A Phase I/II Study of Infusional Vinblastine with the P-Glycoprotein Antagonist Valspodar (PSC 833) in Renal Cell Carcinoma

Susan E. Bates,1 Susan Bakke,1 Min Kang,1 Robert W. Robey,1 Suoping Zhai,1 Paul Thambi,1 Clara C. Chen,5 Sheela Patil,8 Tom Smith,8 Seth M. Steinberg,2 Maria Merino,4 Barry Goldspiel,7 Beverly Meadows,1 Wilfred D. Stein,9 Peter Choyke,6 Frank Balis,3 William D. Figg,1 and Tito Fojo1

1Cancer Therapeutics Branch, 2Biostatistics Branch, 3Pediatric Oncology Branch, and 4Laboratory of Pathology, Center for Cancer Research, National Cancer Institute, Bethesda, Maryland; Departments of 5Nuclear Medicine and 6Radiology and 7Clinical Center Pharmacy, Warren G. Magnuson Clinical Center, NIH, Bethesda, Maryland; 8Novartis Pharmaceutical Company, East Hanover, New Jersey; and 9Hebrew University, Jerusalem, Israel

ABSTRACT

Purpose: P-glycoprotein (Pgp) inhibitors have been under clinical evaluation for drug resistance reversal for over a decade. Valspodar (PSC 833) inhibits Pgp-mediated efflux but delays drug clearance, requiring reduction of anticancer drug dosage. We designed an infusion schedule for valsepodar and vinblastine to mimic infusional vinblastine alone. The study was designed to determine the maximally tolerated dose of vinblastine, while attempting to understand the pharmacokinetic interactions between vinblastine and valsopodar and to determine the response rate in patients with metastatic renal cell cancer.

Patients and Methods: Thirty-nine patients received continuous infusion valsepodar and vinblastine. Vinblastine was administered for 3 days to compensate for the expected delay in clearance and the required dose reduction. Valsopodar was administered initially at a dose of 10 mg/kg/d; the dose of vinblastine varied.

Results: The maximum-tolerated dose of vinblastine was 1.3 mg/m²/d. As suggested previously, serum valsepodar concentrations exceeded those needed for Pgp inhibition. Consequently, the dose of valsepodar was reduced to 5 mg/kg, allowing a vinblastine dose of 2.1 mg/m²/d to be administered. Pharmacodynamic studies demonstrated continued inhibition of Pgp at lower valsepodar doses by functional assay in Pgp-expressing CD56+ cells and by ⁹⁹ᵐTc-sesta-mibi imaging. A 15-fold range in cytochrome P450 activity was observed, as measured by midazolam clearance. No major responses were observed.

Conclusions: These results suggest that the pharmacokinetic impact of cytochrome P450 inhibition by valsepodar can be reduced although not eliminated, while preserving Pgp inhibition, thus separating the pharmacokinetic and pharmacodynamic activities of valsepodar.

INTRODUCTION

Most cancers are either resistant to chemotherapy initially or acquire resistance after exposure to chemotherapy. One mechanism of resistance is the reduction of intracellular concentrations of anticancer agents by drug transporters. P-glycoprotein (Pgp), encoded by the MDR-1 gene and the most extensively studied drug transporter, was first described by Ling et al. (1) in the 1970s. In vitro studies with agents such as verapamil and cyclosporine A showed that Pgp could be inhibited, stimulating interest in clinical trials. However, clinical trials with these agents had limited success initially because of toxicities exacerbated by the high serum concentrations of the drugs required to achieve Pgp inhibition (such as bradycardia with verapamil; Ref. 2) and because of pharmacokinetic interactions with chemotherapy agents (3, 4).

Second-generation Pgp inhibitors such as the cyclosporine D derivative valsepodar (PSC 833) were subsequently developed as nontoxic, more potent agents. However, pharmacokinetic interactions required reductions in the dose of the concurrently administered anticancer agent (4). The pharmacokinetic interactions are likely because of decreased clearance of the anticancer agents, possibly as a result of Pgp inhibition in organs such as the gastrointestinal tract and kidney, as well as inhibition of CYP3A (1, 5–7). Third-generation, highly specific Pgp antagonists such as tariquidar (XR9576), laniquidar (R-101933), zo- suquidar (LY-335979), elacridar (GF120918), and ONT-093 (OC-144-093) are now being developed in an attempt to avoid significant pharmacokinetic interactions (1, 8–12).

We performed a Phase I/II study with infusional valsepodar and infusional vinblastine in patients with renal cell carcinoma. We chose to conduct this study based on promising results in renal cell carcinoma from our previous Phase I study using oral valsepodar and infusional vinblastine administered for 5 days (13). In that trial there were three complete responses and one partial response, all in patients with clear cell carcinoma of the kidney. However, a significant reduction in the vinblastine dose was required. The maximum-tolerated dose (MTD) identified for infusional vinblastine in combination with oral valsepodar was 0.6 mg/m²/d for 5 days, for a total dose of 3 mg/m²/cycle. This is approximately one-third of the 8.5 mg/m²/cycle (1.7 mg/m²/d for 5 days) vinblastine dose that can be administered to patients receiving infusional vinblastine for 5 days without a Pgp antagonist (14).
Several strategies were used to improve upon the results of the Phase I trial. First, we planned to give vinblastine as a 3-day instead of a 5-day infusion to allow safe administration of a higher vinblastine dose. Starting with a vinblastine dose of 1.0 mg/m²/d (total dose 3 mg/m²), our goal was to increase the vinblastine to approach a total dose of 8.5 mg/m². Another strategy to improve the Phase I results was to give valsopodar by continuous infusion. The oral administration of valsopodar in the Phase I trial resulted in wide interpatient variability in valsopodar whole blood concentrations, and periodically, high peak or low trough levels (13). The high peak concentrations were implicated in the transient ataxia observed in some patients, and low trough concentrations potentially reduced Pgp inhibition. We also attempted to determine whether various levels of CYP3A from patient to patient could explain the variability seen in the pharmacokinetic profiles of valsopodar and in the interaction with vinblastine in the previous Phase I trial. The cytochrome P450 (P450) 3A activity level in each patient was estimated with vinblastine in the previous Phase I trial. The cytochrome P450 (P450) 3A activity level in each patient was estimated in the transient ataxia observed in some patients, and low trough concentrations potentially reduced Pgp inhibition. We also attempted to determine whether various levels of CYP3A from patient to patient could explain the variability seen in the pharmacokinetic profiles of valsopodar and in the interaction with vinblastine in the previous Phase I trial. The cytochrome P450 (P450) 3A activity level in each patient was estimated by determining midazolam clearance after i.v. administration (15, 16).

