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ORIGINAL ARTICLE

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Safety and efficacy of the MDR inhibitor Incel (biricodar, VX-710) in combination with mitoxantrone and prednisone in hormone-refractory prostate cancer

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Abstract *Purpose*: VX-710 (biricodar, Incel) restores drug sensitivity to cells expressing P-glycoprotein (P-gp) and multidrug resistance-associated protein (MRP1). MRP1 is expressed in a high proportion of prostate tumors while P-gp expression is variable. Since mitoxantrone (M) and prednisone (P) are substrates for MDR transporters, we initiated a study to evaluate the safety, pharmacokinetics, and efficacy of VX-710 plus M/P in patients with hormone-refractory prostate cancer (HRPC). *Patients and methods*: Eligible patients had progressive HRPC (defined as new lesions, new disease-related pain, or 50% increase in PSA within 6 weeks of entry), testosterone < 30 ng/ml, no prior chemotherapy, ECOG performance status of 0–3, and adequate organ function. Patients received VX-710 (120 mg/m^2 per h) as a 72-h continuous intravenous infusion with intravenous bolus mitoxantrone (12 mg/m^2) administered 4 h after VX-710 was started and prednisone (5 mg twice daily) administered throughout the study treatment. Endpoints included serum PSA response, PSA response duration, time to PSA progression, pain reduction, and quality of life measures. *Results*: Enrolled in the study were 40 patients and 184 courses of VX-710 plus M/P were administered. Intensive pharmacokinetics, which were performed on six patients who received one cycle of M/P alone, followed by VX-710 plus M/P for all other cycles, showed that VX-710 did not alter mitoxantrone clearance. VX-710 blood concentration at the time of mitoxantrone administration averaged 4.52 μg/ml. VX-710 plus M/P was well tolerated. Transient nausea/vomiting and mild neutropenia were the principal treatment toxicities. Five patients experienced an uncomplicated febrile neutropenic episode (12%), three had severe nausea/vomiting, and two experienced transient moderate to severe ataxia. Of the 40 patients, 12 (30%, 95% confidence interval 16–44%) had a reduction in PSA of ≥50% and 9 of the 12 patients (23% overall, 95% CI 10–35%) achieved a reduction in PSA of ≥80% that was sustained for the duration of treatment with M/P plus VX-710. The median time to PSA progression was 41 weeks (95% CI 34–68 weeks). Of the 40 patients, 15 completed treatment with stable disease and 13 had progressive disease with increasing serum PSA during study treatment. Median survival was 48 weeks for the intent-to-treat population of 40 patients. *Conclusions*: The addition of VX-710 to M/P therapy did not appear to increase the proportion of patients with significant serum PSA reductions compared to M/P alone. However, the duration of PSA response observed for the 12 PSA responders suggests that MDR inhibition may benefit some patients with HRPC. In addition to MRP1 or P-gp expression, other mechanisms of drug resistance are probably associated with the relative insensitivity of HRPC to cytotoxic therapy.

Keywords Multidrug resistance · VX-710 · Mitoxantrone · HRPC

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Introduction

Hormone-refractory or hormone-insensitive prostate carcinoma (HRPC) is generally a therapy-resistant disease [25]. Historically, therapy with cytotoxic agents has not provided a survival benefit compared to best supportive care [25, 26]. However, recent advances suggest that chemotherapy may play a role in the management of HRPC [10, 24]. The results from two large randomized phase III studies with mitoxantrone and prednisone (M/P) or mitoxantrone and hydrocortisone (M/H) have demonstrated that chemotherapy provides an increased palliative benefit compared to therapy with the steroid alone [20, 38]. A number of phase II studies with paclitaxel, docetaxel, and etoposide, either as single agents or in combination with estramustine have also shown palliation, objective tumor responses and high PSA response rates [18, 22, 28, 34]. Studies are currently underway to determine if a survival advantage may be associated with the use of one or more of these newer therapies.

A number of molecular mechanisms may be involved in tumor cell resistance to chemotherapy. Mutations in the p53 tumor suppressor gene and increased expression of bcl-2, bax, glutathione-S-transferase π, and topoisomerase IIα or IIβ are some of the genetic alterations identified in prostate cancer specimens [37, 40] that may contribute to drug resistance. Expression of the multidrug transporters P-glycoprotein (P-gp) and the multidrug resistance-associated protein, MRP1, is another mechanism of resistance to anthracyclines, taxanes and other natural product cytotoxic drugs [1, 4, 9]. Expression of P-gp and/or MRP1 is well documented in hematologic malignancies and solid tumors, including primary prostate cancer [17, 35]. The binding of estramustine to P-gp and inhibition of its drug transport function [2, 36, 43] may explain the apparent increase in antitumor activity observed when estramustine is combined with taxanes, vinblastine or etoposide.

