-based gait and/or mild dysdiadochokinesis and difficulty walking heel to toe; grade 3, unable to walk without assistance from another person or a walker and, on examination, a markedly abnormal gait and inability to walk heel to toe; grade 4, unable to walk with assistance.

For grade 3 or 4 ataxia, the PSC 833 dose was reduced to 4 mg/kg, and the patient was observed closely for resolution of toxicity. If grade 3 ataxia still persisted, one PSC 833 dose was held, and the patient restarted at 4 mg/kg. If grade 3 ataxia still persisted, the PSC 833 dose was further reduced to 3 mg/kg.

#### Pharmacokinetic Studies of Doxorubicin, Paclitaxel, and PSC 833

Pharmacokinetic studies of doxorubicin and paclitaxel were performed on 25 patients during their first cycle of doxorubicin and paclitaxel alone, and subsequently during their first cycle with PSC 833. Plasma and urine levels of these drugs were analyzed. Samples of venous blood (2 ml, one pediatric green top tube) were drawn and patients asked to collect all of the urine for 48 h after the initiation of the chemotherapy infusions, in 24-h aliquots. These samples were analyzed for doxorubicin, paclitaxel, and their respective metabolites by HPLC. For the first doxorubicin and paclitaxel cycle, blood samples were drawn just prior to the chemotherapy infusion, and then 0.5, 1, 2, 3, 4, 6, 8, 12, 18, 24, 36, and 48 h after initiation of the paclitaxel infusion.

Venous sampling during the combined treatment with PSC 833 was similar to that for the chemotherapy alone with the addition of limited sampling for peak and trough PSC 833 values. Whole blood PSC 833 concentrations were determined using the ANAWA whole blood RIA kit for PSC 833 (ANAWA laboratories AG 8602; Zurich, Switzerland).

**Doxorubicin Assay.** Doxorubicin and its major metabolite doxorubicinol were analyzed by reverse-phase HPLC using modifications to prior methods (37). Concentrations were calculated using peak area ratios of doxorubicin or doxorubicinol to internal standard in relation to the standard curve. The coefficient of determinations ( $r^2$ ) for all of the standard curves was >0.95. The lower limit of quantitation was 2 nM, and the interday and intraday coefficients of variation were <5.0%.

**Paclitaxel Assay.** Paclitaxel was analyzed by reverse-phase HPLC using modifications to a prior method (38). Retention time of 6-OH-paclitaxel was determined using a supernatant of human liver microsomes containing metabolized paclitaxel (39). Retention times were: 6-OH-paclitaxel (6.5 min), cephalomannine (9.1 min), and paclitaxel (10.9 min). Concentrations were calculated as peak area ratios of paclitaxel or metabolite:internal standard relative to the standard curve. The coefficients of determination

( $r^2$ ) for standard curves were >0.95. The lower limit of quantitation was 0.01 μM, and the interday and intraday coefficients of variation were <8.0%.

#### Pharmacokinetic Analyses

Standard, noncompartmental pharmacokinetic calculations of parameters from doxorubicin and paclitaxel concentration-time data were performed using the XLPHARM M-IND program (Dr. V. K. Piotrovskij, VKPharmacokinetics, Turnhout, Belgium) implemented on Microsoft Excel (version 5.0, Microsoft Corporation, Redmond, WA; Ref. 40). The AUC was normalized for differences in the delivered dose by dividing the AUC by the dose administered in mg/m<sup>2</sup>. Volume of distribution at steady state ( $V_{ss}$ ) and clearance were derived from traditional noncompartmental equations in which:

$$(A) \quad V_{ss} = \text{Dose} \times \frac{AUMC}{AUC} - \frac{\text{Dose} \times T}{2 \times AUC}$$

where  $T$  is the duration of the infusion, clearance is the dose divided by the plasma AUC, and  $AUMC$  is the area under the first moment curve. The peripheral volume of distribution ( $V_p$ ) was calculated by subtracting the central volume of distribution ( $V_c$ ) from  $V_{ss}$ . The MRT was calculated using the equation:

