Safety and Efficacy of the Multidrug-Resistance Inhibitor Biricodar (VX-710) with Concurrent Doxorubicin in Patients with Anthracycline-resistant Advanced Soft Tissue Sarcoma

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ABSTRACT

Purpose: Incel (biricodar, VX-710) restores drug sensitivity to P-glycoprotein and multidrug resistance-associated protein-1-expressing cells. This Phase I/II study evaluated the safety/tolerability, pharmacokinetics, and efficacy of VX-710 plus doxorubicin in patients with inoperable, locally advanced or metastatic, anthracycline-resistant/refractory, soft tissue sarcoma.

Experimental Design: In Phase I, i.v. bolus doxorubicin at 60, 75, or 67.5 mg/m² was administered 8 h after initiation of a 72-h continuous i.v. (CIV) infusion of VX-710 (120 mg/m²/h) to cohorts of patients to establish a maximum tolerated dose. For efficacy evaluations in Phase II, eligible patients had inoperable, locally advanced or metastatic, anthracycline-resistant/refractory soft tissue sarcoma; ≤225 mg/m² cumulative prior doxorubicin; and adequate hematological, liver, and kidney function. Cycles were repeated every 3 weeks.

Results: Fourteen patients were enrolled in Phase I. Myelosuppression was the dose-limiting toxicity with 75 and then 67.5 mg/m² doxorubicin, and the maximum tolerated dose was established at 60 mg/m² with VX-710, 120 mg/m²/h, 72-h CIV. VX-710 had no apparent effect on doxorubicin pharmacokinetics. Twenty-nine patients enrolled in Phase II were treated with VX-710, 120 mg/m²/h 72-h CIV, and 60 mg/m² doxorubicin. Among 26 evaluable patients, minimal activity was noted among 11 patients with gastrointestinal stromal tumors (GISTs); however, in 15 patients with anthracycline-resistant sarcomas of other histologies, 2 achieved partial responses and 7 patients had disease stabilization with an overall median progression-free interval of 3.4 months.

Conclusion: Anthracycline resistance in GISTs appears to be independent of P-glycoprotein or multidrug resistance-associated protein-1 resistance mechanisms. However, the combination of VX-710 and doxorubicin resulted in objective responses or disease stabilization in patients with strictly defined anthracycline-refractory non-GIST sarcomas, which warrants further evaluation.

INTRODUCTION

Soft tissue sarcomas (STSs), which derive from mesenchymal tissue, are aggressive tumors that comprise less than 1% of malignant tumors that affect adults (1, 2). An estimated 8100 new cases and 4600 deaths are predicted in the United States in the year 2000 (1). Five-year survival rates range from 40% to 90% depending on primary disease site (extremity or visceral), histological grade, and presence or absence of metastases at diagnosis (2–4). Standard first-line chemotherapy for patients with primary or metastatic STS is doxorubicin, administered either alone or in combination with ifosfamide (with mesna for bladder protection) and/or DTIC. Doxorubicin and ifosfamide are the two most active single agents in STS, with response rates of 20–25% in previously untreated patients (5). DTIC also has single-agent activity, with an average response rate of approximately 18% (5). Several randomized trials have compared the activity of single-agent doxorubicin with that of doxorubicin/ifosfamide, cyclophosphamide/vincristine/doxorubicin/DTIC (CYVADIC), or the MAID regimen (mesna, doxorubicin, ifosfamide, DTIC). Results show a modest increase in overall response rates (28–34%) and time-to-disease progression with the combination regimens, but no overall increase in response duration or survival compared with that of single-agent doxorubicin (6, 7). Consistent with these data are the results of a

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1 This study was supported by Vertex Pharmaceuticals Incorporated and conducted by the Canadian Sarcoma Group in collaboration with the Dana Farber Cancer Institute.

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3 The abbreviations used are: STS, soft tissue sarcoma; DTIC, dacarbazine [5-(3,3-dimethyl-1-triazenyl)-1H-imidazole-4-carboxamide]; GIST, gastrointestinal stromal tumor; MDR, multidrug resistance; P-gp, P-glycoprotein; MRP1, multidrug resistance-associated protein-1; CIV, continuous i.v.; ULN, upper limit of normal; DLT, dose-limiting toxicity; MTD, maximum tolerated dose; MUGA, multiple gated acquisition; AUC, area under the plasma concentration-versus-time curve; AUMC, area under the first moment curve.
meta-analysis of eight randomized controlled trials (2281 patients) comparing different types of doxorubicin-based combination chemotherapy regimens with single-agent doxorubicin. For response rate, there was a statistically nonsignificant trend favoring combination chemotherapy (odds ratio = 0.78, 95% confidence interval 0.60–1.05, \( P = 0.10 \)), but no difference in overall survival (odds ratio = 0.84, 95% confidence interval 0.67–1.06, \( P = 0.13 \); Ref. 8). Toxicity, however, is significantly greater with the combination regimens, and more than 50% of patients will demonstrate primary resistance or develop acquired resistance to doxorubicin (6–10). Few treatment options exist for patients with advanced, anthracycline-resistant/refractory STS. This is especially so for patients with liver metastases, which are often derived from GISTs or retroperitoneal leiomyosarcomas. Many groups have documented that these are particularly resistant to doxorubicin-based chemotherapy (10–12).