Studies of the expression of MDR1/P-gp and MRP1 at the molecular or protein level have demonstrated low-level expression of both proteins in normal prostate epithelial tissue [14, 23, 37, 40]. In three studies utilizing molecular probes, MDR1 mRNA was detected in 90% of prostate carcinoma specimens. In 38% of specimens, expression levels were higher in tumor tissue than in corresponding normal prostate tissue [19, 31, 32]. However, the results of studies using immunohistochemical methods are more ambiguous. P-gp levels range from below the limit of detection [37, 40] to a heterogeneous cellular distribution and reduced expression level of P-gp in tumor tissue compared to normal prostate tissue [3, 21]. In contrast, MRP1 appears to be the predominant MDR protein in prostate tumor tissue. In several recent studies, MRP1 has been detected in $>90\%$ of primary tumor specimens, with higher expression levels noted in advanced, disseminated cancers that correlated with increasing surgical grade [19, 30, 37, 40].

At present, M/P is the standard therapy approved by the Food and Drug Administration for treatment of HRPC. Addition of a third agent to this well-tolerated regimen is a logical strategy to improve HRPC therapy. Since both mitoxantrone and prednisone are transport substrates for P-gp and possibly MRP1, VX-710 (biricodar dicitrate, Incel), an inhibitor of both P-gp and MRP-1 would be a promising agent to study. VX-710 binds directly to P-gp and MRP1, and concentrations of 0.5 to 2.5 μM are sufficient to fully restore in vitro sensitivity of P-gp- and MRP1-expressing cells to the cytotoxic action of anthracyclines and other cytotoxic drugs associated with the MDR phenotype [15, 16, 42]. Phase I and phase II studies with VX-710 plus doxorubicin have established the safety and pharmacokinetics of VX-710 administered by continuous intravenous infusion for 72–96 h in combination with intravenous bolus doxorubicin at 45 to 75 mg/m^2. A VX-710 blood steady-state concentration of 10 μM was sustained at a dosage of 120 mg/m^2 per h, and VX-710 had no apparent effect on doxorubicin pharmacokinetics or pharmacodynamics [6, 27].

This phase II study was designed to evaluate the combination of M/P and VX-710 in patients with hormone-insensitive stage D prostate cancer. The objectives of this study were to: (1) establish the safety of VX-710 in combination with mitoxantrone and prednisone, (2) compare mitoxantrone pharmacokinetics with and without VX-710, (3) evaluate the activity of this regimen by measuring the proportion of patients who achieved $>50\%$ and $>80\%$ PSA reductions and (4) measure other clinical benefits including pain reduction and improvement in overall quality of life (QOL).

Patients and methods

Patients

Patients eligible for this study had biopsy-confirmed adenocarcinoma of the prostate metastatic to the lymph nodes, bone, liver, lungs, or other soft tissue. Patients were required to have demonstrated progressive disease following a minimum of one trial of hormonal therapy. This was defined as evidence of new metastatic lesions, new disease-related pain, or rising PSA levels. Patients who had increasing PSA in the absence of documented progression of metastatic disease were required to have demonstrated a $>50\%$ increase in PSA over three separate measures taken at 2-week intervals over a minimum period of 6 weeks. Patients were required to have a serum testosterone level <30 ng/ml as evidence of adequate surgical or chemical castration. Patients receiving treatment with LHRH agonists at the time of study entry continued therapy while on study. Patients were required to have stopped antiandrogen therapy (a minimum of 4 weeks for flutamide and 6 weeks for bicalutamide) without evidence of decreasing PSA following withdrawal of antiandrogen therapy.

Patients were required to have an Eastern Cooperative Oncology Group (ECOG) performance status of 0–3, a life expectancy of least 4 months, adequate bone marrow function defined as WBC $\geq 3000/\text{mm}^3$, absolute neutrophil count $>1500/\text{mm}^3$, and platelet count $\geq 100{,}000/\text{mm}^3$, adequate liver function (serum bilirubin levels less than twice the institution's upper limit of normal) adequate renal function (serum creatinine <2.0 mg/dl), and adequate

cardiac function with a left ventricular ejection fraction of ≥50% as determined by echocardiography or radionuclide MUGA scan. Patients were required to be capable of completing pain and QOL scales. A statement of informed consent was obtained according to local Institutional Review Board requirements. However, because pain and QOL were secondary endpoints, the presence of pain was not a requirement for study eligibility.

Exclusion criteria were as follows: continuing antiandrogen therapy, prior chemotherapy, radiation therapy within the last 30 days or strontium-89 therapy within the last 60 days, and CNS metastases or spinal cord compression. Also ineligible were patients with a history of a prior malignancy within the past 5 years (except for non-melanoma skin carcinoma or superficial bladder carcinoma), patients with active cardiac disease (including but not limited to uncontrolled congestive heart failure, a current arrhythmia requiring rate-controlling medication, myocardial infarction within the previous 6 months, angina pectoris requiring antiangina medications, or uncontrolled hypertension), and patients with senile dementia or any psychiatric disorder that prohibited the patient from providing informed consent or understanding the pain and QOL scales. Patients were also ineligible if taking any of the following drugs at the time of enrollment: cimetidine, phenothiazines, calcium channel blockers, phenytoin, cyclosporine A, or other drugs known to be P-gp inhibitors.