$$(B) \quad MRT = \frac{AUMC}{AUC} - \frac{T}{2}$$

where  $T$  is the duration of drug administration. Time above threshold concentrations for paclitaxel (>0.05 or >0.025 μM) were determined by simulating the decay of a terminal first-order time point concentration ( $C$ ) over a range of time points ( $C_{p1}$ – $C_{p2}$ ), using the derived terminal elimination rate constant ( $K_z$ ) value, fitted to the equation:

$$(C) \quad C_{p2} = C_{p1} * \exp - (K_z * T)$$

using Scientist 2.0 (MicroMath Scientific Software, Salt Lake City, UT).

#### Statistical Considerations

Pharmacokinetic data were tested using the Wilk-Shapiro statistic and were found to be abnormally distributed (41). Differences in pharmacokinetic data and parameters between the doxorubicin or paclitaxel groups in the absence and presence of treatment with PSC 833 were compared using the Wilcoxon signed rank test. The *a priori* level of significance was  $P = 0.05$  (two-sided).

## RESULTS

#### Patient Characteristics

Thirty-nine patients with advanced cancers were entered into the trial between June 1995 and June 1998. Six were excluded from this report because they did not receive DPV because of progressive disease ( $n = 3$ ), toxicity ( $n = 2$ ), or patient's choice ( $n = 1$ ). The demographic characteristics of the 33 assessable patients are shown in Table 1. Disease categories of these patients were: ovarian cancer ( $n = 15$ ), lung cancer ( $n = 7$ ), sarcoma ( $n = 3$ ), and 1 each for germ cell, fallopian tube, breast, colon, neuroectodermal, peritoneal, vaginal, and

**Table 1** Demographic and clinical characteristics of the 33 patients registered on this trial

Gender	25 female, 8 male
Median age (range)	53 yr (33–76)
Mean prior chemotherapy regimens	3.4
Tumor types	
Ovarian	15
Lung	7
Sarcoma	3
Other	8
PS, <sup>a</sup> ECOG scale	
PS 0	18
PS 1	12
PS 2	3

<sup>a</sup> PS, performance status; ECOG, Eastern Cooperative Oncology Group.

**Table 2** Hematological toxicities of doxorubicin and paclitaxel coadministered without PSC 833

The MTD of doxorubicin and paclitaxel without PSC 833 was established as shown in cohort II based on the experience with the first 10 patients, of whom 5 had grade 4 neutropenia and two had fever with neutropenia. Subsequently, 16 more patients were enrolled at the MTD for a total of 26 patients.

	Cohort I	Cohort II
Doxorubicin dose, mg/m <sup>2</sup>	40	35
Paclitaxel dose, mg/m <sup>2</sup>	175	150
No. of patients	7	26
Grade 4 <sup>a</sup> neutropenia	6/7	12/26
Grade 3 <sup>b</sup> neutropenia	1/7	11/26
Febrile neutropenia	0/7	5/26
Mean neutrophil nadir/mm <sup>3</sup>	290	930
Mean platelet nadir	162	165

<sup>a</sup> Grade 4 neutropenia was an absolute neutrophil count (ANC) of <500/mm<sup>3</sup>.

<sup>b</sup> Grade 3 neutropenia was an ANC of 500–999/mm<sup>3</sup>.

thymic cancers. The median age was 53 years (range, 33–76 years). The patients were pretreated with a mean of 3.4 regimens (excluding hormonal and radiation therapy) prior to study entry. There were 25 women and 8 men, reflecting the relatively large number of patients with ovarian cancers.

### Determination of MTD

**Doxorubicin and Paclitaxel without PSC 833.** The toxicities for the two dose levels of DP alone are shown in Tables 2 and 3. Seven patients were entered on the starting dose of 40 and 175 mg/m<sup>2</sup>, and six of seven experienced grade 4 neutropenia. Although there were no cases of fever with neutropenia at this dose level, the high rate of grade 4 neutropenia was considered unacceptable without the use of G-CSF. The next dose level was reduced to 35 and 150 mg/m<sup>2</sup>, and this was defined as the MTD because 5 of 10 patients had grade 4 neutropenia with 2 of 10 also experiencing fever. Subsequently, 16 more patients were entered at this dose level. Overall at this dose, 12 of 26 patients had grade 4 neutropenia and 11 of 26 had grade 3 neutropenia. Five of these 26 patients had fever associated with the neutropenia.