Malignant, whether intrinsic or acquired, is a major factor in the failure of cancer chemotherapy. A common form of MDR results from expression of \( MDR1 \), the gene that encodes P-gp (13). P-gp is a 170-kDa transmembrane protein that confers resistance to chemotherapeutic agents by preventing their accumulation in cells via an ATP-dependent process. Overexpression of P-gp in tumor cells is correlated with resistance to the most commonly used chemotherapeutic agents, including anthracyclines, \( Vincr \) alkaloids, epipodophyllotoxins, and taxanes (13–15). MDR is also conferred via another pathway associated with expression of MRP1. MRP1 confers resistance to anthracyclines, \( Vinca \) alkaloids, and epipodophyllotoxins by causing efflux of glutathione-conjugated agents, also by an ATP-dependent process (16).

Several studies have evaluated tumor specimens from patients with STS for expression of \( MDR1 \) and MRP1 mRNA using molecular probes or antibodies to detect P-gp and MRP1 at the protein level. Results of these studies demonstrate expression of these MDR drug efflux pumps in 30–90% of specimens either at diagnosis or after treatment, with the broad range probably reflecting the differences in sensitivity and specificity of the methods used in the analyses (17). In general, P-gp expression has been detected in 30–60% of STS specimens at diagnosis (18–25). MRP1 has been detected in 22 of 39 specimens (56%) with coexpression of MRP1 and P-gp detected in 34–38% of specimens (21, 22). The expression of P-gp or MRP1 appears to be higher in liposarcomas, leiomyosarcomas, and malignant fibrous histiocytomas and to correlate with a higher histological grade (20, 21, 25). An increase in P-gp expression has been observed in biopsies from relapsed patients (18), consistent with the rapid (<1 h) activation of \( MDR1 \) gene expression after exposure of patients to doxorubicin (26). Several recent studies examined possible prognostic correlates, and results suggest that patients with tumors that express P-gp and MRP1 at diagnosis are more likely to have a poor response to treatment (21) and shorter progression-free survival than patients with tumors that do not express P-gp or MRP1 (22–24, 27).

Thus, an agent that modifies intrinsic or acquired MDR could provide substantial clinical benefit for patients with STSs. Many agents have been identified that restore drug accumulation in P-gp-expressing cell lines and overcome MDR in vitro (28), and a few compounds have also been reported to reverse MRP1-mediated MDR. Several agents, notably cyclosporin A, PSC833 (a nonimmunosuppressive cyclosporin analogue), verapamil, and \( R \)-verapamil, have been evaluated in clinical trials (14, 29). Results of studies to date in patients with solid tumors have been largely disappointing, attributable in part to either poor potency of the MDR inhibitor, failure to sustain therapeutic levels of the MDR inhibitor because of dose-limiting toxicity, or pharmacokinetic interactions with cytotoxic agents that complicated interpretation of study data.

VX-710 (biricodar, Incel) is a novel small molecule that inhibits MDR and restores drug sensitivity to cells expressing both P-gp (30) and MRP1 (31) at concentrations that are sustained in vivo with minimal toxicity (32). VX-710 binds directly to P-gp and MRP1, which inhibits pump efflux activity and results in increased intracellular accumulation and retention of cytotoxic agents. At in vitro concentrations of 0.5–2.5 \( \mu M \), VX-710 restores the sensitivity of a variety of P-gp-expressing cells to the cytotoxic action of paclitaxel, doxorubicin, vincristine, and vinblastine (30, 33). Additionally, 2.5–5.0 \( \mu M \) VX-710 restores the sensitivity of MRP1-expressing cells to doxorubicin, vincristine, and etoposide (31). A Phase I study of VX-710 plus doxorubicin previously has established the safety and pharmacokinetics of VX-710 administered by CIV infusion for 96 h in combination with i.v. bolus doxorubicin at a dose of 45 mg/m\(^2\). VX-710 blood steady-state concentrations of 10 \( \mu M \) were sustained at a dosage of 120 mg/m\(^2\)/h, and VX-710 had no apparent effect on doxorubicin pharmacokinetics or pharmacodynamics (34).

Therefore, we initiated a multicenter, open-label Phase I/II study to evaluate the combination of VX-710 (120 mg/m\(^2\)/h continuous infusion) plus doxorubicin in patients with metastatic and/or inoperable locally recurrent STS resistant or refractory to anthracyclines. The objectives of Phase I were to (a) determine the safety and tolerability of VX-710 (120 mg/m\(^2\)/h given as a 72-h CIV infusion) with intercohort doxorubicin doses of 60, 75, and 67.5 mg/m\(^2\), (b) characterize doxorubicin pharmacokinetics for the VX-710/doxorubicin combinations, and (c) determine the optimal doxorubicin dose for the Phase II efficacy portion of the study. Phase II was designed to evaluate the efficacy of VX-710 in combination with doxorubicin in a stringently defined population of patients with anthracycline-resistant STSs and to further establish the safety and tolerability of this combination regimen in this patient population.