Study design and treatment administration

This was an open-label, single arm, multicenter study with a two-stage design. Prednisone was administered at a dose of 5 mg twice daily (10 mg total daily dose) starting on day 1 of study treatment and continuing daily until the end of study treatment. VX-710 was administered by continuous intravenous infusion for 72 h at a dose of 120 mg/m^2 per h. VX-710 was administered through a centrally placed catheter.

VX-710 was provided by Vertex Pharmaceuticals initially in vials containing 3 g 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). A lyophilized formulation (3.25 g/vial) was subsequently developed for reconstitution with 14 ml sterile water for injection to a final concentration of 200 mg/ml. At the time of use, the concentrated drug solution was aseptically mixed with 0.9% normal saline or 5% dextrose in water for administration with a 250-mL PVC infusion set.

Mitoxantrone was administered according to guidelines in the package insert at a dose of 12 mg/m^2 starting 4 h after beginning the VX-710 infusion through a separate catheter. The regimen was repeated every 21 days up to a maximum dose of 130 mg/m^2 mitoxantrone or until evidence of disease progression, intolerable adverse events, or withdrawal of patient consent. Mitoxantrone doses were adjusted based on the degree of myelosuppression. An increase to 14 mg/m^2 was permitted for the second and subsequent cycles if the ANC nadir was $>1000/mm^3$. Mitoxantrone doses were decreased by 2 mg/m^2 if the ANC nadir was $<500/mm^3$ or platelets were $<50{,}000/mm^3$. A maximum of two dose reductions (to a low dose of 8 mg/m^2) were permitted for continued therapy. Retreatment could be delayed for up to 2 weeks until ANC and platelet counts reached $>1{,}500/mm^3$ or $>100{,}000/mm^3$, respectively. Patients with a >10% decrease in absolute left ventricular ejection fraction (LVEF) from their baseline value or an LVEF of <40% had study treatment discontinued.

Pretreatment and follow-up evaluations

Patient history, physical examination, assessment of baseline symptoms, a MUGA scan or echocardiogram, baseline tumor measurements (obtained by computerized tomography, radiography, or physical measurement only for patients with measurable disease) and routine laboratory studies were required within 30 days of starting study treatment. Two blood samples for PSA levels were collected 1 to 7 days apart within 14 days of starting therapy to determine the baseline PSA level. Patients also completed the European Organization for Research and Treatment core questionnaire (EORTC/QLQ-30, version 3.0) with a prostate cancer-specific module. The questionnaire was administered via telephone by a trained interviewer. A member of the clinical study staff administered the six-point McGill-Melzack pain questionnaire and patients also completed the visual analog scale. Patients maintained a diary of analgesic medications starting at baseline. Toxicities were graded according to WHO Common Toxicity Criteria.

Complete blood counts (CBCs) 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 study treatment. A CBC and clinical chemistries (including total protein, creatinine, AST or ALT, total bilirubin, alkaline phosphatase, and electrolytes) and a PSA test were performed on day 1 of cycle 2 and each subsequent course of therapy. The McGill-Melzack pain questionnaire and visual analog scale were also completed on day 1 of all repeat treatment cycles. The EORTC/QLQ-30 questionnaire was completed after cycles 3 and 6 and at study termination. MUGA scans and tumor measurements (for patients with measurable disease) were also obtained after cycles 3, 6 and 9, and at study termination.

Follow-up evaluations were conducted 30 days after study termination for all patients and included a physical examination, assessment of ECOG performance status, a PSA test, adverse event reporting, completion of the McGill-Melzack pain intensity visual analog scale and EORTC/QLQ-30 questionnaires, and recording of analgesic use. Patients who achieved PSA responses were followed at 2-month intervals (e.g., months 3, 5, 7, 9 and 11 following study termination) for PSA levels and survival. All patients were followed at 6-month intervals (or until initiation of an intervening therapy) for survival.

Pharmacokinetic sampling and bioanalytical methods

The first three patients (and three additional patients among the first 20) were assigned to an intensive pharmacokinetic sampling schedule for cycle 1 (mitoxantrone alone) and cycle 2 (VX-710 plus mitoxantrone) to compare mitoxantrone pharmacokinetics. Blood samples (7 ml) were collected at the following time-points: prior to mitoxantrone administration, and 10, 30 and 60 min, and 3, 6, 9, 12, 24, 48 and 72 h after mitoxantrone administration. The 24-, 48- and 72-h samples may have been collected during a 3-h window (e.g., 24 ± 1.5 h) around the specified time-point. VX-710 levels were determined using the pre-mitoxantrone administration sample collected in cycle 2. All other patients were assigned to a limited sampling schedule during cycles 1 and 2 for a population pharmacokinetics analysis. Blood samples (7 ml) were collected prior to mitoxantrone administration and at 10 and 60 min, and 24, 48 and 72 h after mitoxantrone administration. Plasma samples were stored at −70°C prior to analysis.