**Doxorubicin and Paclitaxel with PSC 833.** The toxicities observed for DPV without and with G-CSF are presented in

**Table 3** Nonhematological toxicities (grade 2 and 3) recorded during the first cycle of treatment with the combination of doxorubicin and paclitaxel, without PSC 833<sup>a</sup>

	Cohort I	Cohort II
Doxorubicin dose, mg/m <sup>2</sup>	40	35
Paclitaxel dose, mg/m <sup>2</sup>	175	150
No. of patients	7	26
Stomatitis	1	0
Nausea and vomiting	1	4
Neuropathy	1	1
Fatigue	0	5
Rash	0	2

<sup>a</sup> No grade 4 toxicities were observed.

**Table 4** Hematological toxicities recorded during the first cycle of treatment with the combination of doxorubicin, paclitaxel, and PSC 833

	Cohort I	Cohort II	Cohort III	Cohort IV (+G-CSF)	Cohort V (+G-CSF)
DP doses	10/70	12.5/70	15/80	20/80	20/90
No. of patients	6	7 <sup>a</sup>	8	6	6
Grade 4 neutropenia	2/6	3/6	7/8	1/6	2/6
Grade 3 neutropenia	0/6	3/6	1/8	1/6	3/6
Febrile neutropenia	1/6	2/6	2/8	0/3	2/6
Mean neutrophil nadir/mm <sup>3</sup>	1000	1000	350	2000	1600
Grades 3 and 4 <sup>b</sup> thrombocytopenia	0/6	0/6	0/8	2/6	3/6

<sup>a</sup> Six of seven patients were assessable because one patient died because of progressive disease prior to nadir.

<sup>b</sup> Grade 4 thrombocytopenia was platelet count, <25,000/mm<sup>3</sup>; grade 3 was count, <50,000/mm<sup>3</sup>.

Tables 4 and 5. The MTD of this combination was 12.5 mg/m<sup>2</sup> doxorubicin and 70 mg/m<sup>2</sup> paclitaxel with three of six patients experiencing grade 4 neutropenia, and two with both fever and neutropenia (Table 4). No significant thrombocytopenia (platelets <100,000/mm<sup>3</sup>) was noted in cohorts I through III. It is evident from Tables 2 and 4 that cohort I of DP produced a degree of myelosuppression very similar to that of cohort III of DPV, with mean neutrophil nadir counts of 290 and 350/mm<sup>3</sup>, respectively. Likewise, cohort II of DP was similar in myelosuppression to cohort II of DPV (Tables 2 and 4), with mean neutrophil nadir counts of 930 and 1,000/mm<sup>3</sup>, respectively.

The next twelve patients (cohorts IV and V) were treated with escalating dose of DPV with G-CSF support. DPV was well tolerated by six patients at doxorubicin 20 mg/m<sup>2</sup> and paclitaxel 80 mg/m<sup>2</sup> with one (of six) grade 3 and one (of six) grade 4 thrombocytopenia without evidence of bleeding. Only one grade 4 neutropenia was observed. The use of G-CSF resulted in higher nadir counts (mean/median 2,000/mm<sup>3</sup>) compared with the earlier cohorts. Six patients were treated at the next dose level of 20/90 (cohort V) and this dose defined the MTD because two of six patients had grade 4 neutropenia with fever and two of six patients experienced grade 3 or 4 thrombocytopenia, with a total of three of the six patients experiencing dose-limiting toxicities (Table 4).