**PATIENTS AND METHODS**

**Patient Population.** Patients eligible for Phase I were at least 18 years of age with a histological diagnosis of STS; had bidimensionally measurable lesions (\( \geq 2 \) cm \( \times \) 2 cm by CT scan, \( \geq 1 \) cm \( \times \) 1 cm by chest X-ray, \( \geq 1 \) cm \( \times \) 1 cm skin lesion or node); an Eastern Cooperative Oncology Group performance status of 0–2; adequate hematological (absolute granulocyte count of \( \geq 2.0 \times 10^{9}/\)liter, platelet count of \( \geq 100 \times 10^{9}/\)liter), liver (bilirubin \( \leq 1.5 \times UD \), aspartate aminotransferase \( \leq 2 \times \) ULN (\( \leq 3 \) ULN for patients with documented liver metastases), and renal function (serum creatinine \( \leq 1.5 \times ULN \); and a life expectancy of more than 12 weeks. Eligibility for Phase II required inoperable, locally advanced or metastatic STS; measurable disease; anthracycline resistant/refractory disease (docu-
mented progression on doxorubicin defined as appearance of new lesions, or >25% increase in measurable lesions within 8 weeks of study entry, or chemotherapy-naïve GIST or leiomyosarcoma metastatic to the liver); a minimum prior dose of 60 mg/m² doxorubicin, with a maximum cumulative dose ≤225 mg/m²; left ventricular ejection fraction of ≥50% by MUGA scan; and adequate hematomatological, liver, and renal function as defined above. Patients were ineligible if they had embryonal or alveolar rhabdomyosarcoma, Ewing’s sarcoma, osteosarcoma, carcinomasarcomas, Kaposi’s sarcoma, or any other previous malignancy within the previous 5 years except successfully treated in situ carcinoma or nonmelanoma skin cancer. Previous sarcoma therapy requiring stem cell support or chemotherapy other than a doxorubicin-based regimen was not allowed. Pregnant or lactating women or any individuals with childbearing potential who were unwilling or unable to use effective contraception were ineligible. Additional exclusion criteria were concurrent radiation at the sole site of measurable disease or treatment with other experimental drugs; any other serious illness or medical condition that precluded patient management according to protocol; and concurrent therapy with warfarin or drugs described as P-gp inhibitors or drugs that interfere with cytochrome P-450 enzymes. Informed consent was obtained according to local Institutional Review Board requirements.

Study Design. In Phase I, a minimum of three evaluable patients were enrolled at each doxorubicin dose level (60, 75, and 67.5 mg/m²) to assess safety. Toxicities were evaluated and graded according to the National Cancer Institute of Canada Cancer Treatment Group Expanded Common Toxicity Criteria or the WHO Toxicity Scale after a protocol amendment as described below. DLTs were defined as a grade 3 or higher major organ toxicity, febrile neutropenia requiring i.v. antibiotics, or bleeding because of thrombocytopenia and requiring transfusion; asymptomatic neutropenia or thrombocytopenia did not constitute a dose-limiting toxicity. The MTD of doxorubicin was defined as the dose level below which >2 of up to 6 patients experienced a DLT.

The original design of the study included doxorubicin doses of only 60 mg/m² and 75 mg/m². No DLTs were observed in the three patients treated with 60 mg/m²; however, at 75 mg/m², DLTs were observed in two of three patients. To maximize doxorubicin dose intensity, the protocol was amended to include an intermediate doxorubicin dose of 67.5 mg/m². Safety at this dose level was evaluated during the first cycle of treatment using the WHO Toxicity Scale. Three patients were enrolled at this intermediate dose with the assessment for DLT performed as described above.

During the first cycle, VX-710 was administered as a CIV infusion of 120 mg/m²/h for 72 h. Doxorubicin was given 8 h after the initiation of the VX-710 infusion when the expected VX-710 steady-state concentration had been reached. Pharmacokinetic studies were performed during cycle 1 for all patients in the Phase I portion of the study. For subsequent cycles, the doxorubicin dose could be administered at least 4 but no more than 8 h after the start of the VX-710 infusion. In the Phase II portion of the study, VX-710, 120 mg/m²/h, was administered by CIV infusion for 68–72 h with the MTD of doxorubicin identified in Phase I administered at least 4 and no more than 8 h after the start of the VX-710 infusion. Study treatment was administered every 3 weeks. Antiemetics were allowed at the discretion of the investigator. Administration of granulocyte colony-stimulating factor was not permitted during the first cycle of therapy.

VX-710 was provided by Vertex Pharmaceuticals (Cambridge, MA) in vials containing 3 g of VX-710 as a dicitrate salt in 6 ml of sterile water for injection (500 mg/ml VX-710, which is 300 mg base equivalent). At the time of use, the concentrated drug solution was aseptically mixed with 0.9% normal saline or D5W for administration with a 250-ml polyvinyl chloride infusion set.

Pretreatment and Follow-up Studies. Patient history, physical examination and assessment of baseline symptoms, pretreatment MUGA scan, ECG, baseline tumor measurements, and routine laboratory studies including urinalysis were performed within 7–14 days before starting study treatment. CBCs were drawn on day 1 and then weekly during each cycle. Clinical chemistries and urinalysis were performed on day 1 starting with repeat cycles. MUGA scans were repeated at least 1 week after the cumulative doxorubicin dose exceeded 450 mg/m² and after every treatment cycle thereafter. ECGs were performed when clinically indicated and repeated at the discretion of the investigators. Computerized tomography or plain radiographs were obtained after even-numbered cycles to measure tumor response.

