

# Safety and Efficacy of the Multidrug Resistance Inhibitor Incel (Biricodar; VX-710) in Combination with Paclitaxel for Advanced Breast Cancer Refractory to Paclitaxel

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## ABSTRACT

**Purpose:** VX-710 (biricodar, Incel) restores drug sensitivity to P-glycoprotein (*MDR1*) and multidrug resistance-associated protein (*MRP1*)-expressing cells. This Phase II study evaluated the safety/tolerability, pharmacokinetics, and efficacy of VX-710 plus paclitaxel in women with locally advanced or metastatic breast cancer who were refractory to prior paclitaxel therapy.

**Experimental Design:** Eligible patients had paclitaxel-refractory disease defined as progressive disease after a minimum of two cycles of paclitaxel (weekly or 3-week

schedule) or relapsed/progressive disease within 6 months of prior paclitaxel therapy. Patients received 80 mg/m<sup>2</sup> paclitaxel over 3 h starting 4 h after initiation of a 24-h continuous i.v. infusion of 120 mg/m<sup>2</sup>/h VX-710. Cycles were repeated every 3 weeks.

**Results:** Thirty-seven patients received study treatment and 35 were evaluable for response. VX-710 + paclitaxel therapy was generally well tolerated. Myelosuppression was the principal toxicity, with a median nadir ANC cycle 1 of  $0.76 \times 10^9$  cells/liter and a 40% overall incidence of Grade 4 neutropenia. Nonhematological side effects (asthenia, paresthesia, headache, myalgia, nausea, and diarrhea) were generally mild to moderate and reversible. Paclitaxel AUC ( $16.8 \pm 5.0 \mu\text{g} \times \text{h/ml}$ ) and clearance ( $5.1 \pm 1.3$  liters/h/m<sup>2</sup>) during the first treatment cycle were comparable with standard 175 mg/m<sup>2</sup> paclitaxel administered in a 3-h schedule. Four patients achieved partial responses (three of the four had progressive disease on prior paclitaxel) with a mean response duration of 5.5 months.

**Conclusions:** The 11.4% (4 of 35) objective response rate observed in this study suggests that VX-710 can resensitize a subgroup of paclitaxel-refractory patients to paclitaxel. The safety and pharmacokinetics of the VX-710/paclitaxel regimen support further evaluation in breast cancer patients with initial paclitaxel therapy to prevent emergence of the MDR phenotype in recurrent disease.

## INTRODUCTION

Patients with locally advanced or metastatic breast cancer generally achieve objective responses and substantial palliation after treatment with the currently available chemotherapies. An anthracycline-based regimen or paclitaxel is presently the most common regimen for patients with advanced disease, although treatment with docetaxel, combination or sequential treatment with an anthracycline and a taxane, or therapy with paclitaxel and recombinant humanized anti-HER2 monoclonal antibody are currently alternative treatment approaches (1, 2).

The activity of paclitaxel in advanced breast cancer is well documented. Depending on the paclitaxel dose, infusion schedule, and patient demographics (*e.g.*, chemotherapy-naïve, previously treated, or anthracycline-resistant), response rates range from 23 to 56% (3–10). Similarly, median times to disease progression and survival range from 4 to 9 months, and 9.5 to 21 months, respectively (3–10). Despite the activity of paclitaxel observed in these and other studies, responses are often of short duration, and relapsed patients frequently develop acquired drug resistance, resulting in diminished sensitivity to subsequent treatments. Furthermore, a significant percentage of patients (>40%) are intrinsically resistant to paclitaxel therapy.

There are several potential mechanisms for intrinsic or acquired resistance to paclitaxel (11). Paclitaxel, a diterpene

natural product, acts by binding to the  $\beta$ -subunit of polymerized tubulin (12, 13), promoting assembly of microtubules and inhibiting their depolymerization, which is necessary for mitosis. The ultimate cytotoxic effect of paclitaxel results from the induction of apoptosis (14, 15). MDR<sup>4</sup> associated with expression of the *MDR1* gene encoding P-gp is one potential mechanism for intrinsic or acquired drug resistance. P-gp is an ATP-dependent drug efflux pump that transports paclitaxel, docetaxel, doxorubicin, and other natural product cytotoxic agents from cells that express P-gp (16). Tumor cell resistance to paclitaxel may also result from several mechanisms, including insufficient cellular accumulation, mutations in the  $\beta$ -tubulin binding site, altered  $\beta$ -tubulin isotype expression, or defective apoptotic signaling (17).

