

# Pharmacokinetic and pharmacodynamic study of tariquidar (XR9576), a P-glycoprotein inhibitor, in combination with doxorubicin, vinorelbine, or docetaxel in children and adolescents with refractory solid tumors

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## Abstract

**Purpose** P-glycoprotein (Pgp), an ATP-dependent transport protein, confers multidrug resistance in cancer cells. Tariquidar binds and inhibits Pgp. To assess the toxicity, pharmacokinetics (PK), and pharmacodynamics of tariquidar, we conducted a phase I trial of tariquidar in combination with doxorubicin, docetaxel, or vinorelbine in children and adolescents with recurrent or refractory solid tumors.

**Methods** Patients less than 19 years of age with refractory or recurrent solid tumors were eligible. Tariquidar (1, 1.5, or 2 mg/kg) was administered alone and in combination with doxorubicin, docetaxel, or vinorelbine. PK of tariquidar and cytotoxic drugs was performed. Pgp function

was assessed by a rhodamine efflux assay and <sup>99m</sup>Tc-sestamibi scintigraphy. Tumor Pgp expression was assessed by immunohistochemistry. Response was assessed using Response Evaluation Criteria in Solid Tumors.

**Results** Twenty-nine subjects were enrolled. No tariquidar-related dose-limiting toxicity (DLT) was observed. DLT related to cytotoxic drugs occurred in 12 % of subjects receiving tariquidar 2 mg/kg. When administered in combination with tariquidar, the clearance of docetaxel and vinorelbine was reduced compared to prior studies. Inhibition of rhodamine efflux was dose dependent. After tariquidar administration, <sup>99m</sup>Tc-sestamibi accumulation in tumor increased by 22 %. Objective responses (1 complete, 2 partial) were observed. There was no association between tumor Pgp expression and response.

**Conclusion** A tolerable and biologically active dose of tariquidar was established in children and adolescents. This trial demonstrates that modulators of resistance can be evaluated in combination with chemotherapy, and pharmacokinetic and pharmacodynamic endpoints can be useful in determination of recommended dose in children and adolescents.

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**Keywords** Multidrug resistance · P-glycoprotein · Pediatric cancer · Phase I · Pharmacokinetics

## Introduction

P-glycoprotein (Pgp, ABC-B1), a plasma membrane glycoprotein, functions as an ATP-dependent drug efflux pump. Pgp expression in tumor cells confers multidrug resistance by preventing the intracellular accumulation of chemotherapeutic agents including anthracyclines, taxanes, vinca alkaloids, and epipodophyllotoxins. Inhibition of Pgp may

reverse resistance by increasing tumor intracellular drug accumulation and exposure [18, 22].

Pgp is expressed in childhood cancers, including leukemia, rhabdomyosarcoma, osteosarcoma, retinoblastoma, Ewing sarcoma, and neuroblastoma. In central nervous system tumors, Pgp is expressed in tumor, in tumor blood vessels, and in the epithelium of the choroid plexus [29]. The role of Pgp in treatment outcome is controversial [1, 4, 5, 10]. In retrospective studies of rhabdomyosarcoma, osteosarcoma, retinoblastoma and neuroblastoma, tumor Pgp expression at diagnosis was associated with poor prognosis and undetectable expression correlated with durable remission [11]. However, associations with outcome have not been confirmed in prospective trials [35].

Tariquidar (molecular weight 646.7) inhibits the ATPase activity of the Pgp drug efflux pump. In vitro, tariquidar reverses intrinsic and acquired resistance to doxorubicin, vincristine, and paclitaxel with potency 10-fold greater than first-generation Pgp inhibitors including valspodar [23]. Tariquidar [17, 21] and structural analogs [26] also inhibit Breast Cancer Resistance Protein (BCRP/ABCG2). In adults, tariquidar was well tolerated. At the recommended dose (2 mg/kg IV over 30 min), maximum inhibition of Pgp measured by rhodamine uptake in CD56+ lymphocytes was observed and maintained for 24 h. The peak serum concentration ( $C_{max}$ ) was 2.3  $\mu\text{M}$ , and area under the concentration time curve ( $\text{AUC}_{0-48}$ ) was 12.6  $\mu\text{M h}$ , clearance was 160 mL/min/ $\text{m}^2$ , and half-life was 26 h [7, 32].