Pharmacokinetic Sampling and Assays. To study the pharmacokinetics of doxorubicin, blood samples (2 ml whole blood in pediatric tubes containing EDTA for VX-710; 5 ml whole blood in pediatric tubes containing heparin for doxorubicin in plasma) were drawn from patients via a venous catheter placed in the arm contralateral to the VX-710 infusion. Blood samples were collected for the analysis of VX-710 blood concentrations before the initiation of the infusion and before administration of doxorubicin. For the analysis of doxorubicin, plasma samples were collected before the administration of doxorubicin, immediately after completion of the i.v. bolus injection, and then at 10, 20, and 50 min, 1.5, 4, 16, 40, and 64 h, or just before termination of the VX-710 infusion.

The analytical methods used for extraction of VX-710 or doxorubicin were as follows. VX-710 was recovered from whole blood via a double liquid-liquid extraction procedure using methyl-tert-butyl ether. Subsequently, the extract was subjected to an isocratic reverse-phase HPLC separation using a Selectosil 5 CN column (250 × 4.6 mm, 5 μm) with UV detection at 305 nm for the determination of VX-710 concentration. The linear range was 0.2–12.42 μg/ml. The assay precision and accuracy were 2.6–13.2% and >90%, respectively.

Doxorubicin was recovered from plasma samples using ethyl acetate extraction. Subsequently, the extract was subjected to isocratic reverse-phase HPLC separation using a Phenomenex Selectosil C-18 column (250 × 4.6 mm, 5 μm) with fluorescence detection (excitation wavelength of 470 nm and emission wavelength of 550 nm) for the quantitation of doxorubicin concentration. The linear range of the plasma assay was 5–2000 ng/ml. The assay precision and accuracy were 2.4–28% and >98%, respectively. Concurrent monitoring of plasma concentrations of doxorubicinol, the major metabolite of doxorubicin, was not completely in accordance with the principles of Good Laboratory Practice because of the limited availability of a...
reference standard. Briefly, a calibration curve was generated with the limited amount of doxorubicinol reference standard and subsequently used to quantify the doxorubicinol concentrations in all plasma samples from this study.

**Pharmacokinetic Analyses.** VX-710 blood concentrations were determined from the single sampling time point for each patient. Doxorubicin data were analyzed using the non-compartmental method. The AUC from time 0 to infinity and the AUMC from time 0 to infinity were calculated using the trapezoidal rule. Subsequently, systemic plasma clearance (CLs) was calculated as the ratio of dose:AUC, and volume of distribution at steady state (Vss) was calculated as (dose × AUMC)/AUC (2). The terminal half-life was calculated from the terminal elimination rate constant obtained from fitting the doxorubicin concentration-time data to a biexponential equation using PCNONLIN. The area under the plasma doxorubicinol concentration-versus-time curve was also calculated using the trapezoidal rule. Subsequently, the ratio of doxorubicin AUC:doxorubicinol AUC was calculated.

**Response Criteria.** Standard criteria were used to classify tumor response. A complete response was defined as the total disappearance of all measurable and evaluable evidence of cancer confirmed by two measurements at least 4 weeks apart; a partial response was defined as a reduction of at least 50% in the sum of the products of bidimensionally measurable lesions from two measurements at least 4 weeks apart; progressive disease was defined as an increase of more than 25% in the sum of the products of the bidimensional measurements and/or appearance of new lesions; stable disease was classified as a response that did not meet criteria for a tumor response or progression. An independent radiology review was performed for patients who achieved objective responses, terminated treatment with stable disease, or had an interval of more than 6 weeks with stable disease to confirm the assessments made at individual study centers.

**Statistical Analysis.** All patients in Phase II and those patients in Phase I who met the study inclusion criteria and were treated at the same doxorubicin dose used in the Phase II portion of the study were evaluated for efficacy. Because patients in this study were refractory to doxorubicin, the objective response rates were expected to be low. Therefore, a multivariate end point was used that included both tumor response and disease progression to determine treatment activity using a type I error rate of 5% and a power of 80%; a two-stage design was developed according to the method of Zee et al. (35):

(a) Twenty-five evaluable patients were to be entered into the first stage of the Phase II trial. The treatment would be considered inactive and the trial would be terminated if the following were observed: ≤1 response; ≤2 responses, and ≥12 early progressions; ≤3 responses and ≥13 early progressions; or ≥14 early progressions. Treatment activity was defined as ≥2 responses and ≤9 early progressions; ≥4 responses and ≤11 early progressions; or ≥5 responses.

(b) If the treatment was active during stage 1, 25 more patients were enrolled (total n = 50) in stage 2. Treatment activity was defined as ≥1 response and ≤23 early progressions; ≥3 responses and ≤24 early progressions; ≥5 responses and ≤26 early progressions; or ≥6 responses.

This multivariate procedure tests the null hypothesis that the response rate is 5% with an early progression rate of 60% versus the alternate hypothesis that the response rate is 15% with an early progression rate of 40%:

\[ H_0:1:P1 = 0.05 \text{ versus } H_1:1:P1 = 0.15 \]

\[ H_0:2:P1 = 0.60 \text{ versus } H_1:1:P2 = 0.40 \]

The significance level is 0.004 and the power is 0.795. The expected sample size for the multivariate design is 26.30 when the null hypothesis is true and 29.35 when the alternate hypothesis is true.

Emerging data documenting the striking resistance of GIST to all known chemotherapeutic agents has prompted some investigators to analyze separately or plan parallel Phase II studies for patients with GIST and non-GIST sarcomas (36–38). Although this was not a part of the initial statistical analysis plan, we decided that a post hoc analysis of this issue was appropriate. In addition, we also performed a retrospective analysis of time to disease progression. The time to progression was calculated from the first day of study treatment to the first objective evidence of tumor progression. If progression was not documented by radiological assessment or by worsening symptoms, patients were censored at the start of any intervening therapy (surgery, radiation, or chemotherapy for relapsed disease) or at the time a patient was lost to follow-up.

