A Phase I Trial of a Potent P-Glycoprotein Inhibitor, Zosuquidar Trihydrochloride (LY335979), Administered Intravenously in Combination with Doxorubicin in Patients with Advanced Malignancy

Alan Sandler, 1 Michael Gordon, 1 Dinesh P. de Alwis, 2 Isabelle Pouliquen, 2 Lisa Green, 3 Phil Marider, 3 Ajai Chaudhary, 3 Karen Fife, 1 Linda Battiato, 1 Christopher Sweeney, 1 Christopher Jordan, 3 Michael Burgess, 2 and Christopher A. Slapak 1, 3

1 Department of Medicine, Section of Hematology/Oncology, Indiana University School of Medicine, Indianapolis, Indiana; 2 Lilly Research Laboratories, Indianapolis, Indiana; 3 Lilly Research Laboratories, Indianapolis, Indiana

ABSTRACT

Purpose: Our intention was to (a) to investigate the safety and tolerability of a potent P-glycoprotein modulator, zosuquidar trihydrochloride (LY335979), when administered i.v. alone or in combination with doxorubicin, (b) to determine the pharmacokinetics of zosuquidar and correlate exposure to inhibition of P-glycoprotein function in a surrogate assay, and (c) to compare the pharmacokinetics of doxorubicin in the presence and absence of zosuquidar.

Patients and Methods: Patients with advanced malignancies who provided written informed consent received zosuquidar and doxorubicin administered separately during the first cycle of therapy and then concurrently in subsequent cycles. Zosuquidar was given i.v. over 48 h in a cohort-dose escalation manner until the occurrence of dose-limiting toxicity or protocol specified maximum exposure. Doxorubicin doses of 45, 60, 75 mg/m² were administered during the course of the trial.

Results: Dose escalation proceeded through 9 cohorts with a total of 40 patients. The maximal doses administered were 640 mg/m² of zosuquidar and 75 mg/m² of doxorubicin. No dose-limiting toxicity of zosuquidar was observed. Pharmacokinetic analysis revealed that, in the presence of zosuquidar at doses that exceeded 500 mg, there was a modest decrease in clearance (17–22%) and modest increase in area under the curve (15–25%) of doxorubicin. This change was associated with an enhanced leukopenia and thrombocytopenia but was without demonstrable clinical significance. The higher doses of zosuquidar were associated with maximal P-glycoprotein inhibition in natural killer cells.

Conclusion: Zosuquidar can be safely coadministered with doxorubicin using a 48 h i.v. dosing schedule.

INTRODUCTION

Since the initial description of cancer cells exhibiting multidrug resistance to cytotoxic agents, and the subsequent identification and cloning of the human gene that confers this phenotype, multiple drug resistance 1 (MDR1), much effort has focused on the development of clinically useful compounds capable of modulating this effect (1, 2). Several agents have undergone substantial testing in the clinical trial setting. The results to-date are at best inconclusive and in some cases, disappointing (3). However, few of the first or second-generation MDR-modulating agents have had the requisite potency or selectivity toward the target to adequately assess questions of efficacy in the clinical trial setting.

Zosuquidar trihydrochloride (LY335979) is a third generation modulating agent that was developed specifically as a selective, MDR1/P-glycoprotein (P-gp) inhibitor (4). In vitro concentrations of zosuquidar from 50 to 100 nM are capable of circumventing P-gp-mediated drug resistance in virtually every cell culture system tested (5, 6). In experiments using tumor-bearing mice with syngeneic and human xenografts, zosuquidar was efficacious in restoring drug sensitivity to P-gp expressing implants (5). Moreover, preclinical studies using murine and canine models demonstrated that zosuquidar had no observable effect on the pharmacokinetic profile of coadministered P-gp substrates, including doxorubicin and paclitaxel (5). These data suggest that zosuquidar warrants investigation in clinical trials aimed at reversing drug resistance mediated by P-gp.

We initiated a Phase I trial designed to determine whether pharmacologically effective plasma concentrations of zosuquidar could be safely achieved in cancer patients who received the drug i.v. The study was also designed to evaluate the effects of zosuquidar on doxorubicin pharmacokinetics and toxicity. The results indicate that biologically effective plasma concentrations of zosuquidar are associated with minimal toxicity and without significant alteration of doxorubicin pharmacokinetics.