In adults with refractory solid tumors, tariquidar was administered in combination with vinorelbine. In this combination, the maximum tolerated dose (MTD) of vinorelbine was 20 mg/ $\text{m}^2$ , and the dose-limiting toxicities (DLT) were neutropenia, hypotension, and infection. Non-hematological toxicities were gastrointestinal (abdominal pain, anorexia, constipation, ileus, nausea, vomiting, and diarrhea) and fatigue. One patient with breast cancer had a minor response, and a second with renal cell cancer had a partial response [2]. In a phase II study in women with breast carcinoma, tariquidar was added to the ongoing taxane- or anthracycline-based regimens. One patient experienced severe toxicity. One woman who received tariquidar in combination with docetaxel and trastuzumab had a partial response [28]. Two phase III trials of tariquidar were initiated in patients with non-small cell lung cancer. Tariquidar or placebo was administered in combination with paclitaxel/carboplatin or in combination with vinorelbine. Both studies were closed due to chemotherapy-related toxicity in the tariquidar arm.

Clinical development of first-generation Pgp inhibitors was hampered by pharmacokinetic interactions with cytotoxic chemotherapy [9, 15]. In early clinical trials in adults, minor pharmacokinetic interactions were noted for tariquidar in combination with doxorubicin and paclitaxel,

with systemic exposure increasing 26 and 44 %, respectively. No change in vinorelbine pharmacokinetics was observed. Due to the large inter-patient variability in the pharmacokinetics of these cytotoxic agents alone, the changes were not considered clinically significant [2, 20, 28].

In addition to over-expression in drug-resistant cancer cells, Pgp is present in normal tissues including renal proximal tubules, capillary endothelial cells in the blood–brain barrier, bile canaliculi, bone marrow stem cells, and peripheral blood mononuclear cells (CD56+ PBMC) [6, 13, 14, 24]. Substrates for Pgp are structurally and chemically diverse [25, 36]. In addition to chemotherapeutic agents, Pgp substrates include the fluorescent dye rhodamine and sestamibi. Rhodamine efflux from CD 56+ PBMCs by flow cytometry has been used to assess Pgp function in a number of clinical trials [2, 20, 30, 32].  $^{99\text{m}}\text{Tc}$ -sestamibi scintigraphy, traditionally used to evaluate cardiac function, has been used to assess Pgp function in tumors and normal tissues of patients receiving tariquidar by comparing time activity curves (TAC) in tumor, liver (positive control), lung (negative control), and heart [3]. Cardiac muscle does not express Pgp; therefore, accumulation and retention of  $^{99\text{m}}\text{Tc}$ -sestamibi are not altered by Pgp antagonists, and the heart can be used to normalize TAC between scans. In tissue expressing Pgp, such as normal liver, intestine, or some tumors, the retention of  $^{99\text{m}}\text{Tc}$ -sestamibi increases in the presence of Pgp antagonists [27].

Intrinsic and acquired drug resistance is a significant issue in pediatric solid tumors. Tariquidar is a potent and specific inhibitor of Pgp and has been tolerated in combination with chemotherapy in adults. We conducted a phase I trial of tariquidar in combination with doxorubicin, docetaxel, or vinorelbine in children and adolescents with recurrent or refractory solid tumors. The primary objective was to determine the maximum tolerated or recommended dose and toxicity profile of tariquidar in this population. Secondary objectives were to study the pharmacokinetics of tariquidar alone and in combination with chemotherapy. Pgp function was assessed using the rhodamine efflux flow cytometry assay in PBMC and by functional imaging using  $^{99\text{m}}\text{Tc}$ -sestamibi scintigraphy and immunohistochemical staining of archival tumor samples to examine Pgp expression in tumors.

## Methods

### Eligibility

Children and adolescents >2 years and less than 19 years of age were eligible if they had a solid tumor that was recurrent or refractory to standard treatment. Subjects must have

recovered from the toxic effects of prior therapy and have an ECOG performance status of  $\leq 2$ . Prior to enrollment, subjects must have met criteria for time intervals from prior anticancer and supportive therapy (Supplemental Table 1). Children and adolescents with brain tumors receiving corticosteroids had to be on a stable or decreasing corticosteroid dose. All subjects were required to have an absolute neutrophil count  $\geq 1500/\mu\text{L}$ , hemoglobin  $>8 \text{ gm/dL}$ , and platelet count  $\geq 100,000/\mu\text{L}$ , normal electrocardiogram, bilirubin  $\leq 1.5 \times$  upper limit of normal, alanine aminotransferase (ALT)  $<2.5 \times$  the upper limit of normal, and a normal age-adjusted serum creatinine or a creatinine clearance  $\geq 60 \text{ mL/min/1.73 m}^2$ .