**RESULTS**

**Phase I**

**Patients.** A total of 14 patients were enrolled in the Phase I portion of the study. Patient characteristics are summarized in Table 1, and the distribution of the patients by doxorubicin dose group and a summary of treatment are shown in Table 2. Five additional patients were enrolled in the 60 mg/m² dose group pending approval of the protocol amendment adding the 67.5 mg/m² dose group to this study.

**Safety and Tolerability.** Dose-limiting toxicity (febrile neutropenia) was experienced by two of the three patients en-
rolled in the 75 mg/m² dose group during cycle 1. Febrile neutropenia was also experienced by two of the three patients enrolled in the 67.5 mg/m² dose group, with one episode in cycle 1 for one patient and in cycle 2 for the other patient. Median cycle 1 nadirs for WBCs, absolute neutrophil counts, platelets, and hemoglobin are summarized in Table 3. Results indicate a more significant effect on each of these parameters when increasing the doxorubicin dose from 60 to 75 mg/m². Patients in both the 75 and 67.5 mg/m² dose groups continued study treatment with doxorubicin dose reductions of 20–30% in subsequent cycles. On the basis of these results, the optimal dose combination for the Phase II portion of the trial was established as 120 mg/m²/h 72-h CIV of VX-710 with 60 mg/m² doxorubicin.

**Pharmacokinetic Analyses.** The mean VX-710 blood concentration determined at the time of doxorubicin administration was 5.7 μg/ml (9.5 μM) with a SD of ±2.8 for the 14 patients, which is similar to the VX-710 steady-state concentration determined for the 120 mg/m²/h dose group in the Phase I study (34). Mean doxorubicin concentration-versus-time profiles were similar for all three doxorubicin dose levels (Fig. 1). Doxorubicin pharmacokinetic parameters at each dose level are summarized in Table 4. The doxorubicin area under the concentration-versus time curve (AUC), clearance, and volume of distribution at steady state ($V_{ss}$) are similar for all three doses. In particular, doxorubicin clearance (range of 0.57–0.67 liter/h/kg) is in close agreement with literature values of 0.3–0.8 liter/h/kg (Table 4; Refs. 39–42).

**Phase II**

**Patients.** Twenty-three patients were entered to complete enrollment in stage 1 of the Phase II study, in addition to 6 patients from Phase I who were treated with doxorubicin 60 mg/m² and met the eligibility criteria for efficacy evaluation. Patient characteristics for these 29 patients are summarized in Table 1. A total of 106 courses of VX-710 plus doxorubicin therapy (mean 3.8 courses/patient) were administered to the stage 1 cohort of patients.

**Safety and Tolerability.** A total of 37 patients comprised the safety population for the analysis of nonhematological toxicities, including the 6 patients treated in the 67.5 and 75 mg/m² doxorubicin dose groups in the Phase I portion of the study. The evaluation of hematological toxicity is based on all 31 patients who received 60 mg/m² doxorubicin as their initial dose of chemotherapy. VX-710 in combination with doxorubicin 60 mg/m² was generally well tolerated. Myelosuppression was the principal treatment toxicity. Overall median (range) WBC and absolute neutrophil count nadirs were 1.35 (0.1–3.1) and 0.2 (0.0–1.5) × 10⁹ cells/l, respectively. The overall

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**Table 2** Treatment summary for Phase I

<table>
<thead>
<tr>
<th>Dose level (mg/m²)</th>
<th>Patients ($n$)</th>
<th>Prior doxorubicin ($n$)</th>
<th>Median prior doxorubicin dose, mg/m² (range)</th>
<th>Treatment cycles ($n$)</th>
<th>DLTs</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>8</td>
<td>6</td>
<td>165 (143–225)</td>
<td>27</td>
<td>None</td>
</tr>
<tr>
<td>75</td>
<td>3</td>
<td>1</td>
<td>150</td>
<td>11</td>
<td>2/3</td>
</tr>
<tr>
<td>67.5</td>
<td>3</td>
<td>1</td>
<td>150</td>
<td>11</td>
<td>2/3</td>
</tr>
</tbody>
</table>

**Table 3** Median cycle 1 nadirs for patients treated with 60, 75, or 67.5 mg/m² doxorubicin in combination with VX-710

<table>
<thead>
<tr>
<th>Dose level (mg/m²)</th>
<th>WBCs ($×10⁹$ cells/liter)</th>
<th>ANC ($×10⁹$ cells/liter)</th>
<th>Platelets ($×10⁹$ cells/liter)</th>
<th>Hemoglobin (g/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>2.4 (1.2–4.6)</td>
<td>0.56 (0.048–1.3)</td>
<td>173 (134–374)</td>
<td>100 (79–118)</td>
</tr>
<tr>
<td>75</td>
<td>0.2 (0.17–3.0)</td>
<td>0.0 (0–1.83)</td>
<td>41 (25–150)</td>
<td>81 (64–106)</td>
</tr>
<tr>
<td>67.5</td>
<td>2.1 (0.2–5.5)</td>
<td>0.4 (0.0–2.8)</td>
<td>226 (91–637)</td>
<td>112 (74–119)</td>
</tr>
</tbody>
</table>