Many studies have evaluated expression of MDR1 mRNA or P-gp in breast cancer specimens collected from patients at diagnosis or at relapse after chemotherapy. Trock *et al.* (18) recently conducted a meta-analysis of 31 studies that evaluated tumor specimens from breast cancer patients. Results showed that 41% of breast tumors expressed MDR1 mRNA or P-gp, but with significant heterogeneity across the individual studies, attributable largely to analytical methodology. In the same report, data from nine studies that included patients treated with cytotoxic agents associated with the MDR phenotype showed a significant increase in the proportion of patients with tumors expressing P-gp subsequent to chemotherapy. Furthermore, patients with P-gp expressing tumors were 3-fold less likely to achieve an objective response after treatment compared with patients whose tumors did not express P-gp. Another study of 359 specimens detected P-gp expression in only 11% of untreated patients compared with 30% of treated patients, and the extent of P-gp expression correlated with *in vitro* resistance to doxorubicin and paclitaxel in functional assays (19).

Several investigations have used the radiopharmaceutical agent <sup>99m</sup>Tc-sestamibi (which is a substrate for P-gp transport; Ref. 20) in imaging studies with breast cancer patients to identify a relationship between <sup>99m</sup>Tc-sestamibi accumulation and retention in tumor tissue with expression of P-gp and response to chemotherapy. Three studies reported increased retention of <sup>99m</sup>Tc-sestamibi in breast tumor lesions that expressed low levels of P-gp compared with a higher <sup>99m</sup>Tc-sestamibi washout rate and reduced retention in lesions expressing high levels of P-gp (21–23). Carmiello *et al.* (24) recently reported similar results in locally advanced breast cancer patients. Rapid tumor <sup>99m</sup>Tc-sestamibi washout was observed in 15 of 17 (88%) patients who failed to respond to neoadjuvant epirubicin chemotherapy compared with a poor treatment response in only 8 of 22 (36%) patients with prolonged tumor retention of <sup>99m</sup>Tc-sestamibi. In conclusion, studies that used mRNA detection or immunohistochemistry methods, *ex vivo* functional assays, or *in vivo* tumor imaging all show a strong association between therapy with MDR drugs like doxorubicin and paclitaxel, intrinsic or acquired expression of P-gp, reduced tumor cell drug

retention, and a poor treatment response in breast cancer patients.

VX-710 (biricodar, Incel) is a novel compound that binds directly to P-gp, blocking its efflux activity (25). VX-710 concentrations of 0.5–2.5  $\mu$ M are sufficient to fully restore sensitivity of P-gp-expressing cells to the cytotoxic action of paclitaxel and other cytotoxic drugs associated with the MDR phenotype (25, 26). Results of a Phase I study with VX-710 administered as a single agent and in combination with paclitaxel indicated that VX-710 is well tolerated, with VX-710 steady-state concentrations of  $\sim$ 4.5  $\mu$ g/ml ( $\sim$ 7.5  $\mu$ M) sustained at the 120 mg/m<sup>2</sup>/h dose level chosen for Phase II evaluation (27). VX-710 did reduce paclitaxel clearance, such that a paclitaxel dose of 80 mg/m<sup>2</sup> resulted in exposure comparable with a standard regimen of 175 mg/m<sup>2</sup> paclitaxel administered as a 3-h schedule. Myelosuppression was the principal toxicity with the 120 mg/m<sup>2</sup>/h VX-710 + 80 mg/m<sup>2</sup> paclitaxel dose combination (27).

We initiated a multicenter, open-label, Phase II study to evaluate VX-710 plus paclitaxel in patients with locally advanced or metastatic breast cancer refractory to paclitaxel. The objectives of the study were to (a) evaluate the safety and tolerability of the VX-710 + paclitaxel therapy in this patient population, (b) further characterize the pharmacokinetics of paclitaxel in combination with VX-710, and (c) determine the efficacy of VX-710 + paclitaxel in patients with advanced breast cancer refractory to paclitaxel therapy.

## PATIENTS AND METHODS

**Patient Population.** Patients eligible for this study were at least 18 years of age with histologically confirmed, locally advanced, or metastatic carcinoma of the breast unsuitable for definitive surgical resection. Paclitaxel-refractory disease was defined as progressive disease after a minimum of two doses of paclitaxel given weekly or every 3 weeks, or relapse within 6 months of prior paclitaxel therapy; no more than three prior chemotherapy regimens; bidimensionally measurable lesions ( $\geq$  1 lesion  $\geq$  1 cm  $\times$  1 cm); and an Eastern Cooperative Oncology Group performance status of  $\leq$  2. Patients had adequate bone marrow and major organ function, defined as an absolute granulocyte count (ANC) of  $\geq$  1500 cells/ $\mu$ l and a platelet count of  $\geq$  100,000 cells/ $\mu$ l; serum creatinine within normal limits; bilirubin within normal limits; and aspartate aminotransferase or alanine aminotransferase  $\leq$  1.5 times the upper limit of normal, or  $\leq$  2.5 times the upper limit of normal for patients with documented liver metastasis. Patients with brain metastases had to have clinically controlled neurological symptoms. Pregnant or breast-feeding women or women with childbearing potential who were unwilling or unable to use effective contraception were ineligible. Additional exclusion criteria included prior bone marrow transplantation or radiation to  $>$ 33% of the marrow-containing bone; concurrent serious infections; any unstable or serious concurrent medical condition; a history of prior malignant neoplasms (except patients who have received adequate treatment for basal cell, squamous cell skin cancer, or any *in situ* carcinoma) unless disease-free for  $\geq$  5 years; or a known hypersensitivity to paclitaxel, Cremophor, or any component of VX-710. Patients using any investigational drug or device in the

<sup>4</sup> The abbreviations used are: MDR, multidrug resistance; P-gp, P-glycoprotein; ANC, absolute neutrophil count.