A Phase II trial of infusional vinblastine for 3 days in combination with infusional valsopodar for 6 days was conducted with the primary goals of determining the MTD of vinblastine, attempting to understand the pharmacokinetic interactions between vinblastine and valsopodar and determining the response rate of this regimen in patients with metastatic renal cell cancer.

PATIENTS AND METHODS

Patient Selection. Forty patients were enrolled on trial after written informed consent was obtained (Table 1). Enrollment criteria included age >18 years, Eastern Cooperative Oncology Group performance status score of 0–2, life expectancy of at least 16 weeks, adequate hepatic function (aspartate aminotransferase < 2.5 times upper limit of normal; total bilirubin < 1.5 times upper limit of normal), adequate renal function (serum creatinine < 2 mg/dl or creatinine clearance > 50 ml/min), and normal hematological parameters (absolute neutrophil count > 1500/mm³, platelets > 100,000/mm³). Exclusion criteria included radiation therapy or chemotherapy in the preceding 4 weeks, brain metastases, and ongoing treatment with agents known to increase cyclosporine A blood concentrations.

Drug Supply and Treatment Schema. Valsopodar (Novartis Pharmaceutical Corp., East Hanover, New Jersey) was distributed by the Cancer Therapy Evaluation Program, National Cancer Institute. Valsopodar was supplied in a concentrated solution of 50 mg/ml in vials of 1 ml and 5 ml in a polyoxylated castor oil (Cremophor EL) vehicle. The concentrated solution of valsopodar was used to prepare a 24 h i.v. infusion each day by diluting it in 250 ml or 500 ml of 5% dextrose or 0.9% sodium chloride. In cycle 1, infusional valsopodar (including the loading dose) was initially administered over a 48 h window to allow a 99m Tc-sestamibi imaging and sampling for the CD56+ rhodamine efflux assay. This was followed 4 days later by initiation of the 6-day infusion of valsopodar and then the 3-day infusion of vinblastine, which began 24 h after the initiation of valsopodar. Vinblastine was obtained from commercial sources in vials containing 10 mg of lyophilized powdered. After reconstitution, a 24-h dose was further diluted to 250 ml in 0.9% sodium chloride injection.

After dose-limiting toxicity because of constipation was observed, a prophylactic bowel regimen was administered for 2 weeks after the vinblastine infusion. The regimen consisted of 2 doxycycline sodium or doxycycline calcium tablets administered p.o. twice a day, 2 senokot tablets taken p.o. twice a day, 2 teaspoons of Milk of Magnesia in the evening, and increased oral fluid intake.

The vinblastine dose was escalated in subsequent cycles if no dose-limiting toxicities (DLTs) were encountered. A DLT was defined by first cycle toxicities, based on the National Cancer Institute Common Toxicity Criteria (version 1.0) and included all grade 3 or 4 non-hematological toxicities (except hyperbilirubinemia, ataxia, and constipation), an absolute neutrophil count <500 for >4 days, neutropenic fever, or grade 4 thrombocytopenia. Grade 3 constipation that occurred with an adequate prophylactic bowel regimen was also a DLT but was not considered a DLT if it occurred without an adequate prophylactic regimen. Grade 3 hyperbilirubinemia, a known reversible toxicity because of valsopodar, was considered a DLT only if it did not resolve to <grade 1 within 2 weeks. No patient developed ataxia, the cerebellar toxicity observed with oral valsopodar (13).

Dose escalation followed the levels shown in Table 2. The first patient was given valsopodar for 5 days with vinblastine to ensure that infusional valsopodar would be tolerable. In subsequent cycles for that patient and for all other enrolled patients, valsopodar was given for 6 days. Three patients were enrolled on each dose level (except level 10) and if one of the three patients experienced a DLT, three additional patients were enrolled on that dose level. If two or more patients on a dose level experienced a DLT, then the MTD was exceeded, and patients were added to the next lower dose level to a maximum of six total.

<table>
<thead>
<tr>
<th>Table 1 Patient demographics</th>
<th>No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>11</td>
</tr>
<tr>
<td>Female</td>
<td>29</td>
</tr>
<tr>
<td>Age</td>
<td></td>
</tr>
<tr>
<td>18–30</td>
<td>2</td>
</tr>
<tr>
<td>31–50</td>
<td>13</td>
</tr>
<tr>
<td>51–73</td>
<td>25</td>
</tr>
<tr>
<td>Performance status</td>
<td></td>
</tr>
<tr>
<td>ECOG 0</td>
<td>6</td>
</tr>
<tr>
<td>ECOG 1</td>
<td>31</td>
</tr>
<tr>
<td>ECOG 2</td>
<td>3</td>
</tr>
<tr>
<td>Pathology</td>
<td></td>
</tr>
<tr>
<td>Clear cell</td>
<td>33</td>
</tr>
<tr>
<td>Papillary</td>
<td>1</td>
</tr>
<tr>
<td>Other</td>
<td>6</td>
</tr>
<tr>
<td>Prior therapy</td>
<td></td>
</tr>
<tr>
<td>IL-2 alone</td>
<td>18</td>
</tr>
<tr>
<td>Interferon alone</td>
<td>4</td>
</tr>
<tr>
<td>IL-2/interferon</td>
<td>9</td>
</tr>
<tr>
<td>IL-2/interferon/other</td>
<td>13</td>
</tr>
<tr>
<td>Interferon/other</td>
<td>6</td>
</tr>
<tr>
<td>Chemotherapy/other</td>
<td>23</td>
</tr>
</tbody>
</table>