For participants who received tariquidar in combination with doxorubicin, normal left ventricular ejection fraction was required and the prior cumulative anthracycline dose was restricted. In participants who received prior anthracycline as a bolus injection without a cardioprotectant (e.g., dexrazoxane) or if the subject had mediastinal radiation, the lifetime cumulative dose of anthracycline could not exceed  $300 \text{ mg/m}^2$  at enrollment. For participants who received prior anthracycline as a continuous infusion or with a cardioprotectant and no mediastinal radiation, the cumulative lifetime dose of anthracycline could not exceed  $400 \text{ mg/m}^2$  at enrollment.

Patients receiving other investigational agents were excluded, as were pregnant or breast-feeding females and patients with clinically significant unrelated systemic illness that would compromise data analysis or interpretation.

This trial was conducted under an investigator IND at the Pediatric Oncology Branch of the NCI and approved by the NCI Institutional Review Board. The use of radiation for research was approved by the NIH Radiation Safety Committee. The trial was registered with [clinicaltrials.gov](http://clinicaltrials.gov) (NCT00011414). All subjects or their legal guardians

provided informed consent, and participants provided verbal assent for protocol therapy and research tests.

**Treatment regimen and dose escalation**

Tariquidar (Xenova, LP) was administered intravenously (IV) over 30 min. The starting dose was  $1 \text{ mg/kg/dose}$  with escalation to  $1.5$  and  $2 \text{ mg/kg}$  in subsequent cohorts. During cycle 1, tariquidar was administered as a single agent, and then at least 48 h later, tariquidar was administered 60 min prior to chemotherapy (Fig. 1). For each participant, the chemotherapy regimen was chosen based on the histology of the tumor and their prior therapy. On subsequent cycles, tariquidar was administered in combination with chemotherapy. All participants received filgrastim ( $5 \text{ mcg/kg}$  subcutaneously) beginning 48 h after chemotherapy. Participants receiving doxorubicin received dexrazoxane. For subjects enrolled at the  $1$  or  $1.5 \text{ mg/kg}$  dose level, intra-subject dose escalation was permitted if the participant successfully completed two cycles at the enrollment dose, had at least stable disease, and did not experience dose-limiting toxicity attributable to tariquidar. Treatment cycles were 21 days in duration, but could be extended to 28 days to allow for participants to recover from toxicity. For participants who had stable disease or a response and did not experience toxicity that required discontinuation of protocol therapy, there was no limit on the number of cycles of therapy that could be administered. However, if a subject receiving doxorubicin reached a maximum lifetime cumulative anthracycline dose ( $500 \text{ mg/m}^2$ ) but was otherwise eligible to continue protocol therapy, the subject could switch their cytotoxic agent to docetaxel or vinorelbine for subsequent cycles.

Cohorts of 3–6 subjects were treated at each tariquidar dose level. When a minimum of three subjects who were

**Fig. 1** Chemotherapy schema. Chemotherapy was doxorubicin  $50 \text{ mg/m}^2/\text{dose}$  IV over 15 min once per cycle, docetaxel  $75 \text{ mg/m}^2/\text{dose}$  IV over 60 min once per cycle, or vinorelbine  $20 \text{ mg/m}^2/\text{dose}$  IV over 10 min weekly  $2 \times$  doses per cycle

**Treatment Schema Cycle 1**

Day	-1	0	1	2	3	4	5	6	7	8	9	10	11	12
			TQD		TQD							TQD*		
					DOX		→ GCSF							
					DOCETAXEL		→ GCSF							
					VRB							VRB	→ GCSF	
			pk	pk	pk	pk	pk							
			rh	rh										
	s		s											

TQD = tariquidar IV  
 TQD\* = tariquidar dose in subjects receiving vinorelbine  
 DOX = Doxorubicin ( $50 \text{ mg/m}^2$ )  
 DOCETAXEL = Docetaxel ( $75 \text{ mg/m}^2$ )  
 VRB = Vinorelbine ( $20 \text{ mg/m}^2$  weekly  $\times 2$  doses)  
 GCSF = filgrastim ( $5 \mu\text{g/kg}$  daily until count recovery)

pk = pharmacokinetics  
 rh = Rhodamine-123 assay  
 S- sestamibi scan

evaluable for toxicity completed one cycle of therapy at a dose level without evidence of dose-limiting toxicity, subsequent participants were enrolled at the next higher dose level. At the recommended dose of tariquidar, the study was expanded to treat up to six participants with each chemotherapeutic agent.

Monitoring for treatment-related toxicity included weekly physical examination, serum chemistries, and twice weekly complete blood counts. For participants receiving doxorubicin, echocardiogram was performed at baseline and then prior to each doxorubicin-containing cycle. Disease assessment was performed at baseline, prior to cycle 2, and then prior to every other cycle. After cycle 14, disease assessments were done after every fourth cycle.