28 days before study entry were also excluded. Patients were ineligible if taking any of the following drugs at the time of enrollment: anastrozole, cimetidine, ethynyl estradiol, megestol acetate, phenytoin (Dilantin), quinidine, tacrolimus, toremifene citrate, tamoxifen, astemizol, cyclosporine, ketoconazole, phenobarbital, quercetin, rifampin, terfenadine, and troleandomycin. Informed consent was obtained according to local Institutional Review Board requirements.

**Study Design.** This was an open-label, single-arm, multicenter study with a two-stage design. Because the response of paclitaxel-refractory patients to rechallenge with paclitaxel was assumed to be near zero, a response rate of 15% was considered to be clinically meaningful in this refractory patient population. Stage 1 and Stage 2 sample sizes were determined based on the 95% confidence interval for the estimated response rate ( $p$ )  $\pm$  1.96 times its SE, the square root of ( $p[1 - p]/n$ ). Thus, if no objective responses were observed among the first 19 patients in Stage 1 of the study, it could be concluded that the rejection error was 5% for a response rate of 15% (28). If at least one response were to be observed among the first 19 patients, then Stage 2 would enroll up to 58 additional patients. Enrollment in the study was stopped at 38 patients based on accrual and the number of responses observed.

VX-710 was administered by continuous i.v. infusion for 24 h at a dose of 120 mg/m<sup>2</sup>/h. Paclitaxel (80 mg/m<sup>2</sup> i.v.) was administered over a 3-h period starting 4 h after beginning the VX-710 infusion. All patients received 20 mg of dexamethasone i.v., 50 mg of diphenhydramine i.v., and 50 mg of ranitidine i.v. starting 30 min before initiation of the paclitaxel infusion. The regimen was repeated every 21 days until evidence of disease progression, intolerable adverse events, or withdrawal of patient consent.

VX-710 was provided by Vertex Pharmaceuticals 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 mixed aseptically with 0.9% normal saline or dextrose 5% in water for administration with a 250-ml polyvinyl chloride infusion set.

**Pretreatment and Follow-up Evaluations.** Patient history, physical examination, assessment of baseline symptoms, an EKG, and routine laboratory studies, including a pregnancy test for women of childbearing potential, were required within 2 weeks before study registration. Baseline tumor measurements were obtained (computerized tomography, X-ray, or physical measurement) within 4 weeks before study entry. Complete blood counts were performed weekly after study drug administration. A physical examination including performance status, weight, temperature, blood pressure, and pulse was conducted before each subsequent course of therapy and at the end of treatment. A complete blood count, platelet count, and clinical chemistries (to include at least total protein, creatinine, aspartate aminotransferase or alanine aminotransferase, total bilirubin, alkaline phosphatase, and electrolytes) were performed within 3 working days before beginning each course of therapy. Tumor measurements (computerized tomography, X-ray, or physical measurement) were obtained after every 2 courses for six courses and after every three courses thereafter.

**Pharmacokinetic Sampling and Assays.** A limited blood sampling schedule was used during cycle 1 for a population pharmacokinetics analysis to assess the disposition of paclitaxel. Plasma samples were drawn from all patients within 30 min of completing the paclitaxel infusion, and at 1–2 h, 4–7 h, 13–15 h, and 17–33 h after the end of the paclitaxel infusion. Plasma samples were stored at  $-70^{\circ}\text{C}$  before analysis.

For the determination of paclitaxel concentrations, 2-ml plasma samples were subjected to a double liquid-liquid extraction procedure with 8 ml of methyl-*t*-butyl ether. The dry residue was reconstituted in 1 ml of 40% aqueous acetonitrile and extracted once again through a 500-mg C18 Waters Sep-Pak (Waters Corp., Milford, MA) solid phase extraction cartridge, and the sample was eluted with 5 ml of acetonitrile and then evaporated to dryness under nitrogen at ambient temperature. The dry residue was reconstituted in 200  $\mu\text{l}$  of mobile phase for high-performance liquid chromatography analysis. An isocratic reverse-phase separation on a Phenomenex (Torrance, CA) I B-sil C8 column with UV detection at 227 nm was used to quantitate paclitaxel concentrations. The mobile phase for paclitaxel analysis consisted of 50% acetate buffer (pH 5.0), 35% acetonitrile, and 15% methanol. This assay has a detection limit of 4.5 ng/ml, a quantitation limit of 15 ng/ml, linearity between 5 and 2000 ng/ml, precision of 7.6–16.2% RDS, and an accuracy  $> 85\%$ .