The analytical methods used for extraction and quantitation of VX-710 and mitoxantrone 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 reversed-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 and the assay precision and accuracy were 2.6–13.2% and >90%, respectively. This assay was determined to be selective and specific following the analysis of six independent blood samples.

For the determination of mitoxantrone concentrations, an internal standard (methylene blue) was added to 0.25 ml plasma samples and proteins were precipitated by the addition of four volumes of acetonitrile. The sample was vortexed prior to centrifugation for 10 min at 4°C. The supernatant was then evaporated to dryness under a gentle stream of nitrogen at 40°C. The residue was

reconstituted with mobile phase then injected onto a Zorbax SB-C18 column (250×3.0 mm, 3.5 μm; Hewlett-Packard). Mitoxantrone and the internal standard were eluted from the column using a linear gradient of ammonium acetate buffer (0.05 *M*, pH 4.0) from 90% to 10% over 13 min. Mitoxantrone and internal standard were detected at 662 nm. The linear range was 1.25 to 1000 ng/ml and the assay precision and accuracy were 1.3–9.1% and >86%, respectively. This assay was determined to be selective and specific following the analysis of six independent blood samples.

Pharmacokinetic analysis

A two-compartment pharmacokinetic model was used for fitting as it was found that a three-compartment pharmacokinetic model (generally used to describe mitoxantrone pharmacokinetics in the literature) failed to converge, indicating that the data did not support a more complicated model. The difference in the objective function (O.F.) between two models is a chi-squared random variable with one degree of freedom. A decrease of 3.8 or greater between two models (degrees of freedom 1) corresponds to a significance level of $P < 0.05$ and a decrease of 7.9 or greater corresponds to a significance level of $P < 0.005$.

Response criteria and endpoints

Serum PSA response (>50% decrease from baseline confirmed 3 weeks later) was the primary study endpoint. Duration of serum PSA response and time to PSA progression (defined according to consensus criteria [7]), changes in pain and QOL measures and objective response rate (for patients with measurable disease) were all secondary endpoints. Response criteria for patients with evaluable disease were as follows:

- complete PSA response: complete disappearance of all evaluable disease, normalization of PSA (≤0.1 ng/ml), normalization of pain and discontinued use of all analgesic medications used to treat metastatic disease
- partial PSA response: a >50% decrease in baseline PSA on two measurements at least 3 weeks apart, no development of new or suspicious metastatic disease, and qualitative improvement in pain
- stable disease: a <50% increase or decrease in baseline PSA (both measures), no development of new metastatic disease, and stable or qualitatively improved pain
- progressive disease: a >50% increase from baseline PSA (all measurements), new measurable or evaluable lesions, demonstrated worsening of pain symptoms, or worsening of evaluable lesions (other than lesions on a bone scan)

For patients with measurable disease, standard response criteria based on the decrease in the sum of products of all bidimensionally measurable lesions were used to determine complete (disappearance of all measurable and evaluable lesions) or partial responses (>50% decrease), and stable disease (<50% decrease or <25% increase) or disease progression (>25% increase).

Statistical analysis

This was a two-stage study conducted at six study centers. Sample size calculations were based on the two-stage phase II design methods of Simon [33]. The lower activity level of interest (p_0) was a 35% PSA response rate based on the 33% PSA response rate for mitoxantrone plus prednisone reported by Tannock et al. [38]. The target level of activity (p_1) proposed for the M/P plus VX-710 regimen was a 55% PSA response rate. The α selected for this study was 0.05 and β was 0.10. Accordingly, 20 patients were enrolled in the first stage of the study. If 8 or more patients achieved serum PSA responses, then 39 additional patients would be enrolled. Enrollment in the study was not stopped pending the outcome of the interim analysis. Seven patients discontinued prematurely with only one or no post-treatment PSA determinations and were not considered evaluable for PSA response. A total of 40 patients had enrolled at the time the first 20 patients were evaluable for PSA response. However, study data are summarized by intent-to-treat analysis.

Results

Patients

A total of 40 patients were enrolled in this study. Patient demographics and disease characteristics are summarized in Table 1. The majority of patients had an ECOG performance status of 0 (26/40, 65%) and had received two or more prior regimens of hormonal therapy (23/40, 58%). All 40 patients had increasing PSA levels as the primary indicator of progressive hormone-insensitive prostate cancer. The median (range) baseline PSA level for the 40 patients was 51 ng/ml (8–1980 ng/ml). Seven patients had measurable soft-tissue disease at baseline and the others had evidence of soft-tissue or bone disease. A total of 184 cycles of M/P plus VX-710 therapy were administered to patients in this study, with a range of 1 to 11 cycles and a median of 3 cycles per patient; 15 patients received 6 or more cycles.