**Fig. 1** Mean doxorubicin concentration-versus-time profiles.
incidences of grade 3 and grade 4 neutropenia were 25% (27 of 106 cycles) and 40% (40 of 106 cycles), respectively. Nine patients (29%) had an episode of febrile neutropenia. Seven patients had 20% doxorubicin dose reductions starting with cycle 2, with the dose reductions attributable to febrile neutropenia for six patients. Colony-stimulating factor was administered in later cycles to 12 patients, 3 of whom also had doxorubicin dose reductions. One patient with a retroperitoneal GIST and extensive liver metastases had deteriorating liver function concurrent with the first treatment cycle, became septic, and died despite aggressive antibiotic therapy. VX-710 combined with doxorubicin 60 mg/m² had no significant effect on platelet counts, but 5 patients experienced grade 3/4 anemia.

Other adverse events were generally mild to moderate in severity and were reversible, with incidences similar to those observed after doxorubicin therapy as a single agent. The most common nonhematological adverse events (≥ grade 2 experienced by more than 20% of patients) were asthenia, alopecia, vomiting, fever, stomatitis, and anorexia (Table 5).

During long-term follow-up, two patients developed symptoms of congestive cardiomyopathy 3 months and 7 months after completion of VX-710/doxorubicin treatment. Both patients were diagnosed with cardiomyopathy secondary to doxorubicin chemotherapy. Each patient had received 120 mg/m² doxorubicin before starting treatment, and both patients reached vomiting, fever, stomatitis, and anorexia (Table 5).

Vasodilation 8 (22) 8
Fever 11 (30) 3 7 1
Constipation 12 (32) 2 5 6
Diarrhea 11 (30) 8 2 1 1
Doxol:Dox

* The worst grade experienced by the patient.


doxorubicin combination with VX-710

<table>
<thead>
<tr>
<th>Dose level (mg/m²)</th>
<th>AUC (ng · h/ml)</th>
<th>Clₐ (liters/kg)</th>
<th>Vₐ (liters/kg)</th>
<th>AUCs Doxorubicin</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>3758 ± 1688</td>
<td>0.57 ± 0.27</td>
<td>11.0 ± 9.5</td>
<td>1.04 ± 0.9</td>
</tr>
<tr>
<td>75</td>
<td>4049 ± 2130</td>
<td>0.60 ± 0.29</td>
<td>8.0 ± 5.05</td>
<td>1.73 ± 1.1</td>
</tr>
<tr>
<td>67.5</td>
<td>2878 ± 942</td>
<td>0.67 ± 0.19</td>
<td>16.3 ± 9.2</td>
<td>ND</td>
</tr>
</tbody>
</table>

* AUCs Doxorubicin: Dox, doxorubicin.

Table 6 Tumor responses of Phase II evaluable patients

<table>
<thead>
<tr>
<th>Tumor histology</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
</tr>
<tr>
<td>Non-GIST</td>
<td>15</td>
</tr>
<tr>
<td>GIST</td>
<td>11</td>
</tr>
<tr>
<td>Overall</td>
<td>26</td>
</tr>
</tbody>
</table>

of study treatment) is within the 5–20% range expected after cumulative doxorubicin doses of ≥450 mg/m² (43, 44). However, from this small study it would be premature to draw any conclusion about the cardiac safety of the VX-710/doxorubicin combination.

**Efficacy.** Twenty-six patients were evaluable for treatment response. Three patients were not evaluable for the following reasons: 1 patient described above with extensive hepatic metastases developed sepsis and died during cycle 1; progression on prior doxorubicin therapy could not be documented for another patient; and a histological diagnosis of sarcoma could not be confirmed on subsequent pathological review at the treating institution for a patient with an undifferentiated epithelioid tumor. Response results are summarized in Table 6 for all patients and by subgroups of patients who had either GIST or all other sarcoma histologies, including leiomyosarcomas. Overall, 2 patients achieved partial responses. Both patients had leiomyosarcomas (1 uterine with abdominal metastasis and 1 retroperitoneal with liver metastasis) and had progressive disease after two cycles of 75 mg/m² doxorubicin before starting VX-710/doxorubicin therapy. The patient with uterine leiomyosarcoma received seven treatment cycles but discontinued because of maximum doxorubicin exposure with >70% reduction in tumor burden. The response was sustained for 1 year. The other patient had six treatment cycles and achieved >85% tumor shrinkage that enabled surgical resection with curative intent. The patient had recurrent disease 10 months later.

Among the subgroups, 10 of 11 patients with GIST had progressive disease after two treatment cycles, whereas 7 of the 15 patients with non-GIST had stabilization of their disease for a minimum of 12 weeks. The characteristics of these patients are summarized in Table 7. All these patients with stable disease started therapy with VX-710/doxorubicin within 3–6 weeks of progression on doxorubicin. The median progression-free intervals for all 26 evaluable patients, the subgroup of patients with GIST, and the subgroup of patients with non-GIST sarcomas were 6.3 weeks, 6.1 weeks, and 13.6 weeks, respectively (Fig. 2). Among the eight patients treated in Phase I who are not included in the efficacy analysis above, one patient withdrew consent after treatment cycle 1 and two patients progressed after two and three treatment cycles, respectively. Five other patients received four or five treatment cycles with stable disease, and one of these five patients with GIST and liver metastases achieved a minor response (40% tumor shrinkage) that enabled surgical resection that was not possible before study treatment.
DISCUSSION