**Table 5** Nonhematological toxicities (grade 2 and 3) recorded during the first cycle of treatment with the combination of doxorubicin, paclitaxel, and PSC 833<sup>a</sup>

	Cohort I	Cohort II	Cohort III	Cohort IV	Cohort V
Doses of doxorubicin and paclitaxel, mg/m <sup>2</sup>	10/70	12.5/70	15/80	20/80	20/90
No. of patients	6	7	8	6	6
Ataxia	3	4	7	3	3
Stomatitis	0	0	1	1	0
Nausea and vomiting	2	2	3	0	1
Fatigue	1	0	2	2	2
Hyperbilirubinemia	4	1	3	4	3
Hypertension	1	0	1	0	0
Orthostatic hypotension	0	0	0	0	2
Neuropathy	0	0	1	0	0

<sup>a</sup> No grade 4 toxicities were observed.

### Toxicities

The most frequent toxicity for both DP and DPV was hematological as shown in Tables 2 and 4. Grade 4 neutropenia was observed in 18 of 33 patients treated with DP alone and 12 of 20 patients treated with DPV. Fever with neutropenia was observed in 5 of 33 patients on DP and 5 of 20 treated with DPV. Two patients developed fever and neutropenia despite prophylactic G-CSF in cohort V, Table 4. Significant thrombocytopenia (<50,000/mm<sup>3</sup>) was seen in two of six patients in cohort IV and in three of six patients in cohort V.

Nonhematological toxicities (grades 2 and 3) for DP alone are shown in Table 3 and for DPV in Table 5. No grade 4 toxicities were observed. Transient reversible hyperbilirubinemia was an anticipated side effect of PSC 833 (41) and was seen in 15 of 33 patients. One patient developed a cardiac arrhythmia that required treatment. Other reversible side effects observed were nausea/vomiting ( $n = 5$  for DP;  $n = 8$  for DPV), constipation ( $n = 1$  for DP;  $n = 8$  for DPV), fatigue ( $n = 6$  for DP;  $n = 7$  for DPV), and skin rash ( $n = 2$  for DP and  $n = 1$  for DPV).

Cerebellar neurotoxicity attributed to PSC 833 (42) is shown in Table 6 for each of the cohorts studied. In 9 patients, PSC 833 produced significant (grade 3), but rapidly reversible, ataxia requiring dose reduction of PSC 833 to 4 mg/kg in eight patients and to 3 mg/kg in two patients. Only two of these nine patients had peak PSC 833 blood levels >3000 ng/ml (7020 and 4310 ng/ml, respectively)

### Antitumor Responses

Five partial responses were noted among 33 assessable patients (non-small cell lung, 12, 6, and 4 months; ovarian cancer, 5 months; and fallopian tube cancer, 11 months), as well as two minor responses (ovarian cancer, 6 months; mediastinal germ cell tumor, 4 months). Six patients had StD (three ovarian cancers and one sarcoma for 4 months, two non-small cell lung cancers for 6 and 9 months, respectively).

### Pharmacokinetic Evaluations

The median peak whole blood PSC 833 level in 15 patients evaluated was 3090 ng/ml (range, 1460–7020 ng/ml) with a

**Table 6** Cerebellar ataxia by cohorts of patients treated with doxorubicin, paclitaxel, and PSC 833<sup>a</sup>

Grade	Cohort I $n = 6$	Cohort II $n = 7$	Cohort III $n = 8$	Cohort IV $n = 6$	Cohort V $n = 6$
0	2	1	0	0	0
1	1	2	1	3	3
2	3	3	3	2	0
3	0	1	4	1	3

<sup>a</sup> No ataxia was noted during treatment cycles without valsopodar. In nine patients, PSC 833 produced significant (grade 3), but rapidly reversible, ataxia requiring dose reduction of PSC 833 to 4 mg/kg. Two patients required a dose reduction to 3 mg/kg of PSC 833. No grade 4 ataxia was noted.