### Definition of dose-limiting toxicity (DLT) and maximum tolerated dose (MTD)

In children, the acute toxicity profile of doxorubicin, vinorelbine, and docetaxel are well documented [8, 16, 19, 31]. Known toxicities of these anticancer agents do not overlap with tariquidar toxicity observed in adults. Toxicities attributable to cytotoxic agents that were within the expected severity range were not considered dose limiting. Hematological toxicity was attributed to the anticancer agent.

Clinical and laboratory adverse events were graded according to the NCI Common Toxicity Criteria version 2 (<http://ctep.info.nih.gov>). Hematological dose-limiting toxicity (H-DLT) was defined as grade 4 neutropenia (<500/ $\mu\text{L}$ ) of 5 or more days duration or grade 4 thrombocytopenia (<10,000/ $\mu\text{L}$ ) occurring on 2 or more days of the cycle. Platelet transfusion administered for a platelet count less than 20,000/ $\mu\text{L}$  but greater than 10,000/ $\mu\text{L}$  was considered grade 4 thrombocytopenia. In addition, failure to recover a neutrophil or platelet count to greater than or equal to 1500 or 100,000/ $\mu\text{L}$ , respectively, by day 28 of the treatment cycle was considered H-DLT. Non-hematological DLT included any grade 3 or 4 non-hematological toxicity or failure to recover to grade  $\leq 1$  toxicity or to baseline by day 28 of the treatment cycle.

The maximum tolerated dose (MTD) of tariquidar was determined from DLT occurring during cycle 1. The MTD was the dose level immediately below the dose level at which  $\geq 2$  patients in a cohort (dose level) of 2–6 patients experienced a DLT. The dose was not escalated above the recommended dose in adults (equivalent to 2 mg/kg). In the expanded cohort of subjects treated at the MTD or recommended dose of tariquidar, if less than 20 % experienced a DLT, the tariquidar dose would be considered tolerable in combination with chemotherapy.

Participants who experienced tariquidar-related DLT were offered up to two dose reductions during subsequent

cycles if they had benefited from the prior protocol treatment. The doxorubicin, vinorelbine, or docetaxel dose was reduced by 30 % on subsequent treatments cycles for dose-limiting hematological toxicity or any grade  $\geq 3$  non-hematological toxicity except for grade 3 nausea or vomiting. In addition, vinorelbine was discontinued in participants who experienced grade  $\geq 2$  neuropathy or constipation that did not diminish to grade  $\leq 1$  by day 28 of a treatment cycle. Docetaxel was discontinued in participants who experienced anaphylaxis or grade  $\geq 2$  neuropathy or peripheral edema that did not resolve to grade  $\leq 1$  by cycle day 28.

### Pharmacokinetic and pharmacodynamic studies

Pharmacokinetic evaluation of tariquidar alone (day 1) and in combination with doxorubicin, docetaxel, or vinorelbine (day 3) was conducted. Serial whole blood samples were collected from a site distant from the drug infusion. Samples were placed on ice and centrifuged within 2 h of collection. Plasma was stored at  $-70\text{ }^{\circ}\text{C}$  until analysis.

The plasma concentrations of tariquidar and cytotoxic drugs were measured using sensitive and specific methods (Supplementary data Table 2). Pharmacokinetic data were analyzed using non-compartmental methods. To determine whether tariquidar had a pharmacokinetic interaction with cytotoxic agents, the mean clearance of each cytotoxic agent was compared to published results in children [8, 12, 16, 19, 31, 33].

The rhodamine assay in CD56+ PBMCs was used as a surrogate measure of Pgp function [34]. Peripheral blood (6 mL) was obtained prior to and 24 h after administration of the first dose of tariquidar during cycle 1. Inhibition of rhodamine efflux was measured as previously described [30]. Flow cytometry analysis (FACSCalibur with CellQuest software; Becton–Dickinson, San Jose, CA) was performed using a live lymphocyte gate; 10,000 events per replicate were collected. The geometric mean fluorescence intensity (MFI) of intracellular rhodamine 123 in CD56+ lymphocytes was measured in the participant's sample at baseline (no tariquidar) and 24 h after systemic tariquidar administration and control (participant's baseline sample incubated ex vivo with 3  $\mu\text{M}$  tariquidar). Percent inhibition of rhodamine efflux was calculated as the ratio of the MFI 24 h after systemic tariquidar administration: MFI of control (participant's baseline sample incubated ex vivo with 3  $\mu\text{M}$  tariquidar).