Safety and tolerability

All 40 patients are assessable for safety. The combination of M/P with 120 mg/m^2 per h of VX-710 by 72-h continuous intravenous infusion was generally well tolerated in this patient population. A comparison of nadir absolute neutrophil counts (ANCs) for the six patients who received M/P alone followed by M/P plus VX-710 and for all cycles of M/P plus VX-710 is presented in

Table 1 Patient characteristics

Number of patients enrolled	40
Age (years)	
Median	72
Range	50–85
Baseline ECOG performance status	
0	26
1	12
2	2
Prior treatment history	
Prostatectomy	12
Radiation therapy	23
Hormonal therapy	
One regimen	4
Two regimens	9
More than two regimens	27
Baseline PSA (ng/ml)	
Median	51
Mean	259
Range	8–1980

Table 2 Comparison of absolute neutrophil counts of patients receiving M/P alone followed by M/P plus VX-710 (intensive pharmacokinetic group) with all patients receiving M/P plus VX-710 therapy

No. of patients	Cycle no.	Subject group	Median	Mean (SD)	Range
6	1	M/P alone	2.11	2.14 (0.86)	0.97–3.16
	2[a]	M/P + VX-710	0.78	0.99 (0.84)	0.11–2.59
40[a]	All (184)	M/P + VX-710	0.51	0.70 (0.57)	0.03–2.41

[a]Includes intensive pharmacokinetic group cycle 2 data ($n=6$)

Table 3 Most frequent nonhematologic toxicities by severity (worst grade reported) The values indicate the number(%) of patients ($n=40$)

Adverse event	Total	Grade 1/2	Grade 3	Grade 4
Asthenia	36 (90%)	29	7	–
Nausea	33 (83%)	27	6	–
Anorexia	24 (53%)	24	–	–
Headache	23 (48%)	16	7	–
Vomiting	20 (50%)	12	7	1
Constipation	20 (50%)	20	–	–
Diarrhea	15 (38%)	12	3	–
Dehydration	12 (30%)	11	1	–
Dizziness/ataxia	11 (28%)	11	2	–

Table 2. Median ANCs were more than twofold lower after administration of M/P plus VX-710 (0.78×10^9 cells/l) compared to patients following administration of M/P alone (2.11×10^9 cells/l). The median overall ANC nadir was slightly lower (0.51×10^9 cells/l) for the 184 cycles of M/P plus VX-710 therapy administered in this study. Among the 40 patients, 9 (23%) and 27 (68%) experienced an episode of grade 3 and grade 4 neutropenia, respectively and, three patients discontinued study treatment because of myelosuppression. Six patients (15%) experienced an episode of uncomplicated febrile neutropenia. The mitoxantrone dose was reduced to 10 or 8 mg/m^2 in six patients because of prior neutropenia. No patients were given colony-stimulating factors as prophylaxis for neutropenia. Twelve patients developed anemia (grade 1 or 2 in eight, grade 3 in four) that was treated with packed RBC transfusions or erythropoietin. M/P plus VX-710 therapy had only a mild effect on platelet counts. One patient experienced an episode of grade 4 thrombocytopenia during cycle 1 and discontinued study treatment due to religious beliefs that prohibited blood or platelet transfusions.

Non-hematologic toxicities experienced by 20% or more patients that were at least possibly related to M/P plus VX-710 treatment are summarized in Table 3. Mild to moderate (grade 1 or 2) asthenia, nausea, anorexia, headache, vomiting, diarrhea, constipation, dehydration, and dizziness were the most frequent non-hematologic toxicities (Table 3). Seven episodes of grade 3 nausea/vomiting occurred among five patients that required hospitalization for hydration therapy. Four of the five patients completed subsequent treatment cycles with aggressive antiemetic therapy or prophylactic hydration before mitoxantrone administration and one patient discontinued study treatment because of severe vomiting. Three patients experienced episodes of grade 2 (one patient) or grade 3 (two patients) ataxia within 24–36 h of starting the VX-710 infusion. Symptoms resolved spontaneously within 6–12 h of either discontinuing the VX-710 infusion (one patient) or reducing the VX-710 dose by 50%. Two of these patients subsequently discontinued study treatment because of these events.

Two patients died during the study, one as a result of an apparent myocardial infarction and the second after diagnosis of hepatocellular carcinoma. These events were judged to be unrelated to M/P plus VX-710 treatment. Two patients discontinued treatment (after five and nine cycles, respectively) because of decreased LVEF. However, 12 other patients received total cumulative mitoxantrone doses of 96 to 140 mg/m^2 without any significant decrease in LVEF.