ECOG, Eastern Cooperative Oncology Group; IL-2, interleukin-2.
patients. If no more than one of the six patients developed a DLT, then that dose level was considered the recommended dose. If no DLTs were seen in three patients on a dose level subsequent patients were enrolled at the next higher dose level. Intrapatient dose escalation of vinblastine was allowed. Standard response criteria were used.

**Pharmacokinetics.** Valspodar levels were measured in serum samples collected in parallel with samples drawn for the CD56+ assay. The vinblastine levels were determined by RIA as reported previously (17). Using the RIAPROG program, a linear standard range was constructed with standard concentrations ranging from 37.5 to 1800 ng/ml. A lower limit of quantitation was 1 ng/ml and a valspodar concentration of 3 ng/ml was established.

Serial blood samples were collected for midazolam (MDZ) pharmacokinetic and metabolic analysis. Blood samples were drawn in heparinized tubes at 0.083, 0.25, 0.5, 1, 2, 3, 4, and 5 h after the bolus administration of 0.0145 mg/kg MDZ. Samples were centrifuged immediately, and the plasma was collected and stored at −80°C until analysis.

The concentrations of MDZ and its metabolite, 1-hydroxy (1-OH) MDZ, were determined using a validated liquid chromatography/mass spectrometry method. The lower limits of quantification were 1 and 0.5 ng/ml for MDZ and 1-OH MDZ, respectively. The inter-assay and intra-assay coefficients of variation were within 10% in all concentration ranges.

Vinblastine simulation was performed using ADAPT II version 4 (Biomedical Simulation Resource, University of Southern California, Los Angeles, CA) with the use of a three-compartment model with output in compartment two (18, 19). The pharmacokinetic parameters reported in a study published previously were used as priors for the simulation.

**99mTc-Sestamibi Imaging.** The clearance of 99mTc-sestamibi from the liver and from tumor tissue gives an indirect indication of the degree of Pgp function and its inhibition in both normal and malignant cells. Pre-valsodar and post-valsodar 99mTc-sestamibi imaging studies were obtained 48–96 h apart. The post-valsodar study was performed approximately 24 h after the start of the valsodar infusion. 99mTc-Sestamibi imaging studies were performed as reported in two earlier National Cancer Institute trials (12, 20).

**Rhodamine Accumulation.** Inhibition of rhodamine efflux from CD56+ peripheral cells is recognized as a reliable *ex vivo* surrogate of Pgp inhibition (21–23). In this assay, 15 ml of whole blood was obtained from patients in a heparinized syringe just before treatment with valsodar, then at 24 and 48 h after the start of the valsodar infusion. Rhodamine 123 (Sigma-Aldrich, St. Louis, MO), with or without valsodar, was added to aliquots of whole blood to achieve a final rhodamine concentration of 0.5 μg/ml and a valsodar concentration of 3 μg/ml. The blood was then incubated for 30 min at 37°C in 5% CO2, layered onto lymphocyte separation medium and centrifuged at 2000 rpm for 5 min and assayed as described previously (21).

**Statistical Analysis.** Trends between parameters and valsodar dose level were evaluated for their statistical significance using the Jonckheere-Terpstra trend test (24). A Wilcoxon rank sum test was used to determine whether differences in CD56+ parameters were different statistically between two dose level groupings. The correlation between midazolam pharmacokinetic parameters and cycle 1 or 2 minimum absolute granulocyte count (AGC) was evaluated using Spearman rank correlation. A correlation was considered to be strong if $|r| >$
0.7, moderately strong if $0.5 < |r| < 0.7$, weak to moderate if $0.3 < |r| < 0.5$, and weak if $|r| < 0.3$. All $P$ values are two-sided and have not been adjusted for multiple comparisons.

RESULTS

The demographics are summarized in Table 1. Patients ranged in age from 18 to 73. Prior therapy included a nephrectomy in 37 patients, immunotherapy with interleukin (IL)-2 alone and/or in combination in 36, and IFN alone and/or in combination in 8 patients. Some patients had received more than one IL-2 or IFN regimen. Patients had received a mean of 2.75 regimens (range 0–8) before study entry. Thirty-nine patients received vinblastine with valspodar in cycle 1. One patient who developed chest pain and hypertension during the initial valspodar infusion was never treated with vinblastine and is not included in the analysis. Cardiac work-up revealed coronary artery occlusion. Six additional patients were removed from study before the second cycle because of disease progression (3), toxicity (2), and patient refusal to return (1).

Table 2 provides dose level and DLTs for patients on the trial. Each cycle was 28 days. The continuous i.v. valspodar infusion of 10 mg/kg/d was started at the same time as the loading dose and was maintained for 144 h (days 0–6). Starting with the nineteenth patient, the 10 mg/kg/d continuous i.v. dose of valspodar was reduced incrementally after an amendment to the protocol that added dose levels of 8, 6, 5, and 4 mg/kg/d. Continuous i.v. vinblastine was started 24 h after the initiation of valspodar on day 2 and continued for 72 h (days 2–4). The starting dose of vinblastine of 1.0 mg/m²/d was chosen based on experience in the previous Phase I trial. Because the MTD for vinblastine in that trial was 0.6 mg/m²/d over 5 days, an equivalent total dose of 3.0 mg/m² was chosen as the starting dose for the 3-day infusion.