To describe the relationship of tariquidar dose to Pgp inhibition, the percent inhibition of rhodamine efflux was plotted as a function of dose and the data fit to maximum effect model (MLAB, Civilized Software, Bethesda, MD) using the equation:

$$E(d) = \frac{(E_{\max} \cdot \text{Dose}^h)}{\text{Dose}_{50}^h + \text{Dose}^h} + E_{\min}$$

where  $E_{\max}$  is the maximum effect,  $\text{Dose}_{50}$  is the dose producing 50 %  $E_{\max}$ ,  $h$  is the slope of the sigmoid curve (the Hill Constant), and  $E_{\min}$  is the measured baseline effect.

$^{99\text{m}}\text{Tc}$ -sestamibi scintigraphy, functional imaging to assess Pgp inhibition in normal tissue and tumor [3], was performed at baseline and 48 h after the first dose of tariquidar.  $^{99\text{m}}\text{Tc}$ -sestamibi (0.29 mCi/kg, maximum 20 mCi) was administered and images acquired on

a dual-headed camera (ADAC Laboratories, Milpitas, CA) using low-energy/high-resolution collimators at the 140 keV photopeak of  $^{99\text{m}}\text{Tc}$ . Time activity curves (TAC) were generated in tumors, normal lung, liver, and heart and used to calculate the area under the concentration  $\times$  time curve (AUC) for 0- to 3-h interval. The percent change in tissue:heart  $^{99\text{m}}\text{Tc}$ -sestamibi  $\text{AUC}_{0-3\text{h}}$  was calculated for each tissue (lung, liver, or tumor) as follows:

$$\% \text{ Change} = \frac{[(\text{tissue:heartAUC}_{0-3\text{h}} \text{ postTariquidar}) - (\text{tissue:heartAUC}_{0-3\text{h}} \text{ preTariquidar})]}{\text{tissue:heartAUC}_{0-3\text{h}} \text{ preTariquidar}} \times 100$$

**Table 1** Subject characteristics, tariquidar dose levels, anticancer drug, tumor type, Pgp expression in tumors and response

Tariquidar dose (evaluable/ Subject enrolled)	Subject	Anticancer drug	Tumor type	Pgp tumor expression diagnosis/relapse	Best response [cycle]
1 mg/kg ( $n = 3/4$ )	001 <sup>a</sup>	Doxorubicin	Adrenocortical cancer	3+/2+	PD
	002	Doxorubicin	Adrenocortical cancer	2+/3+	SD [C8]
	003	Doxorubicin	Pleuropulmonary blastoma	1+/1+	PD
	004	Doxorubicin	Osteosarcoma	ND/ND	SD [C3]
1.5 mg/kg ( $n = 6/6$ )	005	Docetaxel	Ewings sarcoma	1+/-	PD
	006	Doxorubicin	Osteosarcoma	-/1+	PD
	007	Docetaxel	Ewings sarcoma	-/-	PD
	008	Doxorubicin	MPNST	1+/1+	PD
	009	Vinorelbine	Pancreatoblastoma	-/3+	CR [C16]
2 mg/kg ( $n = 17/19$ )	010	Docetaxel	Hodgkin's disease	ND	PD
	011	Doxorubicin	Adrenocortical cancer	3+/ND	SD [C1]
	012	Doxorubicin	Ewings sarcoma	-/-	PD
	013	Doxorubicin	NP carcinoma	-/-	SD [C1]
	014	Doxorubicin	Rhabdomyosarcoma	-/ND	PR [C2]
	015	Docetaxel	Ewings sarcoma	-/ND	SD [C1]
	016	Vinorelbine	Synovial cell sarcoma	-/1+	PD
	017	Doxorubicin	Osteosarcoma	-/ND	PD
	018 <sup>a</sup>	None	Pancreatoblastoma	ND	PD
	019	Vinorelbine	Undifferentiated sarcoma	ND	PD
	020	Docetaxel	Hepatoblastoma	ND	PD
	021	Docetaxel	Ewings sarcoma	ND	PD
	022	Doxorubicin	Undifferentiated sarcoma	-/-	PD
	023	Docetaxel	Neuroendocrine carcinoma	1+/1+	SD [C4]
	024 <sup>a</sup>	None	Osteosarcoma	ND	PD
025	Docetaxel	Ewings sarcoma	-/ND	SD [C2]	
026	Docetaxel	Undifferentiated sarcoma	ND	PD	
027	Vinorelbine	Pancreatoblastoma	ND	SD [C2]	
028	Vinorelbine	Ewings sarcoma	ND	PD [C1]	
029	Vinorelbine	Osteosarcoma	ND	PD [C1]	