VX-710 and mitoxantrone pharmacokinetics

The mean VX-710 blood concentration determined at the time of mitoxantrone administration was 4.52 μg/ml (7.5 μ*M*) for 65 treatment cycles administered to 37 patients. This value is similar to the VX-710 steady-state concentration determined for the 120-mg/m^2 per h dose groups in the phase I studies with VX-710 and paclitaxel or doxorubicin [27, 29]. Mitoxantrone pharmacokinetics for six patients following administration of M/P alone (cycle 1) and then M/P plus VX-710 (cycle 2) are summarized in Table 4, and the mean mitoxantrone plasma concentration-versus-time profiles for these six patients are shown in Fig. 1. The data show that the mitoxantrone dose normalized area under the concentration-time curve (AUC), clearance (CL_s), volume of distribution (V_{ss}) and C_{max} were similar for both treatment cycles, indicating that VX-710 does not significantly effect mitoxantrone pharmacokinetics (Table 4).

A population pharmacokinetics analysis was performed for mitoxantrone using a sparse blood sampling schedule for 33 patients during cycles 1 and 2. Mitoxantrone pharmacokinetic parameter estimates for these 33 patients revealed a CL_s of 28.5 l/h and a V_{ss} of 1268 l.

Table 4 Mitoxantrone pharmacokinetic parameter estimates. Values are the medians (range)

Group	C_{max} (ng/ml)	Dose-normalized AUC (ng h/ml)	CL_s (l/h/kg)	V_{ss} (l/kg)
M/P alone	293.2 (209.4–340.7)	0.035 (0.010–0.070)	0.35 (0.13–0.97)	21.47 (10.12–32.75)
VX-710 + M/P	197.6 (132.9–521.8)	0.025 (0.020–0.180)	0.38 (0.12–0.54)	15.24 (7.7–38.16)

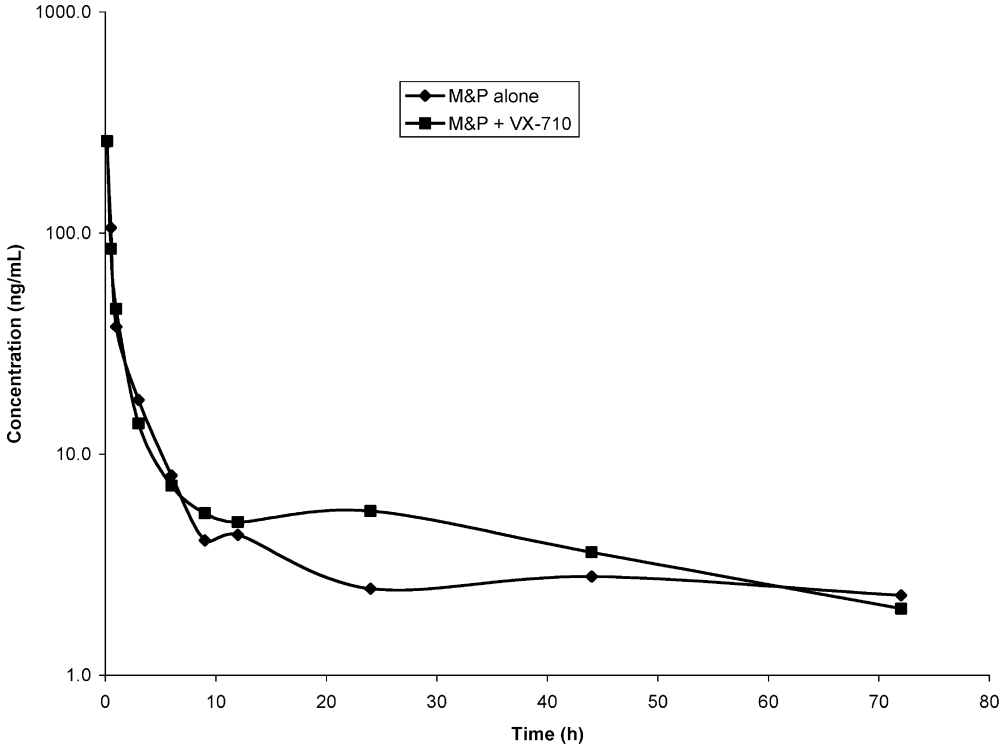

Fig. 1 Mean mitoxantrone plasma concentration-versus-time profiles for six patients given M/P alone in cycle 1 and then in combination with VX-710 in cycle 2

Table 5 Patients who achieved >50% and >80% PSA decreases and disease status at treatment termination for patients receiving M/P plus VX-710 therapy

	Intent-to-treat patients ($n=40$)
PSA decrease (%)	
>50[a]	12 (30%)
>80	9 (22%)[b]
Disease status at treatment termination	
PSA response	12 (30%)
Stable disease	15 (42%)
Progressive disease	13 (37%)

[a]Includes those with a >80% reduction
[b]Three patients had maximum PSA reductions of 68%, 73% and 75%, respectively