DLTs were not observed at a vinblastine dose of 1.0 mg/m²/d. At the 1.3 mg/m²/d level, two episodes of grade 3 constipation were noted. However, because these patients had not received prophylaxis for constipation, the protocol was amended to allow further dose escalation with prophylaxis. In the subsequent cohort treated at 1.3 mg/m²/d, one patient experienced grade 4 neutropenia and grade 4 ileus, and the cohort was expanded to 6 patients. At 1.7 mg/m²/d vinblastine, two episodes of grade 4 neutropenia were observed in the first five patients, and this dose was considered to have exceeded the MTD. Notably, one patient enrolled at the MTD had no toxicity, and the dose was escalated over 18 cycles to a maximum of 4 mg/m²/d.

At this point, vlsapodar dose de-escalation was implemented in an attempt to advance the dose of vinblastine. No DLTs were observed with 1.7 mg/m²/d vinblastine in combination with 8 mg/kg/d vlsapodar. However, with 2.1 mg/m²/d vinblastine, two episodes of febrile neutropenia were observed. Vlsapodar was next reduced to 6 and then to 5 mg/kg/d, allowing the vinblastine dose to be escalated to 2.1 but not 2.6 mg/m²/d. Reduction of vlsapodar to 4 mg/kg/d did not obviate the toxicities in combination with the 2.6 mg/m²/d dose and accrual was stopped. By reducing the dose of vlsapodar to 50% of the starting dose, a total vinblastine dose of 6.3 mg/m² (2.1 mg/m²/d × 3 days) could be safely administered. This was 75% of the 8.5 mg/m² dose recommended without vlsapodar. It should be noted that 5 of the 11 patients who received 2.6 mg/m²/d vinblastine (7.8 mg/m² total dose) in combination with 4 or 5 mg/kg/d vlsapodar were treated without dose-limiting toxicity.

Implicit in the attempt to escalate the vinblastine dose by reducing the vlsapodar dose was our belief that the pharmacokinetic impact of vlsapodar could be reduced while preserving the inhibition of Pgp. To assess the extent of Pgp inhibition during the vlsapodar dose reductions, we obtained circulating mononuclear cells and evaluated inhibition of rhodamine efflux from Pgp-expressing CD56+ cells in a flow cytometric ex vivo assay. The extent of Pgp inhibition was quantitated by calculating the channel shift value, defined as the difference in the mean channel number of rhodamine fluorescence in the absence or the presence of exogenously added vlsapodar. Rhodamine fluorescence in the sample without exogenously added vlsapodar depends on the vlsapodar circulating in the blood of the patient. Fig. 1A demonstrates the results obtained in a patient receiving 8 mg/kg/d vlsapodar. The two top panels show results obtained with cells isolated before the administration of vlsapodar in the clinic (Pre), whereas the two bottom panels show results obtained after the administration of vlsapodar in the clinic. The upper left panel shows the results obtained after the 30 min accumulation period, whereas the other three panels show the results after allowing the cells a 60 min efflux period in rhodamine-free medium. In the lower two panels, inhibition of efflux was equal whether or not vlsapodar was added to the cells ex vivo. In the samples to which vlsapodar was not added ex vivo, levels in the blood at both 2 and 24 h time-points mediate complete inhibition of rhodamine efflux. The calculated difference between the histograms as shown, the channel shift value, was plotted for all patients in Fig. 1B (V–C, top panel; V/E–E, bottom panel).

Channel shift values as a function of vlsapodar blood concentration were fitted to a simple descending hyperbolic saturation curve (Michaelis-Menten type) as shown in Fig. 2. Data obtained at zero time and at both the 2-h and 24-h time points for the accumulation period (Fig. 2A) or the efflux period (Fig. 2B) were utilized. The half-maximal value for inhibition ($K_{\text{m}}$) after the period of accumulation is 118 ng/ml, whereas the $K_{\text{m}}$ after the efflux period is 308 ng/ml. This difference in $K_{\text{m}}$ values was reported previously and may be explained by the fact that vlsapodar is a substrate for Pgp-mediated efflux and is effluxed from the cells during the 60-min incubation (13, 21, 25, 26). Inhibition appears to plateau above a plasma level of 1000 ng/ml.

Mean vlsapodar concentrations at each dose level are provided in Table 3. These samples were obtained simultaneously with those for the CD56+ assay. The 2-h samples were obtained at the end of the loading period, whereas the 24 h samples represent steady-state levels. Vlsapodar concentrations were significantly correlated with dose administered at both time points. Channel shift values for rhodamine accumulation (V–C) at each dose level were also compared. Although some patients receiving 4 or 5 mg/kg/d vlsapodar appeared to have decreased inhibition of rhodamine efflux at the 24-h time point, when the differences between the pretreatment and the 24-h time points were calculated and compared across dose levels, no statistically significant decrease in the
channel shift values could be detected ($P < 0.5$, data not shown). When the 24-h values were compared by trend test across the five dose levels of valspodar, a statistically significant difference emerged ($P < 0.02$; data not shown). Because we had observed a plateau in Pgp inhibition at 1000 ng/ml, we compared the 24-h results in the following two groups: in one, patients who received 6–10 mg/kg/d valspodar had a mean serum valspodar level of 1490 ng/ml, whereas in the other group, patients who received 4–5 mg/kg/d valspodar had a mean serum valspodar level of 887 ng/ml. Comparing the results in the 24-h samples in these two groups, a mean channel shift value of $1.3 \pm 1.5$ was noted in the 6–10 mg/kg/d group; although a mean value of $6.5 \pm 2.4$ was noted in the group treated with 4 or 5 mg/kg/d valspodar. Although the difference between these latter two mean values was statistically significant ($P = 0.0038$), it should be noted that these values are an order of magnitude lower than the 64.4 $\pm 3.4$ mean channel shift value obtained before valspodar administration ($P < 0.001$).