ND not done, MPNST malignant peripheral nerve sheath tumor

<sup>a</sup> Not evaluable for toxicity

**Table 2** Anticancer drug-related toxicity during cycle 1

Tariquidar dose level	Toxicity	Grade	Dose-limiting toxicity	
<i>Doxorubicin-related toxicity</i>				
1 mg/kg ( <i>n</i> = 3)	Neutropenia ( <i>n</i> = 1)	3	No	
	Neutropenia ( <i>n</i> = 2)	4	No	
	Anemia ( <i>n</i> = 2)	3, 4	No	
	Thrombocytopenia ( <i>n</i> = 1)	3	No	
	Fever/neutropenia ( <i>n</i> = 1)	3	No	
	Alopecia ( <i>n</i> = 1)	3	No	
1.5 mg/kg ( <i>n</i> = 2)	Neutropenia ( <i>n</i> = 1)	3	No	
	Neutropenia ( <i>n</i> = 1)	4	Yes <sup>a</sup>	
	Thrombocytopenia ( <i>n</i> = 1)	4	No	
	Fever/neutropenia ( <i>n</i> = 1)	3	No	
	Mucositis ( <i>n</i> = 1)	3	No	
2 mg/kg ( <i>n</i> = 6)	Neutropenia ( <i>n</i> = 1)	4	Yes <sup>a</sup>	
	Neutropenia ( <i>n</i> = 1)	4	No	
	Neutropenia ( <i>n</i> = 2)	3	No	
	Thrombocytopenia ( <i>n</i> = 4)	3	No	
	Anemia ( <i>n</i> = 2)	3	No	
	Fever/neutropenia ( <i>n</i> = 2)	3	No	
	Infection without neutropenia ( <i>n</i> = 1)	3	No	
	Vomiting ( <i>n</i> = 1)	3	No	
	Esophagitis ( <i>n</i> = 1)	3	No	
	Diarrhea ( <i>n</i> = 1)	3	No	
	Hypocalcemia ( <i>n</i> = 1)	4	No	
	Hypomagnesemia ( <i>n</i> = 1)	4	No	
	<i>Docetaxel-related toxicities</i>			
	1.5 mg/kg ( <i>n</i> = 3)	Anemia ( <i>n</i> = 1)	3	No
Thrombocytopenia ( <i>n</i> = 1)		3	No <sup>b</sup>	
2 mg/kg ( <i>n</i> = 6)	Anemia ( <i>n</i> = 2)	3	No	
	Thrombocytopenia ( <i>n</i> = 2)	3	No <sup>b</sup>	
	Pain ( <i>n</i> = 1)	3	No	
	Rash ( <i>n</i> = 1)	2	No	
	Desquamation fingers/hands ( <i>n</i> = 1)	2	No	
<i>Vinorelbine-related toxicities</i>				
1.5 mg/kg ( <i>n</i> = 1)	Neutropenia ( <i>n</i> = 1)	4	Yes <sup>a</sup>	
	Anemia ( <i>n</i> = 1)	3	No	
2 mg/kg ( <i>n</i> = 5)	Neutropenia ( <i>n</i> = 1)	4	No	
	Anemia ( <i>n</i> = 1)	3	No	
	Nausea ( <i>n</i> = 1)	3	Yes <sup>c</sup>	
	Vomiting ( <i>n</i> = 1)	3	Yes <sup>c</sup>	
	Constipation ( <i>n</i> = 1)	3	Yes <sup>c</sup>	
	Sensory neuropathy ( <i>n</i> = 1)	3	No	
	Pain/tumor flare ( <i>n</i> = 1)	3	No	

<sup>a</sup> Not recovered to  $\leq$ grade 1 by day 28

<sup>b</sup> Docetaxel-related thrombocytopenia did not meet criteria for dose limiting; however, grade 3 thrombocytopenia in children receiving docetaxel is unusual

<sup>c</sup> Unable to administered day 8 vinorelbine due to toxicity

P-glycoprotein expression was assessed using immunohistochemistry with JSB-1 (Signet Laboratories, Cambridge MA) in paraffin-embedded formalin-fixed tumor

from previous surgical resections or diagnostic biopsies. Cell lines known to be strongly positive (MCF7ADR) and negative (MCF7) for Pgp expression were used as controls.



Tumor cells with membrane staining were scored by intensity as 0–3+.

## Results

Twenty-nine subjects with median (range) age of 13 (2–18) years, 16 females and 13 males, were enrolled. Twenty-two subjects were Caucasian; 7 were black; 6 were Hispanic; 23 were non-Hispanic. The median (range) number of prior chemotherapy regimens was 3 (1–6); fifteen subjects received prior radiation. Subject characteristics are presented in Table 1. Three participants were not evaluable for toxicity due to rapid tumor progression during cycle 1. One participant who received two cycles of tariquidar in combination with doxorubicin prior to reaching the protocol-specified cumulative lifetime dose of doxorubicin (500 mg/m<sup>2</sup>), therefore, was switched to docetaxel in combination with tariquidar and remained on study for seven additional cycles. Median number of cycles of protocol therapy administered was 1 (range 1–31).