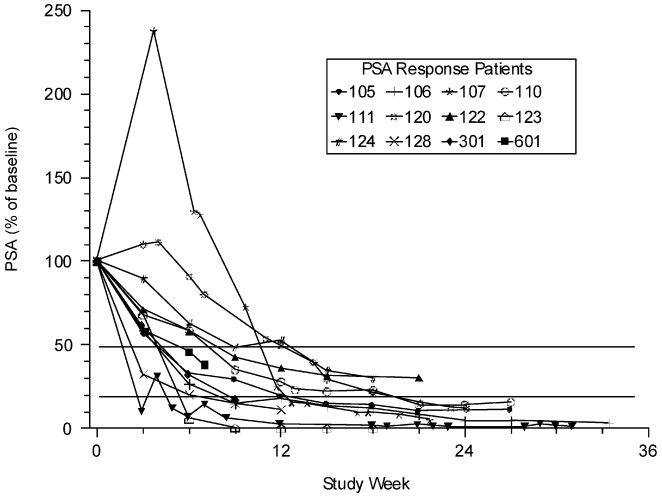

Fig. 2 Time-course profile of plasma PSA levels for 12 patients who achieved 50% reductions in PSA

These values are in close agreement with the estimates determined by intensive sampling for the six patients discussed above. The mitoxantrone pharmacokinetic parameter estimates determined by both intensive and population analyses are within the range of the parameter values reported for mitoxantrone in the literature (CL_s of 26–64 l per h and V_{ss} of 1000–4000 L) [12, 41].

PSA response

PSA response data are summarized in Table 5 for all 40 patients. Of the 40 patients enrolled in this study, 33 completed at least two treatment cycles and had two PSA measurements for response assessment compared to baseline values. Seven patients discontinued from the study with none or only one PSA measurement for PSA response. These patients discontinued for the following reasons: over-sedation with narcotics and diphenhydramine concurrent with VX-710 administration; diagnosis of hepatocellular carcinoma; a pathological hip fracture requiring surgery; decreased performance status; grade 4 thrombocytopenia; and severe nausea and vomiting (two patients). However, all 40 patients were included in an intent-to-treat analysis for overall response.

Among the 40 patients, 12 (30%; 95% confidence interval 16–44%) had a PSA reduction of ≥50% and 9 of the 12 patients (23% overall; 95% CI 10–35%) achieved

a PSA reduction of ≥80% that was sustained for the duration of treatment with M/P plus VX-710 (Fig. 2). The median time to a serum PSA decrease of ≥50% was 45 days (95% CI 41–63 days). In addition to the patients who achieved a PSA reduction, 15 patients completed treatment with stable disease and 13 patients had progressive disease with increasing serum PSA levels (Table 5).

Serum PSA levels in the 12 patients who achieved a serum PSA reduction of ≥50% and ≥80% were obtained 1 month after treatment termination and bimonthly thereafter to determine the duration of the PSA response. The duration of PSA response was defined according to consensus reporting criteria [7] as the time from the first >50% decrease to the first increase of >50% above the nadir value, or an increase of at least 5 ng/ml. The median duration of the PSA response for the 12 patients was 33 weeks (95% CI 26–62 weeks) as determined by the Kaplan-Meier method. The median time to PSA progression for the 12 patients was 41 weeks (95% CI 34–68 weeks). Of the 12 patients, 4 died (range of 11 to 17 months from initial treatment), 3 were lost to follow-up, and 5 were alive at the last follow-up (20.5 to 28 months from initial treatment). The overall median survival for the 40 intent-to-treat patients was 48 weeks.

Measurable disease response

Among the seven patients with measurable disease at baseline, two had 45% reductions after six and nine cycles, respectively. One of these patients achieved a >75% reduction in PSA, while the other had a 32% decrease at cycle 4. Five other patients had no change in the sum of measurable lesions.

Pain and quality of life

The mean (range) pain intensity at baseline was 0.97 (0 to 5.7) on the visual analog scale and nine of the patients reported no pain. Because of the proportion of patients with no pain and overall low baseline pain intensity, an analysis for the minimal perceivable pain relief could not be conducted. Also, the QOL data were not analyzed because most patients had few variables to monitor for assessment of a meaningful impact.

Discussion

In this phase II study, the safety and tolerability, pharmacokinetics, and efficacy of VX-710 plus M/P were evaluated in HRPC. The demographics for patients in this study were typical of a prostate cancer population with stage D hormone-insensitive disease. In particular, patients were similar with respect to age, prior hormonal therapies, sites of metastasis and ECOG performance status to the demographic factors for patients in the two phase III trials with M/P or M/H [20, 38]. The patient population in this study differed from those in the two phase III studies with respect to a lower baseline PSA level (median of 50 ng/ml, Table 1) compared to medians of 150 and 209 ng/ml). Patients without symptomatic pain at baseline were also enrolled in this study, unlike the study by Tannock et al. [38], in which symptomatic pain was a requirement and approximately 60% of patients had discomforting to distressing pain according to the McGill-Melzack pain questionnaire.