This Phase I/II study evaluated the safety, pharmacokinetics, and efficacy of VX-710 combined with doxorubicin in patients with anthracycline-resistant/refractory STS. Phase I established the combination of VX-710 120 mg/m²/h administered by CIV infusion for 72 h and doxorubicin 60 mg/m² as the MTD for the efficacy portion of the study. Myelosuppression was dose-limiting at doxorubicin doses of 67.5 and 75 mg/m² in combination with VX-710, and was increased, although within acceptable limits, at the Phase II dose of 60 mg/m². VX-710 does not appear to significantly affect doxorubicin pharmacokinetics. This conclusion is consistent with results of a study with VX-710 and mitoxantrone in which mitoxantrone clearance and AUC were similar in prostate cancer patients given mitoxantrone alone followed by mitoxantrone with VX-710 (47).

On the basis of an “intent to treat” analysis, there were 2 partial responses and 19 early progressions among 29 patients, which did not meet our preset criteria for pursuing the second stage of the study. However, our patient population differs substantially from that of other sarcoma patients entering Phase II studies of investigational agents. All patients had to have documented progression (>25% increase in the sum of the products of measurable disease and/or appearance of new lesions) in the previous 8 weeks before the start of VX-710 therapy and, for patients with non-GIST sarcomas, progression must have occurred on doxorubicin-based chemotherapy. In this highly resistant population of non-GIST sarcoma subtypes, two patients achieved partial responses (13%) and 7 (47%) had disease stabilization, with a median progression-free interval of 13.6 weeks. Although it is acknowledged that the division of the study population into GIST and non-GIST sarcomas was a post hoc exploratory analysis (see “Patients and Methods”), this degree of activity, demonstrating that VX-710 resensitized refractory patients to doxorubicin and restored doxorubicin anti-tumor activity to some patients with non-GIST sarcomas, indicates that VX-710/doxorubicin therapy merits further exploration in this group of sarcomas.

Ifosfamide and doxorubicin are the only drugs with proven single-agent activity (>20%) in STS (48–51), and much research has focused on improving the therapeutic ratio of these agents. Unlike doxorubicin, the cytotoxic activity of ifosfamide is not influenced by P-gp or MRP1 expression, and theoretically, resistance may be overcome by dose escalation. Single arm studies of high dose ifosfamide (9–18 g/m²) in chemotherapy-naïve or pretreated patients have reported response rates of 16%–38%, including occasional responses in patients who failed lower dose ifosfamide, but with substantial toxicity at the highest doses (52–55). An early Phase II trial (56) with STS patients randomized to 45, 60, or 75 mg/m² doxorubicin suggested a steep dose-response relationship. In a randomized European Organization for Research and Treatment of Cancer study, epirubicin, an active analogue of doxorubicin, showed similar activity with respect to response rates, duration of response, time to progression, and survival, with less toxicity when the two agents were given at equimolar doses (75 mg/m²; Ref. 57). However, a more recent European Organization for

Table 7 Characteristics of patients with disease stabilization

<table>
<thead>
<tr>
<th>Parameters</th>
<th>PT 11-06</th>
<th>PT 12-01</th>
<th>PT 11-09</th>
<th>PT 1113</th>
<th>PT 11-14</th>
<th>PT 11-07</th>
<th>PT 11-24</th>
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<tr>
<td>Histology</td>
<td>Leio</td>
<td>Neuro</td>
<td>MS</td>
<td>Lipo</td>
<td>Syn</td>
<td>Leio</td>
<td>Leio</td>
<td>GI</td>
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<tr>
<td>Prior cumulative DOX (mg/m²)</td>
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<td>225</td>
<td>120</td>
<td>125</td>
<td>130</td>
<td>120</td>
<td>120</td>
<td>120</td>
</tr>
<tr>
<td>Prior tumor progression (%)</td>
<td>23</td>
<td>141</td>
<td>32</td>
<td>29</td>
<td>120</td>
<td>0</td>
<td>120</td>
<td>60</td>
</tr>
<tr>
<td>Metastases</td>
<td>stomach</td>
<td>sm bowel</td>
<td>Ii thigh</td>
<td>rt peritoneum</td>
<td>lung</td>
<td>stomach</td>
<td>peritoneum</td>
<td>abdomen, kidney</td>
</tr>
<tr>
<td>Treatment cycles (n)</td>
<td>4</td>
<td>5</td>
<td>5</td>
<td>None</td>
<td>3</td>
<td>2</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Response status</td>
<td>SD</td>
<td>SD</td>
<td>SD</td>
<td>MR; 5% decrease</td>
<td>PD</td>
<td>10% decrease</td>
<td>SD</td>
<td></td>
</tr>
</tbody>
</table>

*Leio, leiomyosarcoma; Neuro, neurogenic sarcoma; MS, malignant schwannoma; Lipo, liposarcoma; Syn, synovial sarcoma; GI, gastrointestinal; DOX, doxorubicin; sm, small; Ii, left; rt, right; SD, stable disease; PD, progression of disease maintained for ≥6 weeks; MR, minor response <50% decrease.

Fig. 2 Progression-free interval for patients with GIST and non-GIST sarcomas during treatment with VX-710 plus doxorubicin.
Research and Treatment of Cancer study failed to demonstrate superiority of high-dose epirubicin (150 mg/m² on day 1 or 50 mg/m² on days 1, 2, and 3) compared with 75 mg/m² doxorubicin (58). Studies with several other potentially less toxic analogues of doxorubicin have shown disappointing activity in STS patients (59–62).