median trough level of 3400 ng/ml (1460–5500 ng/ml). Paired pharmacokinetic analyses with and without PSC 833 were performed in 18 courses of doxorubicin and in 16 courses of paclitaxel. PSC 833 resulted in substantial drug interaction with both doxorubicin and paclitaxel. In the 18 patients with pharmacokinetic analyses of doxorubicin, an average dose reduction of 65% resulted in an actual doxorubicin AUC decline of 35% during PSC 833 treatment. This was primarily a reflection of a 57% reduction in doxorubicin peak concentration at the end of drug administration. However, PSC 833 treatment resulted in an 86% increase in the dose-normalized AUC of doxorubicin, which correlated with a 44% decrease in CL, a 77% increase in the terminal phase  $t_{1/2}$  and an 84% increase in MRT (Table 7). Despite the lower actual AUC values, the longer  $t_{1/2}$  and MRT values indicate that coadministration with PSC 833 results in a prolonged low-level exposure to the drug as illustrated in the concentration-time profile for a patient receiving doxorubicin  $\pm$  PSC 833 in Fig. 1. PSC 833 had a greater effect on doxorubicin AUC, causing an increase of 259% for this metabolite.

In 16 patients with pharmacokinetic analyses of paclitaxel, coadministration of PSC 833 treatment resulted in a 158% increase in paclitaxel  $V_{ss}$ , a 213% increase in ( $V_p$ ), a 96% increase in the terminal phase  $t_{1/2}$ , and a 31% increase in time >0.025 mM concentration (Table 8). There was no apparent effect on the AUC of paclitaxel. However, the longer  $t_{1/2}$  and MRT values indicate that PSC 833 prolongs the duration of exposure to paclitaxel as illustrated in the typical concentration-time profile for a patient receiving paclitaxel with and without PSC 833 in Fig. 2. PSC 833 had a greater effect on 6-OH-paclitaxel, causing a 222% increase in the AUC of this metabolite.

### DISCUSSION

*MDR1* gene expression is seen in a variety of malignancies, both at initial diagnosis and at time of relapse (1, 12), and is one of the mechanisms implicated in clinical drug resistance. Modulation of MDR with inhibitors of P-gp may improve the efficacy of chemotherapy (12, 22). Doxorubicin and paclitaxel are substrates of P-gp (the protein encoded by the *MDR1* gene) and are active agents in many solid tumors. Therefore, modulation of resistance to these drugs is an attractive concept. A large number of clinical trials of reversal of MDR have been undertaken with drugs that are relatively weak P-gp inhibitors, often producing limiting toxicities at doses below those necessary to inhibit MDR (11–12). PSC 833 is 2- to 10-fold more potent than

Table 7 Effect of PSC 833 on pharmacokinetic (PK) parameters of doxorubicin in patients ( $n = 18$ ) treated sequentially over two cycles with DP and then with DPV

The data are means ( $\pm$ SD).

PK parameter <sup>a</sup>	DP	DPV	% change	$P^b$
Dose, $\mu\text{mol}/\text{m}^2$	63 (4)	22 (4)	-65	0.0002
$C_{\text{max}}$ , $\mu\text{M}$	0.17 (0.05)	0.07 (0.03)	-57	0.0003
$t_{1/2}$ , h	23 (7)	40 (13)	+77	0.0004
AUC, $\text{h}\cdot\mu\text{M}$	1.6 (0.5)	1.1 (0.4)	-35	0.0006
AUC per $\mu\text{mol}$ dose	2.6 (0.7)	4.8 (1.5)	+86	0.0003
CL, <sup>c</sup> liter/h/m <sup>2</sup>	42 (12)	23 (10)	-44	0.0002
$V_{\text{ss}}$ , liter/m <sup>2</sup>	1149 (556)	1078 (330)	-6	0.7
MRT, h	27 (8)	50 (17)	+84	0.0004
AUC, $\text{h}\cdot\mu\text{M}$	1.3 (0.6)	1.6 (0.6)	+18	0.13
AUC/ $\mu\text{mol}$ dose	0.03 (0.01)	0.05 (0.02)	+259	0.0002

<sup>a</sup> Pharmacokinetics are based on 48 h of sampling after doxorubicin infusion. Analyses are from 18 patients receiving 18 courses of DP and 18 courses of DPV, without G-CSF.