Because it became apparent we were reaching the final dose levels, 99m Tc-sestamibi imaging was added as a second cell shift value could be detected ($P > 0.5$, data not shown). When the 24-h values were compared by trend test across the five dose levels of valspodar, a statistically significant difference emerged ($P = 0.02$; data not shown). Because we had observed a plateau in Pgp inhibition at 1000 ng/ml, we compared the 24-h results in the following two groups: in one, patients who received 6–10 mg/kg/d valspodar had a mean serum valspodar level of 1490 ng/ml, whereas in the other group, patients who received 4–5 mg/kg/d valspodar had a mean serum valspodar level of 887 ng/ml. Comparing the results in the 24-h samples in these two groups, a mean channel shift value of $-1.3 \pm 1.5$ was noted in the 6–10 mg/kg/d group; although a mean value of $6.5 \pm 2.4$ was noted in the group treated with 4 or 5 mg/kg/d valspodar. Although the difference between these latter two mean values was statistically significant ($P = 0.0038$), it should be noted that these values are an order of magnitude lower than the 64.4 $\pm 3.4$ mean channel shift value obtained before valspodar administration ($P < 0.001$).

Because it became apparent we were reaching the final dose levels, $^{99m}$Tc-sestamibi imaging was added as a second cell shift value could be detected ($P > 0.5$, data not shown). When the 24-h values were compared by trend test across the five dose levels of valspodar, a statistically significant difference emerged ($P = 0.02$; data not shown). Because we had observed a plateau in Pgp inhibition at 1000 ng/ml, we compared the 24-h results in the following two groups: in one, patients who received 6–10 mg/kg/d valspodar had a mean serum valspodar level of 1490 ng/ml, whereas in the other group, patients who received 4–5 mg/kg/d valspodar had a mean serum valspodar level of 887 ng/ml. Comparing the results in the 24-h samples in these two groups, a mean channel shift value of $-1.3 \pm 1.5$ was noted in the 6–10 mg/kg/d group; although a mean value of $6.5 \pm 2.4$ was noted in the group treated with 4 or 5 mg/kg/d valspodar. Although the difference between these latter two mean values was statistically significant ($P = 0.0038$), it should be noted that these values are an order of magnitude lower than the 64.4 $\pm 3.4$ mean channel shift value obtained before valspodar administration ($P < 0.001$).

Because it became apparent we were reaching the final dose levels, $^{99m}$Tc-sestamibi imaging was added as a second
surrogate to confirm continuing Pgp inhibition. Increased hepatic accumulation of $^{99m}$Tc-sestamibi has been used previously as a surrogate assay of efficacy for Pgp inhibitors (12, 20, 27–29). As described previously, area under the curve (AUC) ratios were obtained by dividing the tissue or tumor $^{99m}$Tc-sestamibi AUC by the $^{99m}$Tc-sestamibi AUC of the heart. Patients who received 4 or 5 mg/kg/d valspodar had increases in 99mTc-sestamibi accumulation increased 53% in the liver and 34% in a lung metastasis after valspodar. Table 4 compares the increases in uptake determined among the three studies.

Valspodar and many anticancer agents share not only their interaction with Pgp but also metabolism by the 3A isozyme of P450 (6). The benzodiazepine midazolam (MDZ) has been used to assay interpatient variability in 3A activity (15, 16). Because we previously noted large interpatient variability in chemotherapy tolerance and wanted to evaluate the contribution of P450 activity, pharmacokinetic sampling for MDZ and its 1-OH metabolite was performed in 31 patients before treatment with vinblastine and valspodar (15). The calculated clearance of midazolam ranged from 3.06 to 47.4 l/h (mean ± SD, 27.8 ± 11.77 l/h). The half-life ranged from 1.1 to 4.03 h (mean ± SD, 2.69 ± 0.87 h). This represents a 15-fold range in clearance and a 4-fold range in half-life. Four MDZ pharmacokinetic parameters [MDZ AUC$_{0-\infty}$, half-life, volume of distribution (Vd), and clearance] and 1-OH MDZ/MDZ ratios determined 30 min after injection were correlated with the nadir AGC using a Spearman correlation. No correlation was found with 1-OH MDZ/MDZ ratios. As shown in Table 5, the cycle 1 nadir AGC was correlated only weakly with MDZ half-life. The cycle 2 nadir AGC correlated weakly to moderately well with Vd and clearance. These results suggest an impact of P450 3A activity on the toxicity of the vinblastine and valspodar combination. A second set of pharmacokinetic blood samples was obtained in six patients in a subsequent cycle. Three of the six patients had