MATERIALS AND METHODS

Patient Selection. Patients who were at least 18 years of age and met all of the following criteria were eligible for the
cycle 2 and subsequent cycles; Table 1). The initial zosuquidar
tered in the study in cohorts of three and received treatment
University School of Medicine. approved by the Institutional Review Board at the Indiana
ing to institutional, state, and federal guidelines. This study was
a
a
b
b

Table 1 Dose escalation cohorts

<table>
<thead>
<tr>
<th>Cohort number</th>
<th>n</th>
<th>Zosuquidar dosea,b (mg/m²/day)</th>
<th>Doxorubicin dosea (mg/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
<td>20</td>
<td>45</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>20</td>
<td>60</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>80</td>
<td>60</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>160</td>
<td>60</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>320</td>
<td>60</td>
</tr>
<tr>
<td>7</td>
<td>6</td>
<td>640</td>
<td>60</td>
</tr>
<tr>
<td>8</td>
<td>4</td>
<td>640</td>
<td>75</td>
</tr>
<tr>
<td>9</td>
<td>12</td>
<td>480</td>
<td>75</td>
</tr>
</tbody>
</table>

a The starting dose of zosuquidar or doxorubicin assigned to the
cohort.
b The dose was given for 2 days. Multiply by two to get the total zosuquidar dose/cycle.

study: (a) histological or cytological diagnosis of metastatic or
locally advanced cancer (must have failed conventional therapy,
have disease considered refractory to standard chemotherapy
regimens, or have disease for which no standard chemotherapy
was available); (b) prior radiation therapy and chemotherap
completed at least 3 weeks before study enrollment (6 weeks if
prior treatment was nitrosourea or mitomycin C); (c) lifetime
cumulative anthracycline dose must have not exceeded the
doxorubicin equivalent of 300 mg/m²; (d) resting blood pool
scan with a cardiac ejection fraction of >45%; (e) performance
status of 0 to 2 on the Eastern Cooperative Oncology Group
scale; (f) estimated life expectancy of at least 12 weeks; (g) and
adequate organ function (granulocytes ≥ 1.5 × 10⁹ cells/liter,
platelets ≥ 100 × 10⁹/liter, hemoglobin ≥ 9 g/liter, bilirubin ≤
upper limit of normal, alanine transaminase and aspartate trans-
aminase ≤ 2.5 times normal, serum creatinine ≤ 1.5 mg/dl).
Written informed consent was obtained from all patients accord-
ing to institutional, state, and federal guidelines. This study was
approved by the Institutional Review Board at the Indiana
University School of Medicine.

Treatmen and Clinical Evaluation. Patients were en-
tered in the study in cohorts of three and received treatment
during multiple cycles of either 35 days (cycle 1) or 21 days
(cycle 2 and subsequent cycles; Table 1). The initial zosuquidar
dose was chosen based on studies in the dog; a 2-week i.v.
infusion demonstrated that a dose of 10 mg/kg/day produced
peak plasma concentrations that exceeded 1000 nm and was
without toxicity (7). The starting dose of zosuquidar was 20
mg/m²/day. It was administered as a continuous i.v. infusion via
central venous access over 48 h beginning on day 1 of every
cycle. A 48-h infusion was chosen to balance the anticipated
half-life of zosuquidar in man, the duration of effective P-gp
inhibition, and the known pharmacokinetic parameters of doxo-
rubicin. In the first two cohorts, this same dose of zosuquidar
was administered and the doxorubicin dose increased. For each
subsequent cohort, the zosuquidar dose was escalated by a
maximum of 100%. Dose escalation was to cease in any of the
following circumstances: (a) the plasma concentration of zosu-
quidar reached or exceeded a predefined upper limit (defined as
2000 nm or approximately 1000 µg/liter); (b) toxicity of any
type (Common Toxicity Criteria version 1 grade 2 or higher
except nausea, vomiting, or alopecia) attributed to zosuquidar
alone was observed in cycle 1, or (3) unacceptable toxicity of
zosuquidar in combination with doxorubicin occurred in cycle 2.
There was no intrapatient dose escalation of zosuquidar.

In cycle 1 only, doxorubicin was administered 14 days after
zosuquidar. For cycle 2 and subsequent cycles, doxorubicin was
administered on day 2; which was 24 h after the start of the
zosuquidar infusion. The first cohort received 45 mg/m² of
doxorubicin administered i.v. over 30 min. Subsequent cohorts
received 60 mg/m² of doxorubicin administered i.v. over 30
min. If the target plasma level of zosuquidar was reached
without defining maximum tolerated dose, doxorubicin was
dose escalated to 75 mg/m² in an additional cohort. This last
cohort was to be expanded to a total of 10 patients, provided that
dose-limiting toxicity had not been reached.