**Population Pharmacokinetic Analysis.** Pharmacokinetic analysis was performed using the NONMEM (Version V) software package (NONMEM Project Group, University of California, San Francisco, CA) for nonlinear mixed effect modeling of the data. A log-linear plot of the raw data revealed that a two- or three-compartment pharmacokinetic model would be required to best fit the data. Thus, for the basic model, a two-compartment model was used with interindividual variability ( $\eta$ ) on all four basic parameters, [volumes of the central (V1) and peripheral compartments (V2), total clearance (CL), and distributional clearance (Q)]. A combination of an additive and a proportional error model was used to model residual variability. The infinitesimal value of the variance ( $\omega^2$ ) associated with the  $\eta$  on V1 indicated that this parameter could be eliminated. The final model included a power model for weight on clearance.

**Response Criteria and End Points.** The primary end point of the study was tumor response, with response duration and performance status as secondary end points. Patients were considered evaluable for response assessment if they received at least two doses of therapy and completed the first reevaluation or if disease progression (*e.g.*, brain metastasis) was documented at any time point before the first reevaluation. The following standard criteria were used to classify tumor response. A complete response (CR) was defined as the total disappearance of all measurable and evaluable evidence of cancer confirmed by 2 measurements at least 4 weeks apart; a partial response (PR) was defined as a reduction of at least 50% in the sum of the products of bidimensionally measurable lesions from 2 measurements at least 4 weeks apart; progressive disease (PD) was defined as an increase of more than 25% in the sum of the products of the bidimensional measurements and/or the appearance of new lesions; stable disease (SD) was classified as a response that did not meet criteria for a tumor response or

Table 1 Patient demographic and disease characteristics

Number of patients	38
Median age (range)	49 (28–68)
ECOG performance status	
0	10
1	25
2	3
Prior adjuvant chemotherapy ( <i>n</i> )	24
Hormonal therapy	12
Paclitaxel as 1st-line chemotherapy for metastatic disease	21
Paclitaxel as 2nd- or 3rd-line chemotherapy for metastatic disease	17
Previous best response to most recent paclitaxel	
Complete response (CR)	1
Partial response (PR)	4
Stable disease (SD)	11
Progressive disease (PD)	22

progression. Response duration was calculated from the time objective response criteria were first met until the date of documented progression.

## RESULTS

**Patients.** A total of 38 patients were enrolled in this study. Patient demographics, prior treatment history, and disease characteristics are summarized in Table 1. Thirty seven patients had metastatic disease. Twenty-four patients had received adjuvant chemotherapy and 21 patients received paclitaxel as their first therapy for advanced disease, whereas 17 patients received paclitaxel as second- or third-line therapy. Twenty-two of the patients demonstrated primary resistance to paclitaxel (*e.g.*, had progressive disease while receiving paclitaxel). Eleven patients had stable disease as the best response to their prior paclitaxel therapy; only 5 of the 38 patients achieved a partial ( $n = 4$ ) or complete response ( $n = 1$ ) to prior paclitaxel therapy, but had progressive or relapsed disease within 6 months. A total of 132 cycles of VX-710 + paclitaxel therapy were administered to patients in this study, with a range of 1–18 cycles and an average of 3.5 cycles/patient.

**Safety and Tolerability.** Thirty-seven patients are assessable for safety (one patient assigned a study number withdrew consent before administration of study treatment). The combination of 120 mg/m<sup>2</sup>/h VX-710 with 80 mg/m<sup>2</sup> paclitaxel was generally well tolerated in this patient population. Myelosuppression was the primary treatment toxicity. Cycle 1 WBC and ANC are summarized in Table 2. WBC and ANC nadirs generally occurred between 10 and 14 days after treatment (median WBC and ANC values of 2.1–2.8 and 0.76–0.79 × 10<sup>3</sup> cells/mm<sup>3</sup>, respectively), and counts returned to baseline values by week 3 (Table 2). Grade 4 neutropenia was observed in 27 (73%) patients and in 40% (52 of 132) of treatment cycles. Overall, there was no evidence of prolonged or cumulative myelosuppression.

Eleven patients received prophylactic colony-stimulating factor starting in cycle 2 or subsequent cycles and 2 patients had paclitaxel reduced to 70 mg/m<sup>2</sup> because of neutropenia experi-

enced in cycle 1. Ten patients (27%) experienced 11 episodes of febrile neutropenia (8% overall incidence). All episodes resolved without complications. Although there was no consistent effect of the VX-710/paclitaxel regimen on RBCs or hemoglobin, 12 patients experienced anemia (Grade 2 for 9 patients and Grade 3 for 3 patients) and 5 patients required transfusions with packed RBCs. The VX-710/paclitaxel regimen had only a mild effect on platelet counts.