<sup>b</sup>  $P$ s are Wilcoxon rank-sum test, two-tailed, doxorubicin alone versus doxorubicin plus PSC 833.

<sup>c</sup> CL, clearance;  $V_{\text{ss}}$ , volume of distribution at steady state.

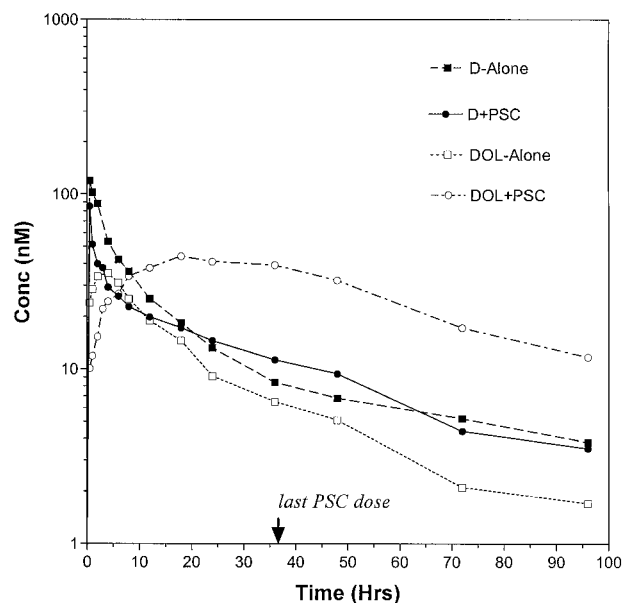


Fig. 1 Drug concentration and time plots of doxorubicin (D) and doxorubicinol (DOL) in the same patient treated sequentially without and with PSC 833 (PSC), with 96-h sampling. The doses of doxorubicin were 35  $\text{mg}/\text{m}^2$  without and 20  $\text{mg}/\text{m}^2$  with PSC 833.

its parent compound cyclosporine for modulating MDR *in vitro* and *in vivo* (23–24). In this study, target levels of PSC 833 capable of MDR1 modulation ( $>1000$  ng/ml) were achieved in all of the 15 patients thus evaluated.

On the basis of our previous experience and those of others using these agents, we anticipated significant pharmacokinetic interactions between PSC 833 and the MDR-related agents, and, therefore, used lower initial doses of doxorubicin and paclitaxel when given with PSC 833 (26, 29, 30, 43). The MTD of DP alone was 35  $\text{mg}/\text{m}^2$  and 150  $\text{mg}/\text{m}^2$  respectively. The addition of PSC 833 to DP resulted in substantial hematological toxicity even at the lowered doses, and, as a result, the MTD of the combination was 12.5  $\text{mg}/\text{m}^2$  doxorubicin and 70  $\text{mg}/\text{m}^2$  pacli-

taxel. This degree of dose reduction is not surprising. Previous studies with paclitaxel or doxorubicin when administered as single agents combined with cyclosporine or PSC 833 also produced marked alterations in pharmacokinetics, necessitating 50 and 60% dose reductions, respectively (12, 28–30). With the addition of G-CSF, we were able to escalate doses of doxorubicin only marginally because of thrombocytopenia. The MTD of the combination with G-CSF support was 20  $\text{mg}/\text{m}^2$  doxorubicin and 90  $\text{mg}/\text{m}^2$  paclitaxel.

The addition of PSC 833 significantly prolonged the plasma  $t_{1/2}$  and MRT of both doxorubicin and paclitaxel. The observed increase in myelosuppression when PSC 833 was combined with doxorubicin and paclitaxel is most likely related to these pharmacokinetic effects. The pharmacokinetic interactions of these drugs are consistent with clinical, laboratory, and animal model observations (26, 44–45) and corroborate the need for dose reduction of doxorubicin and paclitaxel when they are combined with PSC 833.