The maximum tolerated dose was defined as one dose level
lower than the dose level at which at least 2 of 6 patients
experienced unacceptable toxicity. If unacceptable toxicity oc-
curred in 1 of the first 3 patients at a given dose-level, 3
additional patients were treated at that dose level. If no other
patient experienced unacceptable toxicity, dose escalation con-
tinued. If unacceptable toxicity occurred in 2 of 6 patients, dose
escalation was stopped. Unacceptable toxicity for zosuquidar
given in combination with doxorubicin (determined in cycle 2) was
defined as Common Toxicity Criteria grade 4 hematologi-
cal toxicity lasting for 5 days or longer or grade 3 or higher
nonhematologic toxicity (excluding alopecia, nausea, or vomit-
ing).

Doxorubicin doses were adjusted for neutrophil and plate-
let nadirs occurring during the preceding course of therapy, as
previously described (8). The doxorubicin dose was also mod-
ified if patients experienced Common Toxicity Criteria grade 2 or
higher neurological toxicities or hyperbilirubinemia. The
doxorubicin administration was discontinued if there was clin-
cal congestive heart failure or if the left ventricular ejection
fraction fell, on the gated blood pool scan, to less than 45% or
if the total decrease was ≥10% from baseline.

Antitumor response was evaluated, where appropriate, af-
ter the third cycle of therapy and after every other subsequent
cycle. Responses were quantified by either physical examination

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Median 57</td>
</tr>
<tr>
<td></td>
<td>Range 27–72</td>
</tr>
<tr>
<td>Sex</td>
<td>Males 20</td>
</tr>
<tr>
<td></td>
<td>Females 20</td>
</tr>
<tr>
<td>ECOG performance status</td>
<td>Median 0</td>
</tr>
<tr>
<td></td>
<td>Range 0–2</td>
</tr>
<tr>
<td>Disease state</td>
<td>Locally advanced 4</td>
</tr>
<tr>
<td></td>
<td>Metastatic 36</td>
</tr>
<tr>
<td>Prior therapy</td>
<td>Surgery 32</td>
</tr>
<tr>
<td></td>
<td>Chemotherapy 21</td>
</tr>
<tr>
<td></td>
<td>Radiation therapy 14</td>
</tr>
</tbody>
</table>

a ECOG, Eastern Cooperative Oncology Group.
or by appropriate imaging studies according to World Health Organization criteria (9).

**Statistical Analysis.** The data for hematological parameters were analyzed using a linear-mixed-effects model with cycle as a fixed effect and subject as a random effect. This technique accounted for any incomplete data and repeated measures on each patient. The dose level of zosuquidar and the baseline value were included as covariates. Observations corresponding to doxorubicin dose reductions were excluded. The raw data were log-transformed before analysis because the data on this scale better satisfied the normality assumption underlying the analysis (10).

Least square means (LS means) and 95% confidence intervals were generated from the model for each cycle. Ratios of the LS means and their 95% confidence intervals were derived to compare means between cycles. A 95% confidence interval that excludes 1 is equivalent to statistical significance at the 5% level. The percentage decrease in blood counts was obtained using the following formula: \( \frac{(1 - \text{ratio LS means})}{100} \).

**Pharmacokinetic Analysis.** Plasma samples were obtained predose and serially up to 96 h after dose to determine concentrations of zosuquidar, doxorubicin, and the metabolite doxorubicinol when the drugs were administered separately (cycle 1) and in combination (cycle 2).

Zosuquidar and doxorubicin and doxorubicinol concentrations were analyzed using validated high-performance liquid chromatography methods which have been previously described (8).

Plasma pharmacokinetic parameters of zosuquidar, doxorubicin, and doxorubicinol were evaluated using non-compartmental methods (WinNonlin Professional version 2.1). Plasma area under the curve (AUC), clearance (CL), volume of distribution at steady state (\( V_{ss} \)), and terminal half-life \( t_{1/2} \) were calculated for doxorubicin and plasma AUC and \( C_{max} \) (observed maximum plasma concentration) for doxorubicinol as follows:

\[ \text{AUC} = \text{AUC}(0 - t_a) + \frac{C(t_a)'}{\lambda_z}, \]

where \( t_a \) is the last time point where the plasma concentration is above the limit of quantification, \( C(t_a)'/\lambda_z \) is the prediction for the concentration at the last quantifiable time point, and \( \lambda_z \) is the calculated terminal rate constant;

\[ \text{CL} = \frac{D}{\text{AUC}}, \text{where } D = \text{dose}. \]

\[ V_{ss} = \text{CL} \times \text{MRT}_{iv}, \text{where} \]