Nonhematological toxicities were generally mild to moderate (Grade 1 or 2) in severity and reversible. Asthenia, nausea, fever, myalgia, paresthesia, and headache were the most commonly reported adverse effects (Table 3). Eight episodes of Grade 3 toxicity (asthenia, fever, paresthesia, nausea) and 1 episode of fever were reported in five patients (Table 3). VX-710 did not appear to exacerbate peripheral neuropathy in patients who enrolled with baseline symptoms of peripheral neuropathy attributable to their prior paclitaxel therapy.

**Pharmacokinetic Analysis.** Paclitaxel pharmacokinetics were determined from blood samples collected from 31 patients during cycle 1. A plot of the paclitaxel plasma concentrations for individual patients determined from sparse sampling time points and a plot of the simulated paclitaxel plasma concentration-time curve based on median pharmacokinetic parameter estimates is presented in Fig. 1. The mean paclitaxel plasma concentration peaked at approximately 2500 ng/ml and was sustained above 50 ng/ml for approximately 38 h. Pharmacokinetics parameter estimates for the paclitaxel area under the concentration-*versus*-time curve (AUC), volume of distribution at steady state ( $V_{ss}$ ), systemic clearance ( $CL_s$ ), and half-life ( $t_{1/2\beta}$ ) determined by population pharmacokinetic modeling are summarized in Table 4. Parameter estimates for breast cancer patients treated with 80 mg/m<sup>2</sup> paclitaxel in combination with 120 mg/m<sup>2</sup>/h VX-710 are similar to those obtained by intensive pharmacokinetics sampling in the phase I study (27) and consistent with values in previous studies with 175 mg/m<sup>2</sup> paclitaxel (Table 4; Refs. 29 and 31). The principal difference, as expected, is an approximately 50% reduction in paclitaxel clearance (5.1 ± 1.3 liters/h/m<sup>2</sup>) compared with single-agent paclitaxel (9.6–12.6 liters/h/m<sup>2</sup>; Table 4), because clearance is estimated from the AUC-dose relationship. Thus, VX-710 plus paclitaxel 80 mg/m<sup>2</sup> results in a paclitaxel exposure comparable with 175 mg/m<sup>2</sup> paclitaxel administered in a 3-h infusion schedule.

**Efficacy.** Among the 37 patients who received study treatment, 35 patients were evaluable for response assessment. Two patients were not evaluable for the following reasons: one patient with a history of chronic obstructive pulmonary disease developed pneumonia during cycle 1 and withdrew consent, and independent radiology review could not identify bidimensional indicator lesions for a second patient. Four patients achieved confirmed partial responses, for an objective response rate of 11.4%. Response durations were 1.5, 3.5, 5.5, and 13 months, with a median response duration of 5.8 months. Three of the four patients had exhibited progressive disease during their paclitaxel treatment before this study and then had an objective response to VX-710 + paclitaxel therapy. The cycle 1 paclitaxel AUC values for these four patients (9.9, 13.8, 15.6, and 19.0 μg ×

Table 2 Summary of cycle 1 WBC counts and ANC's

Parameter	Statistic	Cell counts ( $\times 10^3/\text{mm}^3$ )			
		Baseline	Week 1	Week 2	Week 3
WBC	<i>N</i>	38	35	34	6
	Mean	6.48	2.27	3.66	7.38
	Median	6.25	2.1	2.8	5.86
	SD	2.73	1.12	2.42	4.12
	Range	2.5–17.0	0.7–6.6	1.0–10.2	2.2–10.2
ANC	<i>N</i>	36	33	32	6
	Mean	4.45	0.87	1.66	5.12
	Median	4.07	0.79	0.76	3.93
	SD	2.58	0.58	1.98	3.04
	Range	1.6–15.7	0.1–2.5	0.1–7.1	2.0–9.6

Table 3 Most frequent nonhematological toxicities by severity

Adverse event	Number (%) of patients ( <i>N</i> = 38)		
	Total	Grade 1/2	Grade 3
Asthenia	25 (68)	23	2
Nausea	17 (46)	16	1
Myalgia	15 (40)	15	
Fever	15 (40)	14	1
Paresthesia	14 (38)	13	1
Headache	14 (38)	14	
Arthralgia	8 (22)	8	
Hypesthesia	8 (22)	8	
Vomiting	7 (19)	7	

h/ml) are similar to the median estimate of  $16.8 \mu\text{g} \times \text{h/ml}$  determined for the study population. Two additional patients had durable minor responses, with tumor reductions of 38% and 45%, respectively. Prior treatment history, site(s) of disease, and summary of study treatment and response for the six patients with evidence of antitumor activity are summarized in Table 5.