Studies with liposomal formulations are another approach to increasing the activity of anthracyclines in sarcoma patients. Preclinical studies with liposomal doxorubicin demonstrated selective, increased delivery of doxorubicin to tumor tissue compared with normal tissues (e.g., heart, bone marrow, and gastrointestinal tract), attributed to the leaky neovasculature and poor lymphatic system in solid tumor tissue (63, 64). In addition, liposomal formulations of doxorubicin restore drug accumulation and partially overcome MDR pump-mediated drug resistance in studies of P-gp-resistant cell lines in vitro (64, 65). Clinical studies have also demonstrated a significant increase in the plasma circulation time of liposomal doxorubicin (e.g., 55-h elimination half-life) compared with free doxorubicin (66). The therapeutic benefit achieved from the altered pharmacokinetic behavior and biodistribution of liposomal doxorubicin has been demonstrated in results of studies in patients with Kaposi’s sarcoma and refractory ovarian carcinoma (67–69). However, studies of liposomal doxorubicin in STS have produced conflicting results. Three small Phase I/II studies in either untreated patients (70, 71) or doxorubicin-treated patients (72) failed to detect any objective responses. In contrast, another study in heavily pretreated, anthracycline-refractory patients reported three responses among 25 patients (73). Finally, a randomized study in chemotherapy-naïve patients did show an improved safety profile for liposomal doxorubicin with less severe neutropenia and gastrointestinal toxicities, but the objective response rate was comparable with single-agent doxorubicin (74).

Several newer agents active in other settings have been evaluated in STS. These studies tested agents that are (paclitaxel, docetaxel, topotecan, irinotecan; Refs. 75–83) or are not (tomudex, temozolomide, gemcitabine; Refs. 84–87) influenced by P-gp or MRP1 expression. Results of these studies failed to demonstrate significant activity for any of the agents in patients with STS.

The finding that VX-710/doxorubicin therapy was not active against GIST is an interesting observation. These sarcomas are the predominant gastrointestinal mesenchymal tumors of the stomach, small intestine, or esophagus. Histologically, GIST generally bear little resemblance to differentiated smooth muscle cells and usually express CD34, but stain negative for desmin and smooth muscle actin in contrast to leiomyosarcomas (88). Recently, expression of CD117 has been shown to be a more specific marker for GIST (89). CD117 encodes the proto-oncogene receptor tyrosine kinase c-kit, which binds stem cell factor and appears to be functionally important in proliferation and differentiation of select germ cells including the interstitial cells of Cajal.

Leiomyosarcomas do not express c-kit (89). Hirota et al. (90) and Lasota et al. (91) have identified mutations in the c-kit gene from patients with GIST in exon 11, the region of the gene coding for the juxtamembrane and tyrosine kinase domains. The mutations result in a “gain-of-function” and were found in both sporadic and familial GIST, suggesting that the c-kit mutations and constitutive activation of the tyrosine kinase are associated with the tumorigenesis of GIST. This is also supported by the dramatic activity of the selective inhibitor of tyrosine kinase phosphorylation, STI571 (Imatinib mesylate) in GIST (88–91).

The lack of activity observed with the combination of VX-710 with doxorubicin in this study suggests that either constitutive activation of c-kit or alternative biochemical mechanisms of drug resistance render GIST nonresponsive to doxorubicin cytotoxicity. Nonetheless, it is important to continue to study these mechanisms, because even STI571 has not yielded complete responses in any patients with GIST, and identification of resistance mechanisms will remain an important and relevant area of research. Additionally, it is of note that STI571 itself appears to be a substrate for efflux pumps such as the product of MDR1 (92–96).

In conclusion, doxorubicin-based chemotherapy for STS results in objective responses in only 25–30% of patients, and responses are often of short duration. Expression of MDR1/P-gp and MRP1 by tumor cells has been correlated with resistance to chemotherapy and poor clinical outcome in several studies. Evaluation of high-dose chemotherapy regimens and studies with anthracycline analogues, liposomal doxorubicin, and many newer chemotherapy agents has not identified a clearly superior treatment option. Thus, a treatment strategy that may overcome or prevent the emergence of drug resistance is urgently needed. This study demonstrated that VX-710 can resensitize strictly defined, anthracycline-resistant sarcomas to doxorubicin, resulting in a 3.4-month progression-free interval and objective responses in 13% of patients with non-GIST sarcomas. These findings support the proof of principal for MDR reversal as a feasible treatment strategy for patients with anthracycline-refractory sarcomas. Additional studies that incorporate VX-710 in combination with or sequential to doxorubicin/ifosfamide regimens, or with liposomal doxorubicin may be appropriate in a refractory patient setting. More importantly, the established safety of VX-710 with 60 mg/m² doxorubicin and unaltered pharmacokinetics support studies that incorporate VX-710 in first-line therapy for STS. Such studies could test the ability of VX-710 to increase the objective response rate and extend the progression-free interval, which might translate into improved survival and quality of life for patients with non-GIST sarcomas.

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Safety and Efficacy of the Multidrug-Resistance Inhibitor Biricodar (VX-710) with Concurrent Doxorubicin in Patients with Anthracycline-resistant Advanced Soft Tissue Sarcoma

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