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Dose (mg/m²/day)</th>
<th>n</th>
<th>( C_{max} ), ( \mu g/\text{liter} ) (range)</th>
<th>AUC, a ( \mu g \text{*h/} \text{liter} ) (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1–3</td>
<td>20–60</td>
<td>9</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>4</td>
<td>80</td>
<td>3</td>
<td>64 (54–75)</td>
<td>ND</td>
</tr>
<tr>
<td>5</td>
<td>160</td>
<td>3</td>
<td>119 (67–157)</td>
<td>5890 (2820–7800)</td>
</tr>
<tr>
<td>6</td>
<td>320</td>
<td>3</td>
<td>234 (196–283)</td>
<td>10,960 (8,600–14,050)</td>
</tr>
<tr>
<td>7</td>
<td>640</td>
<td>6</td>
<td>786 (637–954)</td>
<td>33,790b (24,550–43,520)</td>
</tr>
<tr>
<td>8</td>
<td>640</td>
<td>6</td>
<td>790 (566–951)</td>
<td>35,260 (26,450–39,030)</td>
</tr>
<tr>
<td>9</td>
<td>480</td>
<td>10</td>
<td>427 (247–581)</td>
<td>19,400 (10,320–24,950)</td>
</tr>
</tbody>
</table>

a AUC, area under the curve; ND, not determined.

b \( n = 5 \) as post-infusion data for one patient was incomplete.

c \( n = 10 \) since two patients received less than required dose.

---

**Fig. 1** Individual plots of zosuquidar clearance versus administered zosuquidar dose after an i.v. infusion of zosuquidar in the absence (cycle 1) or presence (cycle 2) of doxorubicin. CL, clearance.

•, cycle 1: absence of doxorubicin; solid line, regression line for cycle 1; ○, cycle 2: presence of doxorubicin; dotted line, regression line for cycle 2.
Zosquidar Plus Doxorubicin Phase I Trial

Table 4 The arithmetic mean (SD) of various pharmacokinetic parameters for doxorubicin and doxorubicinol, both in the presence and absence of zosuquidar

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>60 mg/m² No Z</th>
<th>60 mg/m² Z</th>
<th>75 mg/m² No Z</th>
<th>75 mg/m² Z</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td></td>
<td>21</td>
<td>19</td>
<td>9</td>
<td>4</td>
</tr>
<tr>
<td>Arithmetic mean (SD) for doxorubicin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC₀→∞</td>
<td>µg*h/liter</td>
<td>1483 (307)</td>
<td>1848 (607)</td>
<td>2613 (741)</td>
<td>3003 (454)</td>
</tr>
<tr>
<td>Clearance</td>
<td>Liter/h</td>
<td>78 (19)</td>
<td>64 (19)</td>
<td>60 (15)</td>
<td>46 (14)</td>
</tr>
<tr>
<td>V₁/₂</td>
<td>Liter</td>
<td>3009 (1077)</td>
<td>2295 (701)</td>
<td>3028 (1058)</td>
<td>1741 (672)</td>
</tr>
<tr>
<td>Arithmetic mean (SD) for doxorubicinol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC₀→∞</td>
<td>µg*h/liter</td>
<td>757 (584)</td>
<td>999 (837)</td>
<td>1763 (1435)</td>
<td>1979 (528)</td>
</tr>
<tr>
<td>Cmax</td>
<td>µg/liter</td>
<td>16 (10)</td>
<td>21 (13)</td>
<td>27 (12)</td>
<td>35 (12)</td>
</tr>
</tbody>
</table>

* Z, zosuquidar; AUC₀→∞ area under the curve between zero and infinity; V₁/₂, volume of distribution at steady state.

\[ \text{MRT}_{1/2,v} = \frac{\text{AUC}}{\text{AUC}_0} - \frac{T}{2} \]

with T the infusion time and

\[ \text{AUMC} = \text{AUMC}(0 - t_0) + \frac{C(t_0)}{\lambda_c} \times t_0 + \frac{C(t_0)}{\lambda_c^2} \]

where AUMC is area under the moment curve.

\[ t_{1/2} = \ln 2/\lambda_c \]

For zosuquidar, when it was not possible to estimate the terminal half-life, \( t_{1/2,\text{max}} \) was assessed instead. Only \( C_{\text{endinf}} \), \( t_{1/2,\alpha} \) and \( \lambda_c \) were reported for these subjects. \( C_{\text{endinf}} \) was determined directly from the observed concentration-time data. It is defined as the concentration observed at the end of the infusion schedule to occur at 48 h for zosuquidar and 0.5 h for doxorubicin.