No DLT related to tariquidar was observed. Tariquidar-related toxicities observed during cycle 1 were grade 1 pruritus ( $n = 1$ ) at dose level 2 and grade 1 nausea ( $n = 1$ ), grade 1 dysgeusia ( $n = 3$ ), grade 2 hypotension ( $n = 2$ ), and grade 2 peripheral edema ( $n = 1$ ) at dose level 3. No

maximum tolerated dose was achieved. One participant met the criteria for intra-subject dose escalation, his tariquidar dose was increased from 1 mg/kg to 1.5 mg/kg, and he tolerated the increased dose without toxicity.

Serious toxicities related to each of the anticancer drugs during cycle 1 are presented in Table 2. Overall, DLT related to cytotoxic agents administered in combination with tariquidar (2 mg/kg) was 12 % (2/17); 20 % (1/5) for vinorelbine; 17 % (1/6) for doxorubicin; and 0 % (0/6) for docetaxel. Unexpected thrombocytopenia was observed in three participants receiving docetaxel in combination with tariquidar [8]. During cycle 2, subject 009 received tariquidar (1.5 mg/kg) in combination with vinorelbine and experience delayed neutrophil recovery, and the vinorelbine dose was reduced by 30 % (14 mg/m<sup>2</sup>). This subject received tariquidar in combination with vinorelbine for 30 additional cycles without recurrent or cumulative toxicity.

## Pharmacokinetic and pharmacodynamic studies

Plasma pharmacokinetic parameters for tariquidar alone (day 1) and tariquidar in combination with anticancer agents (day 3) are presented in Table 3 along with the parameters for the chemotherapeutic agents administered with tariquidar. Pharmacokinetic parameters were highly variable. At all dose levels, the tariquidar  $C_{max}$  when administered

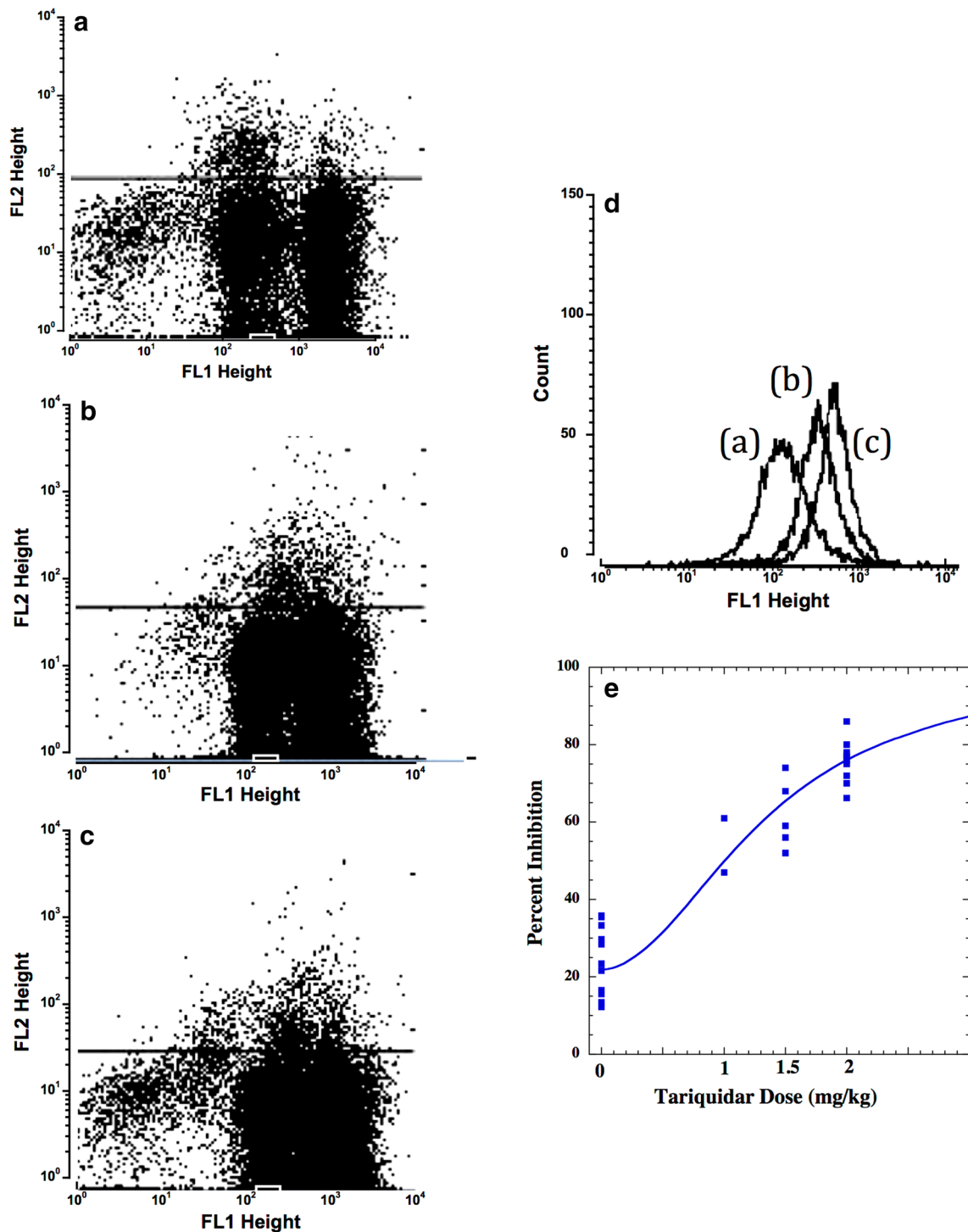
**Table 3** Plasma pharmacokinetics (mean  $\pm$  SD) of tariquidar (day 1 alone and day 3 with chemotherapy) and cytotoxic agents