Table 3 Serum valspodar concentrations and CD56+ surrogate studies

<table>
<thead>
<tr>
<th>Serum valspodar concentrations</th>
<th>Number</th>
<th>Mean ± SEM</th>
<th>Min</th>
<th>Med</th>
<th>Max</th>
<th>$P$ value ($P_a$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-h loading dose: 2 ng/ml</td>
<td>17</td>
<td>357 ± 340</td>
<td>1800</td>
<td>3400</td>
<td>6610</td>
<td>0.0005$^a$</td>
</tr>
<tr>
<td>2-h loading dose: 1 ng/ml</td>
<td>9</td>
<td>1684 ± 204</td>
<td>983</td>
<td>1680</td>
<td>2890</td>
<td></td>
</tr>
<tr>
<td>24-h CIV dose: 10 ng/ml</td>
<td>3</td>
<td>1683 ± 244</td>
<td>1290</td>
<td>1630</td>
<td>2130</td>
<td></td>
</tr>
<tr>
<td>24-h CIV dose: 8 ng/ml</td>
<td>5</td>
<td>1472 ± 147</td>
<td>1040</td>
<td>1370</td>
<td>1890</td>
<td></td>
</tr>
<tr>
<td>24-h CIV dose: 6 ng/ml</td>
<td>3</td>
<td>1330 ± 391</td>
<td>550</td>
<td>1680</td>
<td>1760</td>
<td>0.0034$^b$</td>
</tr>
<tr>
<td>24-h CIV dose: 5 ng/ml</td>
<td>5</td>
<td>992 ± 107</td>
<td>638</td>
<td>1080</td>
<td>1200</td>
<td></td>
</tr>
<tr>
<td>24-h CIV dose: 4 ng/ml</td>
<td>4</td>
<td>758 ± 179</td>
<td>410</td>
<td>687</td>
<td>1250</td>
<td></td>
</tr>
<tr>
<td><strong>CD56+ Surrogate studies:</strong> $\overline{V - C}$ values$^c$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-therapy</td>
<td>31</td>
<td>64 ± 3.4</td>
<td>28</td>
<td>71</td>
<td>95</td>
<td>$&lt;0.001^d$</td>
</tr>
<tr>
<td>2-h loading dose: 2 ng/ml</td>
<td>19</td>
<td>−3.3 ± 1.4</td>
<td>−13.2</td>
<td>−3.9</td>
<td>9.7</td>
<td></td>
</tr>
<tr>
<td>2-hour loading dose: 1 ng/ml</td>
<td>10</td>
<td>1.5 ± 1.8</td>
<td>−5.8</td>
<td>0.4</td>
<td>12.7</td>
<td>0.037$^e$</td>
</tr>
<tr>
<td>24-h CIV dose: 6, 8, or 10 ng/ml</td>
<td>14</td>
<td>−1.3 ± 1.5</td>
<td>−16.4</td>
<td>0.3</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>24-h CIV dose: 4 or 5 ng/ml</td>
<td>10</td>
<td>6.5 ± 2.4</td>
<td>−4.0</td>
<td>5.1</td>
<td>24.5</td>
<td>0.0038$^a$</td>
</tr>
</tbody>
</table>

$^a$ Wilcoxon rank sum $P$ value ($P_a$) comparing the two parameters in the indicated 2 rows.
$^b$ Jonckheere-Terpstra trend test $P_b$ value across all valspodar doses.
$^c$ (V − C) = (valspodar − control) is a measure of rhodamine efflux. Higher values = higher efflux = higher PGP activity.
$^d$ Wilcoxon rank sum $P$ value comparing pre-therapy values with all post-therapy values.

Fig. 3 Imaging with $^{99m}$Tc-sestamibi was performed at baseline (Pre Valspodar) and 24 h after starting the infusion of 5 mg/kg valspodar (Post Valspodar). Note the prolonged retention of activity in the liver in the presence of valspodar. Arrow indicates a metastasis in the left lung. Radionuclide accumulation was increased by 53% in the liver and by 34% in the lung metastasis.

Downloaded from clinicalcancerres.aacrjournals.org on October 15, 2017. © 2004 American Association for Cancer Research.
Vinblastine with Valspodsar in Renal Cell Carcinoma

Table 4  
<table>
<thead>
<tr>
<th>Trialb</th>
<th>% Increase in liver 99mTc-sestamibi AUC ratios</th>
</tr>
</thead>
<tbody>
<tr>
<td>Previous valsopodar trial (13, 18)</td>
<td></td>
</tr>
<tr>
<td>Phase I: p.o. valsopodar + CIV vinblastine</td>
<td>9 118 28 52 89 278</td>
</tr>
<tr>
<td>Current trial</td>
<td></td>
</tr>
<tr>
<td>Phase II CIV valsopodar + CIV vinblastine</td>
<td>6 67 15 13 68 120</td>
</tr>
<tr>
<td>Both valsopodar trials</td>
<td>15 97 19 13 83 278</td>
</tr>
<tr>
<td>Tariquidar trial (20)</td>
<td></td>
</tr>
<tr>
<td>Phase I i.v. tariquidar + D1/D8 vinorelbine</td>
<td>25 130 15 14 114 278</td>
</tr>
</tbody>
</table>

a Pgp, P-glycoprotein; CIV, continuous i.v. infusion.
b Comparisons were performed using the Wilcoxon rank sum test. Although the difference between B and C approached significance, none of the following comparisons met statistical significance: A vs. B, \( P = 0.22 \); A vs. C, \( P = 0.43 \); B vs. C, \( P = 0.052 \); A + B vs. C, \( P = 0.096 \).

increased MDZ clearance after \( \geq 3 \) cycles of treatment. In one, MDZ clearance increased from 28.13–100.12 l/h after 15 cycles, whereas in another, MDZ clearance increased from 17.01–58.87 l/h after 3 cycles.

Although the dose de-escalation ultimately became an important component of this trial, the major goal was to improve upon a regimen of oral valsopodar with infusional vinblastine that in a previous Phase I trial resulted in four durable responses in patients with clear cell renal cell carcinoma (13). Although 33 of the 40 patients enrolled on this study had clear cell histology, minimal clinical activity was observed. One patient remained on study for 18 cycles over 17 months and had a mixed response. Three pulmonary lesions decreased by 69%, whereas one 4-cm pleural-based mass remained unchanged. Four patients exhibited stable disease at four cycles of therapy; three of these developed progression of disease by the sixth cycle.