**Evaluation of P-gp Function in Peripheral Blood Cells.**

A surrogate assay of P-gp function in patients was used that has been previously described using peripheral blood natural killer lymphocytes that express P-gp (6, 8).

\[
\% \text{inhibition} = 100 \times \frac{[\text{MFI}_{\text{post}}(\text{buffer}) - [\text{MFI}_{\text{post}}(\text{spike})/\text{MFI}_{\text{pre}}(\text{spike})]]}{[\text{MFI}_{\text{pre}}(\text{spike}) - [\text{MFI}_{\text{pre}}(\text{buffer})/\text{MFI}_{\text{post}}(\text{spike})]]}
\]

The pharmacokinetic/pharmacodynamic modeling of zosuquidar plasma concentrations versus % inhibition of rhodamine 123 efflux (relative to spike) were carried out using WinNonlin (professional version 2.1).

**RESULTS**

**Dosing Assignments and Patient Characteristics.**

A total of 41 patients were entered into the study, one of whom died before receiving study-directed therapy and is therefore excluded. Forty patients received zosuquidar and doxorubicin in nine cohorts as defined by the protocol and outlined in Table 1. Patients in cohorts 1 and 2 and cohorts 7 and 8 received the same zosuquidar dose (20 mg/m²/day and 640 mg/m²/day, respectively), in each case this was to allow the doxorubicin dose to be increased in a step-wise manner. Because preliminary pharmacokinetic analysis of zosuquidar suggested that some patients who received 640 mg/m²/day may have reached or exceeded plasma levels of 2000 nM, cohort 9 (the final cohort) received 480 mg/m²/day.

The demographics of the 40 patients are summarized in Table 2. Patients were enrolled with a variety of tumor types, the most common being soft tissue sarcoma (15) and renal cell carcinoma (6). Twenty-one patients had prior chemotherapy, six had prior doxorubicin.

**Pharmacokinetics and Pharmacodynamics.**

The non-compartmental pharmacokinetic parameters of zosuquidar are shown by cohort in Table 3. Pharmacokinetic parameters could not be calculated for cohorts 1–3 because most values were below the limit of quantification. For subsequent cohorts mean \( C_{\text{max}} \) and AUC values were generally proportional to dose with the possible exception of cohorts 7 and 8 (both 640 mg/m²/day) in which there appears to be a greater than proportional increase. However, zosuquidar plasma clearance was shown to be independent of administered dose (Fig. 1).

The arithmetic means and SDs for selected pharmacokinetic parameters of doxorubicin and doxorubicinol both in the presence and absence of zosuquidar are shown in Table 4. Across the study as a whole, in the presence of zosuquidar, there was a 17% decrease in CL and 25% increase in AUC of doxorubicin (60 mg/m²) and a similar change (22% and 15% in CL and in AUC, respectively) with the higher dose of doxorubicin (75 mg/m²; Table 5). However, doxorubicin CL decreased with increasing zosuquidar dose in an exponential manner (Fig. 2, upper panel). Thus, when comparing the doxorubicin CL in cycle 1 to that in cycle 2, deviation from the line of identity was expected for the higher doses where the impact on CL was most marked. This trend was observed when the total dose of zosu-
Zosuquidar administered over the 48 h period was ≥500 mg (Fig. 2, lower panel).

Doxorubicin is metabolized to the primary metabolite doxorubicinol. Similarly, in the presence of zosuquidar there was an increase in AUC and $C_{\text{max}}$ for doxorubicinol (32% and 12% for CL and 26% and 30% for AUC after administration of 60 mg/m$^2$ and 75 mg/m$^2$, respectively; see Table 5).

Doxorubicin did not have any effect on the pharmacokinetic parameters calculated for zosuquidar (Table 6). In addition, the interindividual variability in both CL and $V_{ss}$ were similar for cycles 1 and 2.

The effect of zosuquidar on P-gp function was determined using natural killer (CD56$^+$) cells collected at predetermined times in an ex vivo assay (11, 12). A direct reversible concentration-effect relationship was observed (Fig. 3). Increasing concentrations of zosuquidar resulted in greater inhibition of P-gp function until maximum inhibition was attained at concentration in excess of 200 μg/liter. Because zosuquidar demonstrated linear pharmacokinetics, this concentration corresponded to an absolute dose of 500 mg. Hence, as the majority of patients received this dose or greater, maximum inhibition of function was readily achieved.

**Table 6** Mean pharmacokinetic parameters for zosuquidar in the presence and absence of doxorubicin

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Zosuquidar alone ($n = 28$)</th>
<th>Zosuquidar plus doxorubicin ($n = 20$)</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>$CL^a$ (liter/h)</td>
<td>96 (±36)</td>
<td>93 (±38)</td>
<td>−3</td>
</tr>
<tr>
<td>$V_{ss}$ (liter)</td>
<td>1190 (±500)</td>
<td>1120 (±680)</td>
<td>−6</td>
</tr>
<tr>
<td>$t_{1/2}$ (hrs)</td>
<td>17 (±7.3)</td>
<td>17 (±12)</td>
<td>0</td>
</tr>
</tbody>
</table>

$CL$, clearance; $V_{ss}$, volume of distribution at steady state.
Toxicity. The most common toxicities associated with zosuquidar alone were reversible neurological symptoms. The most common was tremor (eight patients) occurring only at doses of \( \geq 480 \text{ mg/m}^2 /\text{day} \). An associated symptom of dizziness was reported for five patients and again mainly at higher doses. None of these episodes were severe (Common Toxicity Criteria grade 1 or 2 only). Symptoms typically became apparent on the second day of dosing and all resolved within a few hours after the conclusion of the infusion.