Previously, Gianni *et al.* (46) reported a correlation of the degree of neutropenia to the duration of paclitaxel concentration above 0.05  $\mu\text{M}$ . In paired analyses, we found no difference in time above 0.05  $\mu\text{M}$  of paclitaxel for DP alone versus DPV, despite the fact that the dose of paclitaxel was 2-fold higher in the DP cycle. The prolongation of the elimination phase of paclitaxel by PSC 833 was manifested by a significant increase in time above 0.025  $\mu\text{M}$  for paclitaxel in the DPV cycle. These effects of PSC 833 on the disposition and distribution of paclitaxel are likely to have contributed to the relatively high myelosuppression seen with the low dose of doxorubicin and paclitaxel in DPV.

There was a marked effect of PSC 833 on the disposition of the major metabolites doxorubicinol and 6-OH-paclitaxel. This is consistent with our previous observations with cyclosporine and doxorubicin in humans (47) and with *in vitro* studies of paclitaxel and cyclosporine (39). However, the clinical importance of the interaction between PSC 833 and the metabolites is likely to be less than for the parental drugs, because these metabolites are markedly less cytotoxic *in vitro* (48–49).

The formulation of paclitaxel contains cremophor EL, which has been shown to cause alterations in the pharmacoki-

Table 8 Effect of PSC 833 on pharmacokinetic (PK) parameters of paclitaxel in patients ( $n = 16$ ) treated sequentially with DP and DPV  
The data are means ( $\pm$ SD).

PK Parameter <sup>a</sup>	DP	DPV	% change	P <sup>b</sup>
Dose, $\mu\text{mol}/\text{m}^2$	188 (15)	86 (6)	-55	0.0003
$C_{\text{max}}$ , $\mu\text{M}$	10.6 (6.4)	3.6 (2.8)	-66	0.0008
$t_{1/2}$ , h	8 (4)	16 (9)	+96	0.0003
AUC, $\text{h}\cdot\mu\text{M}$	20 (9)	8 (4)	-58	0.0003
AUC per $\mu\text{mol}$ dose	0.10 (0.04)	0.10 (0.04)	0	0.5
CL, $\text{liter}/\text{h}/\text{m}^2$	10.7 (3.4)	11.9 (4.7)	+11	0.5
$V_{\text{ss}}$ , $\text{liter}/\text{m}^2$	45 (26)	116 (66)	+158	0.0005
$V_{\text{p}}$ , $\text{liter}/\text{m}^2$	30 (19)	97 (64)	+220	0.0003
MRT, h	4 (2)	11 (7)	+156	0.0003
$h > 0.05 \mu\text{M}$	23 (10)	25 (12)	+9	0.5
$h > 0.025 \mu\text{M}$	31 (13)	41 (19)	+31	0.014
6-OH-paclitaxel				
AUC, $\text{h}\cdot\mu\text{M}$	1.7 (1.5)	5.3 (3.3)	+222	0.003
AUC per $\mu\text{M}$ dose	0.01 (0.01)	0.06 (0.04)	+589	0.0005

<sup>a</sup> Analyses are from 16 patients receiving 16 courses of paclitaxel alone and 17 courses of paclitaxel plus PSC 833.

<sup>b</sup> Ps are Wilcoxon rank-sum test, two-tailed, paclitaxel without versus paclitaxel with PSC 833.

<sup>c</sup> CL, clearance;  $V_{\text{ss}}$ , volume of distribution at steady state;  $V_{\text{p}}$ , volume of peripheral compartment.