DISCUSSION

This trial was written as a Phase I study to define the safe dose of valsopodar and vinblastine, when both drugs are administered by continuous i.v. infusion. Patients with renal cell carcinoma were studied because of favorable responses observed in a Phase I trial combining oral valsopodar with infusional vinblastine. At the initial dose of infusional valsopodar, 10 mg/kg/d, the MTD for vinblastine was identified as 1.3 mg/m\(^2\)/d. Because previous studies suggested that blood valsopodar concentrations exceeded that needed for Pgp inhibition, we sequentially reduced the dose of valsopodar and attempted to increase the vinblastine dose. This strategy allowed safe administration of 2.1 mg/m\(^2\)/d vinblastine for 3 days (6.3 mg/m\(^2\) total dose) with 5 mg/kg valsopodar in five of six patients. Pharmacodynamic studies examining Pgp-expressing CD56 cells found a discernible difference between residual Pgp-mediated rhodamine efflux at valsopodar doses of 6–10 mg/kg/d, compared with the residual efflux at doses of 4–5 mg/kg/d; however, rhodamine efflux at all valsopodar doses was an order of magnitude less than that in the absence of valsopodar. Furthermore, in the patients who received 4 and 5 mg/kg/d valsopodar, increases in 99mTc-sestamibi accumulation in the liver could not be distinguished from those observed in other trials, indicating that even with reduced doses of valsopodar, substantial inhibition of Pgp was still present. We conclude that valsopodar doses substantially lower than those recommended previously are sufficient to inhibit Pgp-mediated transport. Reductions in the valsopodar dose allowed us to administer higher doses of chemotherapy without significantly impairing Pgp inhibition. This was most likely a result of reduced inhibition of drug metabolism, principally that mediated by P450.

The clinical development of Pgp inhibitors such as valsopodar has been rendered more difficult by pharmacokinetic interactions requiring reduction of anticancer drug doses. Putative mechanisms for this drug interaction include inhibition of drug metabolism by the P450 3A subfamily and impaired drug and/or metabolite excretion, secondary to impaired Pgp-mediated drug excretion, or inhibition of other transporters. Two major liabilities can be foreseen in relying on dose reduction to achieve equitoxicity where drug clearance is impaired by a pharmacokinetic interaction. One is that equal AUCs have not been equated to equal efficacy. Longer terminal half-lives for anticancer drugs in the presence of valsopodar may allow a calcul
lated AUC to be comparable with that obtained without valspodar, whereas the concentrations in the terminal portions of the curve may be sub-therapeutic. The second is that marked interpatient variability in the drug interaction of valspodar has been documented. This is likely multifactorial but attributable in part to a previously reported 20-fold range in valspodar clearance and variable P450 function (30). The troubling aspect of this interpatient variability is that it could be manifested as increased chemotherapy toxicity in some and decreased efficacy in others. Patients with low P450 activity could have increased toxicity despite dose reductions. For example, the high mortality seen in the valspodar AML trial may be explained in part by increased toxicity because of the pharmacokinetic interaction (31). Conversely, patients in whom clearance is not substantially impaired will have a reduced AUC if the dose is lowered in all patients in anticipation of a pharmacokinetic interaction. Both our previous study combining valspodar with paclitaxel (32) and the study by Dorr et al. (33) combining valspodar and daunorubicin estimated that approximately one-third of patients would be under-treated when uniform dose reductions are used.

Because the addition of valspodar delays vinblastine clearance, we used pharmacokinetic modeling to guide the trial design. We estimated that with the addition of valspodar, reducing the duration of the vinblastine infusion from 5 to 3 days would result in drug exposure more comparable with that observed when vinblastine was administered alone over 5 days. Because the low vinblastine concentrations achieved by the infusion could not be reliably determined, the drug concentration-time profiles for the relevant doses and schedules were simulated based on pharmacokinetic data reported previously (34). We assumed vinblastine clearance would be reduced by 50%, based on published data for valspodar with either etoposide or paclitaxel (17, 32, 35).

Comparable estimated values for AUCs (0–∞) of 218 and 200 ng/mlh are obtained with a simulated 5-day infusion at the standard dose of 1.7 mg/m²/d vinblastine, with normal clearance, and a simulated 3-day infusion at a dose of 1.3 mg/m²/d with a 50% reduction in clearance (Table 6). Thus, the 3-day regimen chosen for the present study could result in comparable exposure to vinblastine. This is also reflected in the concentration-time profiles modeled in Fig. 4. When the 3-day model was provided with the reverse information, an AUC of 218 (based on 1.7 mg/m²/d × 5 days with a normal clearance) and a dose of 2.1 or 2.6 mg/m²/d administered over 3 days (the vinblastine doses administered with the reduced valspodar dose), the estimated clearance was projected to be 82% and 96%, respectively (Table 7). These projections support our interpretation of the clinical data, which suggests that reducing the valspodar dose led to a reduced inhibition of vinblastine clearance.

To address the issue of interpatient variability, we prospectively measured midazolam clearance as a measure of P450

### Table 6 Simulation of vinblastine area under the curve

<table>
<thead>
<tr>
<th>Duration (days)</th>
<th>Dose (mg/m²/d)</th>
<th>Clearance (percent)</th>
<th>Estimated values AUC₀–∞ [ng/mlh]</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>1.7</td>
<td>100</td>
<td>218</td>
<td>Standard dose without valspodar</td>
</tr>
<tr>
<td>5</td>
<td>0.6</td>
<td>50</td>
<td>154</td>
<td>MTD of 5-day vinblastine with oral valspodar</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>50</td>
<td>151</td>
<td>Starting dose of 3-day vinblastine with CIV valspodar</td>
</tr>
<tr>
<td>3</td>
<td>1.3</td>
<td>50</td>
<td>200</td>
<td>MTD of 3-day vinblastine with 10 mg/kg/d CIV valspodar</td>
</tr>
<tr>
<td>3</td>
<td>1.7</td>
<td>50</td>
<td>261.7</td>
<td></td>
</tr>
</tbody>
</table>

* AUC, area under the curve; MTD, maximum-tolerated dose; CIV, continuous i.v. infusion.
activity, in each patient before treatment with valspodar or vinblastine. The results show that P450 activity as measured by midazolam varied 15-fold, confirming a large interpatient variability. This is consistent with previous reports that implicated variable hepatic enzyme activity secondary to both differential expression of P450 3A and variable activity related to single nucleotide polymorphism (15, 36–38). This is also comparable with the 10-fold variation in clearance cited for drugs metabolized by the cytochrome P450 3A subfamily (39, 40). There was a weak to moderate correlation between midazolam clearance and granulocytopenia in the second cycle when both vinblastine and valspodar were administered concurrently after dose adjustment, suggesting but not proving, that P450 3A activity was important in the toxicity of the combination.