## DISCUSSION

This Phase II study evaluated the safety, pharmacokinetics, and efficacy of VX-710 combined with paclitaxel in women with advanced paclitaxel refractory breast cancer. Our results demonstrate that it is possible to induce objective responses in strictly defined paclitaxel-refractory patients by addition of the MDR inhibitor VX-710 to relatively low-dose paclitaxel therapy. Four of 35 evaluable patients (11.4%) achieved objective responses, and 3 of these 4 patients had experienced disease progression during their paclitaxel therapy before the start of VX-710 and paclitaxel treatment in this study.

The pharmacokinetics analysis summarized in Table 4 shows that the  $120 \text{ mg/m}^2/\text{h}$  VX-710 +  $80 \text{ mg/m}^2$  paclitaxel regimen provides a paclitaxel exposure (AUC of  $16.8 \pm 5.0 \mu\text{g} \times \text{h/ml}$ ;  $t_{1/2\beta}$  of 11.6 h) comparable with the standard  $175 \text{ mg/m}^2$  paclitaxel on a 3-h schedule (29–31). The pharmacokinetic interaction of VX-710 with paclitaxel did result in an approximately 50% reduction in paclitaxel clearance, in agreement with phase I study results. However, according to the simulation in Fig. 1, the paclitaxel plasma concentration was sustained above 50 nm for approximately 38 h compared with

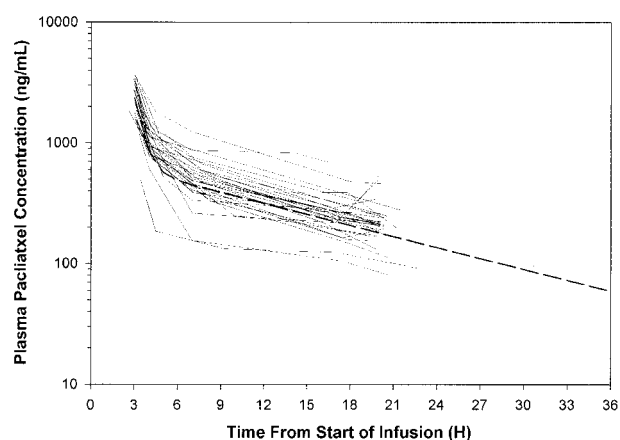


Fig. 1 Individual patient paclitaxel plasma concentrations from actual sparse sampling time point measurements (—) and a simulated paclitaxel plasma concentration-time curve (----) determined from median pharmacokinetic parameter estimates.

28 h in the previous Phase I study with the same VX-710 and paclitaxel dose combination (27). This difference may reflect the small number of patients ( $n = 5$ ) in the Phase I analysis. One possible explanation for the extended duration above 50 nm may be an increase in the distribution of paclitaxel in the peripheral compartment, attributable at least in part to longer retention in P-gp-expressing tissues. Such a hypothesis is consistent with restored antitumor activity of paclitaxel in paclitaxel-refractory patients resulting from VX-710 inhibition of paclitaxel efflux and its increased retention in tumor tissue.

VX-710 + paclitaxel combination therapy was generally safe and well tolerated. Neutropenia was the predominant treatment toxicity, with a 40% overall incidence of Grade 4 neutropenia. Neutropenia was also the dose-limiting toxicity observed in the Phase I study with VX-710 and paclitaxel in a more heavily pretreated population (27). The VX-710 + paclitaxel regimen did not cause cumulative myelosuppression and had only a mild effect on hemoglobin or platelet counts. Although patients were treated with an AUC-normalized paclitaxel dose, the incidence of Grade 4 neutropenia observed with VX-710 is similar to  $225\text{--}250 \text{ mg/m}^2$  paclitaxel over 3 h, or  $135\text{--}175 \text{ mg/m}^2$  paclitaxel over 24 h (5, 8, 32), and higher than the 8–20% incidence after 3-h 175

**Table 4** Comparison of paclitaxel pharmacokinetic parameters for an 80 mg/m<sup>2</sup> dose administered in combination with VX-710 to pharmacokinetics for single-agent paclitaxel at 175 mg/m<sup>2</sup>

Parameter	VX-710 (120 mg/m <sup>2</sup> /hr) + paclitaxel			
	Prior Phase I	Phase II	Huizing <i>et al.</i> (31)	Gianni <i>et al.</i> (29)
Paclitaxel dose (mg/m <sup>2</sup> )	80	80	175	175
Number of patients	5	34	5	3
Infusion duration (h)	3	3	3	3
<i>T</i> <sub>1/2 β</sub> (h)	6.8 <sup>a</sup>	11.6 <sup>b</sup>	14.2	6.5
<i>CL</i> <sub>s</sub> (liters/h/m <sup>2</sup> )	5.9 (1.1) <sup>c</sup>	5.1 (1.3)	12.6 (2.6)	9.6 (1.5)
<i>V</i> <sub>ss</sub> (liters/m <sup>2</sup> )	40.7 (5.6)	50.7 (13.0)	99.2 (59.6)	ND <sup>d</sup>
AUC (μg × h/ml)	14.4 (3.2)	16.8 (5.0)	14.4 (3.0)	15.8 (2.6)

<sup>a</sup> Estimated using noncompartmental methods.