Overall there were 23 episodes of grade 3/4 neutropenia in 16 patients and 2 of grade 3/4 thrombocytopenia (both in the same patient) in cycles 1 and 2. Eight occurred after doxorubicin alone resulting in a dose reduction in four patients, and 17 occurred in cycle 2 leading to 13 dose reductions. All patients with grade 3/4 neutropenia in cycle 1 experienced the same toxicity in cycle 2. A total of six patients required hospitalization for neutropenic fever, three of the patients after cycle 1, and three patients after cycle 2. Three of the episodes occurred in patients who had received a doxorubicin dose of 75 mg/m\(^2\). The overall incidence of neutropenic fever was 4.9% (6 of 122 cycles of doxorubicin or doxorubicin/zosuquidar). All patients received antibiotics, and all recovered without sequelae.

The analysis of the hematological toxicity and assessment of the impact of zosuquidar is complex because doxorubicin was given repeatedly and, in some cases, the dose of both agents was increased between cohorts. The nadir blood counts both in the presence and absence of zosuquidar are shown in Table 7 and Fig. 4. Overall, it was estimated that, on average, WBC decreased by 32% (95% CI, 21–42%) between cycle 1 and 2; absolute neutrophil count by 61% (95% CI, 42–74%) and platelets by 23% (95% CI, 8–35%) as derived from the ratio of LS means. However, as described above, the decrease in doxorubicin CL was most marked when the total dose of zosuquidar was \( \geq 500 \text{ mg} \) corresponding to cohort 5 (group B). In this group there is an average decrease in absolute neutrophil count of 84% (95% CI, 72–91%) compared with 61% (95% CI, 42–74%) overall and

![Fig. 3 The observed pharmacokinetic-pharmacodynamic relationship for zosuquidar as determined in the ex vivo rhodamine 123 CD36 cell assay. ▲, observed values.](image)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group(^{b})</th>
<th>Cycle 1 LS mean (95% CI)</th>
<th>( n )</th>
<th>Cycle 2 LS mean (95% CI)</th>
<th>( n )</th>
<th>Ratio of LS means (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC</td>
<td>Overall</td>
<td>3.0 (2.5–3.7)</td>
<td>40</td>
<td>2.1 (1.7–2.5)</td>
<td>27</td>
<td>0.68 (0.58–0.79)(^{c})</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>3.7 (2.7–5.0)</td>
<td>14</td>
<td>3.7 (2.7–5.1)</td>
<td>14</td>
<td>1.0 (0.81–1.3)</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>2.5 (2.0–3.2)</td>
<td>26</td>
<td>1.1 (0.88–1.5)</td>
<td>13</td>
<td>0.45 (0.36–0.56)(^{c})</td>
</tr>
<tr>
<td>ANC</td>
<td>Overall</td>
<td>1.3 (0.88–1.9)</td>
<td>40</td>
<td>0.5 (0.33–0.8)</td>
<td>26</td>
<td>0.39 (0.26–0.58)(^{c})</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>1.8 (0.95–3.5)</td>
<td>14</td>
<td>1.7 (0.91–3.3)</td>
<td>14</td>
<td>0.96 (0.55–1.7)</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>0.9 (0.59–1.5)</td>
<td>26</td>
<td>0.15 (0.09–0.27)</td>
<td>12</td>
<td>0.16 (0.09–0.28)(^{c})</td>
</tr>
<tr>
<td>Platelets</td>
<td>Overall</td>
<td>218 (189–251)</td>
<td>40</td>
<td>169 (144–198)</td>
<td>27</td>
<td>0.77 (0.65–0.92)(^{c})</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>225 (179–284)</td>
<td>14</td>
<td>215 (171–271)</td>
<td>14</td>
<td>0.95 (0.74–1.2)</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>212 (179–250)</td>
<td>26</td>
<td>133 (107–165)</td>
<td>13</td>
<td>0.63 (0.49–0.79)(^{c})</td>
</tr>
</tbody>
</table>

\(^{a}\) LS, least square; CI, confidence interval; ANC, absolute neutrophil count.