We hypothesized that the inhibition of P450 could be separated from that of Pgp by reducing the valspodar dose. Results from the previous oral valspodar study led to the hypothesis that valspodar concentrations exceeding 1000 ng/ml were probably unnecessary, identifying a K\textsubscript{m} range of 29–181 ng/ml for Pgp inhibition (assayed in CD56\textsuperscript{+} cells). The current study, correlating serum concentrations of valspodar and Pgp inhibition, again documented a plateau beginning at approximately 1000 ng/ml with a calculated K\textsubscript{m} of 118 ng/ml. As the dose of valspodar administered to patients was reduced, serum valspodar concentrations fell, and a minimal loss in the inhibition of rhodamine efflux was observed. Although this difference was statistically significant, the magnitude of the change was small. Rhodamine efflux was inhibited 90% and 84% at the higher and lower valspodar concentrations, respectively, such that rhodamine efflux at all doses was almost 10- to 50-fold less than that in the absence of valspodar administration.

The major limitation of the CD56\textsuperscript{+} assay is that it reflects Pgp inhibition in the blood and not in the tumor. In contrast, \textsuperscript{99m}Tc-sestamibi, a radionuclide cardiac imaging agent and Pgp substrate, was developed as a strategy to determine whether Pgp inhibitors were able to inhibit Pgp in normal tissues and tumors (41). Increased retention has been observed after biricodar, valspodar, and tariquidar in both normal liver and tumor tissue (12, 20, 29). \textsuperscript{99m}Tc-Sestamibi imaging was added to this study to lower doses of valspodar began to be administered. Although the increases in hepatic retention were statistically similar to those quantitated previously in patients receiving either full dose valspodar or tariquidar (12, 20), the mean increase for the six patients receiving the lowest doses of valspodar was less. Because the imaging studies in the current report were performed in patients with levels of valspodar <1000 ng/ml, these results are compatible with some loss of Pgp inhibition.

We would argue that these results suggest a window exists for valspodar in which Pgp inhibition can be achieved with a minimized P450 interaction. At the initial MTD in combination with 10 mg/kg/d valspodar, a total valspodar dose of 3.9 mg/m\textsuperscript{2} (1.3 mg/m\textsuperscript{2}/d) could be administered. After reduction of the valspodar dose, a total valspodar dose of 6.3 mg/m\textsuperscript{2} (2.1 mg/m\textsuperscript{2}/d) could be administered. Indeed, five patients (not including the patient who received 4 mg/m\textsuperscript{2} in combination with 10 mg/kg/d valspodar) were able to tolerate \(\geq2.6\) mg/m\textsuperscript{2}/d vinblastine, once valspodar had been reduced to 4 or 5 mg/kg/d. These latter doses compare favorably with the 8.5 mg/m\textsuperscript{2} that would be infused if vinblastine were given alone over 5 days. At the lowest doses of valspodar, this was achieved with substantial inhibition of Pgp still evident in the majority of patients both in CD56\textsuperscript{+} cells and in the liver as measured by \textsuperscript{99m}Tc-sestamibi retention.

Although Pgp antagonists such as valspodar lack intrinsic antitumor activity and were designed to modulate drug resistance, their clinical development has followed the traditional anticancer drug template. Modulators were combined with established anticancer agents, administering both at their respective MTDs. As the results from this and other trials demonstrate, this approach may not have been optimal. We would argue that the use of uniform dose reductions builds in disparities in efficacy and toxicity because of interpatient differences in P450 inhibition. Furthermore, no studies have proven that equivalent calculated AUCs provide equivalent efficacy. The present results suggest that with valspodar, inhibition of P450 and Pgp could be dissociated, and that doses sufficient only to inhibit Pgp could and should be used. Although one can argue that what occurs at the level of circulating CD56\textsuperscript{+} cells may not accurately represent the dynamics in a solid tumor, note that CD56\textsuperscript{+} cells have Pgp levels that are higher than in the majority of refractory solid tumors and that \textsuperscript{99m}Tc-sestamibi scans performed in this and other studies have shown effective Pgp inhibition in both normal tissues and solid tumors.

In the end, one must wonder if the lack of efficacy and the toxicity encountered during the development of valspodar would have been different had another approach to dose selection been implemented. The broader lesson is that in the era of targeted therapies, prudence demands that clinical trials be guided by surrogate assays of efficacy.

**ACKNOWLEDGMENTS**

We thank Dr. Isagani Chico for help in writing and implementing the valspodar/vinblastine trial in its earliest stages, Dr. Jerry Collins for invaluable help in the design of the trial, and Dr. Percy Ivy for untiring help and encouragement in the conduct of the clinical trial.

**REFERENCES**


A Phase I/II Study of Infusional Vinblastine with the P-Glycoprotein Antagonist Valspodar (PSC 833) in Renal Cell Carcinoma

Susan E. Bates, Susan Bakke, Min Kang, et al.


Updated version
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/10/14/4724

Cited articles
This article cites 37 articles, 18 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/10/14/4724.full#ref-list-1

Citing articles
This article has been cited by 6 HighWire-hosted articles. Access the articles at:
http://clincancerres.aacrjournals.org/content/10/14/4724.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.