<sup>b</sup> Estimated using nonlinear mixed effects modeling.

<sup>c</sup> All estimates are presented as the mean (±STD).

<sup>d</sup> ND, not determined.

**Table 5** Treatment histories of patients responding to VX-710 plus paclitaxel

Patient No.	Prior chemotherapy, no. of cycles, and best response to prior therapies	Interval before start of VX-710 therapy	Sites of disease	No. of cycles of VX-710 + paclitaxel	Best response	Response duration (mo)		
08	CAF <sup>a</sup>	2	SD <sup>b</sup>	2 wk	Liver	8	PR <sup>c</sup>	2.5
	Paclitaxel (175 mg/m <sup>2</sup> q 3 wk)	2	PD					
030	Neoadjuvant AC	4	CR	2 mo	Breast, chest wall	4	PR <sup>d</sup>	1.5
	Paclitaxel (175 mg/m <sup>2</sup> q 3 wk)	6	PD					
031	Adjuvant 5-FU Epirubicin + paclitaxel <sup>e</sup>	4	CR PR	2 wk	Liver	13	PR <sup>c</sup>	13
038	AC	4	CR	3 mo 3 mo	Paratracheal Hilar Subcarinal LN	5	PR <sup>c</sup>	5
	Paclitaxel (175 mg/m <sup>2</sup> q 3 wk)	2	PD					
032	CAF	5	SD	2 wk	Liver	11	MR (45%)	6
	Paclitaxel + marimastat (135 mg/m <sup>2</sup> q 3 wk)	6	SD					
028	Adjuvant AC	4	CR	<1 mo	Breast	6	MR (33%)	4.5
	Paclitaxel (175 mg/m <sup>2</sup> q 3 wk)	2	PD					

<sup>a</sup> A, adriamycin; C, cyclophosphamide; F or 5-FU, 5-fluorouracil.

<sup>b</sup> PD, progressive disease; PR, partial response; SD, stable disease; MR, minor response.

<sup>c</sup> Response status confirmed by independent radiological review.

<sup>d</sup> Response duration was calculated from the time objective response criteria were first met until the date of documented progression. Lesions for Patient 030 were monitored by direct caliper measurements.

<sup>e</sup> Patient 031 received a total of 310 mg paclitaxel in combination with epirubicin, but the administration schedule was not identified in the study record.

mg/m<sup>2</sup> paclitaxel (29, 30, 33–35). The time that paclitaxel plasma concentrations remain >50 nM is a parameter correlated with neutropenia (31), and the approximate 38-h duration suggested by the simulation (Fig. 1) is one explanation for the higher incidence of neutropenia observed in this study. However, preliminary pharmacodynamic modeling evaluated patient demographics and laboratory parameters, and the population pharmacokinetics data. Results suggest that baseline ANC<sub>0</sub> were the one parameter predictive of grade 3/4 neutropenia, not the paclitaxel plasma concentration time above 50 nM.<sup>5</sup> Alternatively, CD34+ hematopoietic

precursor cells express P-gp (36, 37), and it is possible that increased neutropenia during therapy with an MDR inhibitor may be an expected pharmacodynamic effect attributable to increased paclitaxel retention by the progenitor cells. Indeed, more significant neutropenia has been observed in other studies with MDR inhibitors, even when reduced doses of the cytotoxic agents were administered to compensate for pharmacokinetic interactions (38, 39).

Mild to moderate asthenia, nausea, myalgia, and paresthesia were the predominant nonhematological adverse events reported by patients, all with an incidence and severity similar to those of single-agent paclitaxel therapy (40). Mild to moderate headache appears to be the only nonhematological adverse event that may be more frequent with VX-710 + paclitaxel therapy.