Since an effect of VX-710 on mitoxantrone pharmacokinetics could potentially increase toxicity and complicate interpretation of efficacy data, six patients received M/P alone in cycle 1 and then with VX-710 in their second cycle. This allowed an intrapatient comparison of mitoxantrone pharmacokinetics, which showed that VX-710 did not significantly alter the AUC, clearance or terminal elimination half-life of mitoxantrone (Table 4). The absence of a pharmacokinetic interaction between VX-710 and mitoxantrone is consistent with results of studies with VX-710 and doxorubicin [6, 27]. Plasma concentrations of VX-710 ($>7\ \mu M$) were sustained in patients at levels two- to threefold higher than concentrations necessary to inhibit P-gp and MRP1 drug efflux in vitro [15, 16] and increase liver retention of the MDR substrate ^{99m}Tc-sestamibi in vivo [27]. Thus, the drug efflux activity of P-gp or MRP1 should have been adequately inhibited in prostate tumor tissue-expressing these multidrug transporters.

Overall, the VX-710 plus M/P regimen was generally well tolerated and most adverse events were mild to moderate and reversible. Asthenia was the most common nonhematologic adverse effect, with 90% of patients reporting grade 1/2 (73%), or grade 3 (17%) asthenia during study treatment. However, the degree of asthenia patients reported may have been acute or subacute and transient since most patients were at their baseline level prior to each repeat treatment cycle.

Other adverse effects attributable to VX-710 included headache, nausea, vomiting, and dizziness/ataxia. Mild headaches have been a consistent patient complaint in other studies with VX-710 and paclitaxel or doxorubicin [6, 27, 29, 39], but several patients in this study reported more severe headaches. Several patients also experienced more severe nausea and vomiting than is typically associated with M/P alone [38]. Aggressive antiemetic use and prophylactic hydration therapy was effective is managing these patients during subsequent treatment cycles. Two patients had episodes of ataxia or an unsteady gait, which has not been observed in previous studies with VX-710. One of these patients was 82 years old, frail (49 kg), and had a history of chronic obstructive pulmonary disease. The other patient was moderately dehydrated, and these factors may have contributed to the events observed. The nausea and vomiting or ataxia/unsteady gait symptoms observed in these patients typically occurred during day 1 or day 2 of the VX-710 infusion and resolved over several hours

if the VX-710 infusion was temporarily discontinued. Several patients resumed treatment with VX-710 dose reductions of 25% or 50% without a recurrence of these symptoms. The basis for the increased severity of these toxicities compared to results from other studies with VX-710 is not clear. There was no association of these symptoms with other concomitant medications and the affected patients did not appear to have any significant predisposing conditions.

Neutropenia was the predominant hematologic toxicity, which appeared to be increased by the addition of VX-710 to the M/P regimen. The median ANC nadir was more than 2.5-fold lower for the six patients receiving VX-710 with M/P compared to the median ANC nadir for the same six patients following therapy with M/P alone (Table 2). The overall incidence of grade 3/4 neutropenia (91%) was higher compared to 25–68% incidence reported for HRPC patients in other studies [20, 38]. However, the neutropenia was generally brief and manageable without the use of colony-stimulating factors and the number of episodes of neutropenic fever or sepsis was similar to the incidence observed for M/P alone. Because VX-710 did not increase mitoxantrone exposure or reduce clearance and $CD34^+$ hematopoietic precursor cells express P-gp [8, 11], one hypothesis is that neutropenia was due to increased mitoxantrone retention by the progenitor cells. Indeed, more significant neutropenia has been observed in other studies with MDR inhibitors despite dose reductions to compensate for pharmacokinetic interactions with the cytotoxic agents [5, 13].

The percentage of evaluable patients who achieved $>50\%$ and $>80\%$ PSA reductions (30% and 23%; Table 5) during VX-710 plus M/P treatment is similar to the results observed in CALGB study 9182 (38% and 19.6%), and the median survival was also similar [20]. The patients who had $>50\%$ and $>80\%$ PSA reductions sustained their response without any increase in PSA during study treatment, with a 45-week median duration of PSA response. A randomized trial is necessary to determine if addition of VX-710 to M/P therapy could result in a significant increase in PSA response duration and overall time to PSA progression compared to M/P alone.

In conclusion, VX-710 does not affect mitoxantrone pharmacokinetics and the VX-710 plus M/P regimen was well tolerated by the majority of patients treated in this study. The overall PSA response rate did not appear to increase with addition of VX-710 to M/P therapy, despite VX-710 exposure that should have inhibited P-gp and MRP1. Thus, it is possible that P-gp or MRP1 expression does not contribute significantly to in vivo resistance of prostate cancer to mitoxantrone. Other mechanisms, including p53, bcl-2 and other antiapoptotic molecules, or alterations in growth factor signaling pathways, may be of greater significance in determining sensitivity of androgen-independent prostate cancer cells to cytotoxic therapy.

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