\(^{b}\) Group A, total zosuquidar dose received <500 mg; group B, total zosuquidar dose >500 mg.

\(^{c}\) The 95% confidence interval of the ratio of the LS means does not include 1, hence the difference is significant at the 5% level.
only 4% (95% CI, −68% to 45%) when the dose of zosuquidar was <500 mg (group A). Similar trends are shown for WBC and platelet count. In group A, there is no difference between cycle 1 and 2; thus the differences seen in the patients overall is heavily influenced by group B. This latter group makes up the majority of the patient population that received the highest doses of doxorubicin. The impact of the higher dose of doxorubicin in group B is shown by the LS means for cycle 1 where doxorubicin was given alone.

As per protocol specification, six patients were discontinued because of decreased cardiac function as assessed by gated blood-pool imaging studies (Table 8). Patients experienced ejection fraction decreases of 10% to 18% from baseline. Their baseline levels ranged from 60% to 74%, and their levels at discontinuation ranged from 42% to 62%. Lifetime exposures for doxorubicin were 120–360 mg/m². There were no instances of congestive heart failure or other symptomatic cardiac disease. Of note, five of the six patients had ejection fractions above 50% at the time of discontinuation from the study.

**Antitumor Efficacy.** Formal evaluation of efficacy was not the intent of this Phase I study; however, 32 patients were evaluated for efficacy. Two patients had a partial response to therapy, one as assessed by bi-dimensional tumor measurements, the other by a serum tumor marker, Prostate-specific antigen decreased by >50%. The latter patient remained on trial for 12 cycles. An additional 12 patients had stable disease as their best response, with six of the patients having stable disease documented for at least 5 cycles.

The patient with a measurable partial response was a male with metastatic breast cancer. He received 480 mg/m²/day zosuquidar for six cycles before being discontinued having achieved maximal clinical response. His doxorubicin dose was initially 75 mg/m², but it was decreased twice because of leukopenia. Before entering the study, this patient had received prior therapy with docetaxel and estramustine.

**DISCUSSION**

Effective pharmacological inhibition of P-gp function in the clinical setting has previously been achieved at the expense of the delivered dose of the coadministered cytotoxic agent, mostly as a consequence of pharmacokinetic interactions (6, 13, 14). It has been difficult to evaluate the contribution of pharmacodynamic effects as this has been masked by the larger effect of the pharmacokinetic interactions. Ideally, if pharmacokinetic interactions could be minimized, full-dose antitumor therapy could be administered with an MDR modulator and thereby isolate and more adequately assess the effect of P-gp inhibition on clinical outcomes (15). The development of highly selective, potent inhibitors of P-gp, such as zosuquidar, may help achieve this goal.

This Phase I study demonstrated that zosuquidar is well tolerated, exhibiting minimal toxicity, at concentrations in excess of those required to maximally inhibit P-gp function. The only toxicity attributed to i.v. zosuquidar as a single agent is reversible grade 2 neurotoxicity, primarily cerebellar-related tremor. Whether this toxicity occurred as a result of P-gp inhibition at the blood-brain barrier could not be assessed directly in this trial (16). However, when zosuquidar was administered orally, a more frequent and severe (grade 3/4) cerebellar toxicity was evident at lower plasma concentrations where maximal P-gp inhibition may not have been achieved (8). These data

![Graph showing WBC and platelet nadirs in cycles 1 and 2 by dose level of zosuquidar. Dashed line, zosuquidar dose < 500 mg (Group A); solid line, zosuquidar dose ≥ 500 mg (Group B).]

**Table 8  Summary of patient characteristics for those discontinuing due to decreased ejection fraction**

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Cohort</th>
<th>Number of cycles completed</th>
<th>Cum dox (mg/m²)</th>
<th>Cum Z (mg/m²)</th>
<th>Prior chemo/immu/horm therapy</th>
<th>Prior radiotherapy</th>
<th>EF start</th>
<th>EF final</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>3</td>
<td>4</td>
<td>211</td>
<td>321</td>
<td>None</td>
<td>None</td>
<td>62%</td>
<td>52%</td>
</tr>
<tr>
<td>11</td>
<td>4</td>
<td>6</td>
<td>314</td>
<td>960</td>
<td>Paclitaxel, carboplatin</td>
<td>Scapula</td>
<td>60%</td>
<td>42%</td>
</tr>
<tr>
<td>12</td>
<td>4</td>
<td>2</td>
<td>122</td>
<td>325</td>
<td>None</td>
<td>Iliac lymph node</td>
<td>71%</td>
<td>57%</td>
</tr>
<tr>
<td>13</td>
<td>4</td>
<td>6</td>
<td>360</td>
<td>960</td>
<td>Interleukin-2, interferon α, RhuMab VEGF</td>
<td>None</td>
<td>64%</td>
<td>52%</td>
</tr>
<tr>
<td>16</td>
<td>5</td>
<td>2</td>
<td>120</td>
<td>638</td>
<td>None</td>
<td>None</td>
<td>74%</td>
<td>62%</td>
</tr>
<tr>
<td>27</td>
<td>8</td>
<td>4</td>
<td>282</td>
<td>5137</td>
<td>None</td>
<td>Thigh</td>
<td>69%</td>
<td>53%</td>
</tr>
</tbody>
</table>