Several other studies have evaluated MDR inhibitors in

<sup>5</sup> E. Ette, personal communication.

advanced breast cancer patients. A placebo-controlled randomized Phase III trial evaluated the addition of quinidine to epirubicin (100 mg/m<sup>2</sup>) + prednisolone (25 mg twice a day) in patients who had not received prior chemotherapy for advanced disease (41). No difference in response rate or survival was noted between the two treatment arms. However, the median quinidine plasma concentration achieved in patients (5.5 μM) is only marginally effective in reversing MDR *in vitro*. Thus, inadequate dosing is one likely explanation for the ineffectiveness of quinidine in this trial. In a Phase I crossover study with the maximum tolerated dose of r-verapamil (225 mg/m<sup>2</sup> every 4 h) and 200 mg/m<sup>2</sup> paclitaxel, only 2 of 14 patients achieved minor responses despite a 2-fold increase in the paclitaxel AUC (42). Two studies have been performed with r-verapamil in combination with anthracyclines. In one study, patients who progressed or had stable disease after two cycles of epirubicin crossed over to combination therapy (120 mg/m<sup>2</sup> epirubicin + 300 mg r-verapamil once daily). Although 4 of 23 patients (17%) achieved objective responses, the patients in this study were not strictly refractory to their prior anthracycline therapy (43). In another study that enrolled strictly defined anthracycline-refractory patients, 2 of 20 patients achieved partial responses after addition of r-verapamil (180 mg/m<sup>2</sup> once daily) to either doxorubicin- or epirubicin-based therapy (44). The results of this study are similar to our results with VX-710 in a strictly defined paclitaxel-refractory patient population.

In addition to MDR inhibitors, other agents and strategies to overcome paclitaxel resistance have been evaluated in breast cancer patients. Weekly vinorelbine + granulocyte colony-stimulating factor or capecitabine resulted in 20–25% response rates in paclitaxel-refractory patients (45, 46). Single-agent therapy with anti-HER2 monoclonal antibody resulted in a 15% response rate in anthracycline- and taxane-refractory patients (47). Because paclitaxel activity is schedule dependent, another strategy evaluated the effect of a 96-h paclitaxel infusion in patients who failed shorter paclitaxel infusion schedules (35). The 96-h infusion resulted in a 27% response rate, but with increased myelosuppression and mucosal toxicity. Finally, a study with docetaxel in paclitaxel-refractory patients resulted in 8 responses among 44 patients (18%; Ref. 48).

The modest activity (10–27% response rates) observed in the studies summarized above underscores the complexity of the potential mechanisms associated with paclitaxel resistance. In addition to P-gp, other drug transport proteins expressed in breast cancer are MRP1, LRP, and BCRP (49, 50). VX-710 inhibits MRP1 drug efflux (51), but activity against LRP- and BCRP-expressing cell lines has not been tested *in vitro*. However, MRP1 and BCRP expression appears to alter accumulation and sensitivity to anthracyclines but not paclitaxel, whereas LRP may be associated with altered nuclear transport of both classes of compounds (52–54).

Studies with cell lines after single-step selection for paclitaxel resistance suggest that altered tubulin levels and tubulin mutations may also contribute to the drug resistance phenotype. Alterations in β-tubulin isotype expression have been identified in paclitaxel-resistant human sarcoma, prostate, ovary, and lung cancer cell lines, in transfected cell lines that overexpress EGFRvIII or HER2, and in tumor specimens from paclitaxel-refractory ovarian cancer patients (55–58). Studies with pacli-

taxel-resistant Chinese hamster ovary and human ovarian cancer cells have identified mutations in a leucine cluster in the β1-tubulin gene and within the probable tubulin binding site for paclitaxel (59, 60). Altered expression of β-tubulin isotypes or specific mutations probably result in destabilization of microtubules, diminishing the action of paclitaxel. A recent study used PCR and DNA sequencing to analyze β-tubulin expressed in normal tissue and tumor biopsy specimens obtained from non-small cell lung cancer patients before initial chemotherapy (61). β-Tubulin mutations were detected in 16 of 49 patients, and none of these patients responded to paclitaxel therapy. In contrast, among 33 patients who did not harbor β-tubulin mutations, 13 patients achieved objective responses to paclitaxel therapy. Finally, cellular apoptosis proceeds through a highly complicated program involving multiple components and signaling pathways (62). Altered expression of p53, bcl-2, bcl-xL, caspases, and stress- or mitogen-activated kinases (c-Jun NH<sub>2</sub>-terminal kinase/stress-activated protein kinase, MAPKs) have all been associated with paclitaxel resistance (63–65).

In the context of a multifactorial basis for paclitaxel resistance, the 11% response rate observed in this study with VX-710 in paclitaxel-refractory patients may reflect the proportion of breast cancer patients in whom P-gp expression is the major drug resistance determinant in the refractory setting. The safety and pharmacokinetics of the VX-710 + paclitaxel regimen are now well established. One strategy for further evaluation would assess the activity of VX-710 + paclitaxel as initial therapy in breast cancer patients whose tumors express P-gp to overcome intrinsic drug resistance. Another strategy would treat patients whose tumors did not express P-gp to prevent the emergence of acquired drug-resistant disease. In either approach, VX-710 may increase the objective response rate to paclitaxel and extend the progression-free interval, leading to longer survival and improved quality of life for breast cancer patients. We believe the data from this study support further evaluation of MDR inhibition with VX-710 as a therapeutic strategy to improve clinical outcome in patients with breast cancer and other solid tumors.

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