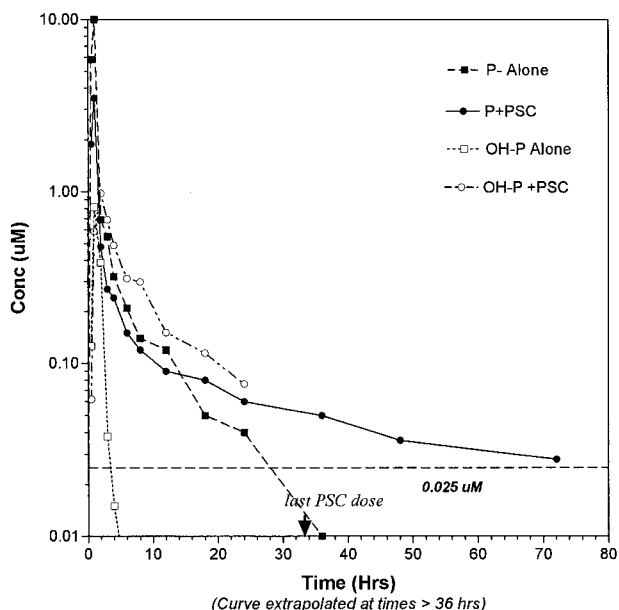


Fig. 2 Drug concentration and time plots of paclitaxel (P) and its 6-OH metabolite in the same patient without and with PSC 833 (PSC). The doses of paclitaxel were  $150 \text{ mg}/\text{m}^2$  without and  $70 \text{ mg}/\text{m}^2$  with PSC 833.

netics of the doxorubicin (50, 51, 52). In this study, because the paclitaxel dose was lowered with PSC 833, the decreased amount of cremophor EL would have had minimal effects during the PSC 833 cycle.

Recently, Gianni *et al.* (53), using a schedule of administration similar the one in our study, *i.e.*, a bolus of doxorubicin, followed 15 min later with a 3-h infusion of paclitaxel, found a modest interaction when doxorubicin administration preceded paclitaxel. These effects were observed during the  $\beta$   $t_{1/2}$  phase with no significant change in the terminal ( $\gamma$ )  $t_{1/2}$ , which suggests that the observed changes could be attributable to alterations in pharmacokinetics when the paclitaxel vehicle, cremophor EL, was at its maximal concentrations. In contrast, we

found significant increase in dose-normalized AUC (86%) and terminal elimination  $t_{1/2}$  (77%), as well as other parameters (Table 7). Similar findings were observed with the major metabolite doxorubicinol. These data support the contention that cremophor EL did not contribute significantly to the observed effects on doxorubicin pharmacokinetics.

Paclitaxel was well tolerated as a 1-h infusion as has been reported previously (54). The main toxicity of both DP and DPV was hematological (Tables 2 and 4). PSC 833 caused a reversible grade 3 ataxia in nine patients, which required dose reduction to 4 mg/kg in eight patients, and 3 mg/kg in two patients. The mechanism of the cerebellar toxicity of PSC 833 is unknown, but is probably not related directly to inhibition of P-gp, because some other inhibitors of P-gp including cyclosporine do not cause ataxia, and P-gp knockout mice are not ataxic (55). The transient hyperbilirubinemia produced by PSC 833 is similar to that of high-dose cyclosporine (42) and is likely attributable to inhibition of the canalicular multispecific organic anion transporter (cMOAT/MRP2; Refs. 56, 57), and/or other transporters of bilirubin and bilirubin glucuronide in the biliary canaliculi.

Tumor regression was observed in 7 of 33 patients and another 6 patients had stabilization of disease for at least 4 months. The design of this study did not allow us to assess what role MDR modulation may have played in these responding patients. Whether these responses are attributable to reversal of *MDR1* or intrinsic sensitivity to doxorubicin and paclitaxel cannot be determined from the design of this Phase I trial. Moreover, treatment of P-gp-negative tumors with inhibitors of P-gp may result in clinical benefit by preventing the development of MDR. This has been demonstrated in a laboratory model of a human sarcoma cell line, in which PSC 833 reduced the mutation rate for doxorubicin resistance significantly, and suppressed the activation of *MDR1* (58). These findings suggest that treatment of drug-sensitive, P-gp-negative malignancies with effective MDR modulation may suppress the emergence of resistant *MDR1* positive subclones and result in improved outcome (1, 14).

In conclusion, PSC 833 can be administered safely in combination with doxorubicin and paclitaxel with appropriate dose modification of the cytotoxic drugs. PSC 833 significantly increases the paclitaxel and doxorubicin exposure secondary to decreased clearance, accounting for the need to reduce doses ~2-fold to achieve equivalent myelosuppression. Additional Phase II/III studies are required to determine the clinical role of this intriguing combination.

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