* Cum dox, cumulative doxorubicin; Z, zosuquidar; EF, ejection fraction; VEGF, vascular endothelial growth factor.
suggest a mechanism other than P-gp inhibition may contribute to the observed neurotoxicity.

Analysis of the pharmacokinetic data demonstrated that the primary effect of zosuquidar on doxorubicin appeared to be a decrease in clearance. This most likely represented inhibition of P-gp in bile canaliculi impeding biliary excretion. This was further supported because there was no decrease in the AUC of doxorubicinol, as would be expected if decreased metabolism had accounted for the change in doxorubicin clearance. In fact, AUC was found to increase in the presence of zosuquidar, which again was consistent with the inhibition of canalicular P-gp.

Trials with other MDR modulators, such as valspodar, when combined with doxorubicin have resulted in larger increases in AUC and Cmax than have been demonstrated with zosuquidar (13). This effect may be attributed, in part, to the specificity of zosuquidar for P-gp compared with other ATP-binding cassette transporters. Because doxorubicin is transported by both P-gp and multidrug resistance protein 2, the impact of a less selective modulator may be significant. Additionally, the impact on doxorubicinol AUC is influenced markedly by the period of P-gp inhibition. In the studies with valspodar, the period of P-gp inhibition after doxorubicin infusion was substantially longer (approximately 96 h) than in this study.

The pharmacokinetic data also revealed an unexpected decrease in the Vss of doxorubicin in the presence of zosuquidar. The mechanisms responsible for this effect are unknown. Recent publications have shown decreases in Vss of P-gp substrates such as talinol and docetaxel by other P-gp inhibitors (Verapamil and R101933, respectively), suggesting the effect may be directly related to P-gp inhibition (17, 18).

The clearance of zosuquidar was independent of BSA, hence dosing using this paradigm is not beneficial. In this study, an absolute dose of ≥500 mg administered over 48 h was associated with both a greater impact on doxorubicin clearance and maximal P-gp inhibition in NK cells. This dose is predicted to give peak plasma concentrations ≥200 μg/liter, the threshold for maximum P-gp inhibition.

Despite the modest changes in the pharmacokinetics of the doxorubicin, there were no clinical sequelae observed in this Phase I trial sufficient to define a dose-limiting toxicity, at the maximal delivered doses of zosuquidar (480–640 mg/m2) and doxorubicin (75 mg/m2/day). However, there was a statistically significant decrease in absolute neutrophil count in the presence of zosuquidar. This decrease was more evident at doses resulting in maximal P-gp inhibition. Because there is a minor decrease in the elimination of doxorubicin and importantly doxorubicinol, which is pharmacologically active, these cannot be ascribed solely to a pharmacodynamic effect on bone marrow stem cells.

Another important effect that potentially would limit the ability to administer a full course of treatment is cardiotoxicity (19). From this study there is no evidence to suggest that cardiac function is adversely affected from the combination although the sample size is small. Further analysis of this potential toxicity would necessitate longer treatment durations in a larger patient number in a randomized setting.

In summary, zosuquidar can be administered safely at doses compatible with maximal inhibition of P-gp function. Some pharmacokinetic interaction was observed that may be explained by inhibition of P-gp in the bile canaliculi reducing elimination. This resulted in an exacerbation of hematological toxicity as determined from nadir blood counts. The combination warrants further exploration in Phase II trials.

REFERENCES

A Phase I Trial of a Potent P-Glycoprotein Inhibitor, Zosuquidar Trihydrochloride (LY335979), Administered Intravenously in Combination with Doxorubicin in Patients with Advanced Malignancy

Alan Sandler, Michael Gordon, Dinesh P. de Alwis, et al.


Updated version
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/10/10/3265

Cited articles
This article cites 16 articles, 8 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/10/10/3265.full#ref-list-1

Citing articles
This article has been cited by 7 HighWire-hosted articles. Access the articles at:
http://clincancerres.aacrjournals.org/content/10/10/3265.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, use this link:
http://clincancerres.aacrjournals.org/content/10/10/3265.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.