Tariquidar dose level (mg/kg)	Tariquidar				Doxorubicin				Doxorubicinol	
	Day	$C_{max}$ ( $\mu$ M)	$AUC_{0-48}$ ( $\mu$ M h)	CL (ml/min/m <sup>2</sup> )	$N$	$C_{max}$ ( $\mu$ M)	$AUC_{0-48}$ ( $\mu$ M h)	CL (ml/min/m <sup>2</sup> )	$C_{max}$ ( $\mu$ M)	$AUC_{0-48}$ ( $\mu$ M h)
1	1	1.2 $\pm$ 0.9	4 $\pm$ 2.8	260 $\pm$ 180						
	3	2.2 $\pm$ 2.1	5.4 $\pm$ 4	180 $\pm$ 90	4	3.0 $\pm$ 0.5	2.3 $\pm$ 0.5	500 $\pm$ 90	0.03 $\pm$ 0.01	1.0 $\pm$ 0.6
1.5	1	2 $\pm$ 1.1	7.3 $\pm$ 2.5	160 $\pm$ 100						
	3	2.3 $\pm$ 2.8	10.5 $\pm$ 1.2	100 $\pm$ 20	2	3.5 $\pm$ 1.9	2.2 $\pm$ 1.1	275 $\pm$ 150	0.05 $\pm$ 0.05	1.2 $\pm$ 0.9
2	1	2.8 $\pm$ 0.9	11.3 $\pm$ 5.6	120 $\pm$ 100						
	3	2.5 $\pm$ 0.3	11.9 $\pm$ 5.5	120 $\pm$ 60	2	2.3 $\pm$ 0.9	1.6 $\pm$ 0.06	390 $\pm$ 20	0.03 $\pm$ 0.01	0.9 $\pm$ 0.7
Overall				166 $\pm$ 102	8	3.0 $\pm$ 1.2	2.1 $\pm$ 0.6	400 $\pm$ 190	0.03 $\pm$ 0.02	1.0 $\pm$ 0.6
Reference CL				160 $\pm$ 105 [2, 7]				420 $\pm$ 90 [33]		

Tariquidar dose level (mg/kg)	Docetaxel				Vinorelbine			
	$N$	$C_{max}$ ( $\mu$ M)	$AUC_{0-48}$ ( $\mu$ M h)	CL (ml/min/m <sup>2</sup> )	$N$	$C_{max}$ ( $\mu$ M)	$AUC_{0-48}$ ( $\mu$ M h)	CL (ml/min/m <sup>2</sup> )
1								
1.5								
	2	3.4 $\pm$ 1.3	5.6 $\pm$ 3.4	310 $\pm$ 170				
2								
	4	3.8 $\pm$ 1.4	5.3 $\pm$ 3.1	340 $\pm$ 150	4	5.1 $\pm$ 0.4	2.5 $\pm$ 1.1	185 $\pm$ 90
Overall	6	3.7 $\pm$ 1.3	5.4 $\pm$ 2.8	330 $\pm$ 140	4	5.1 $\pm$ 0.4	2.5 $\pm$ 1.1	185 $\pm$ 90
Reference CL				550 [12]				564 $\pm$ 148 [2]

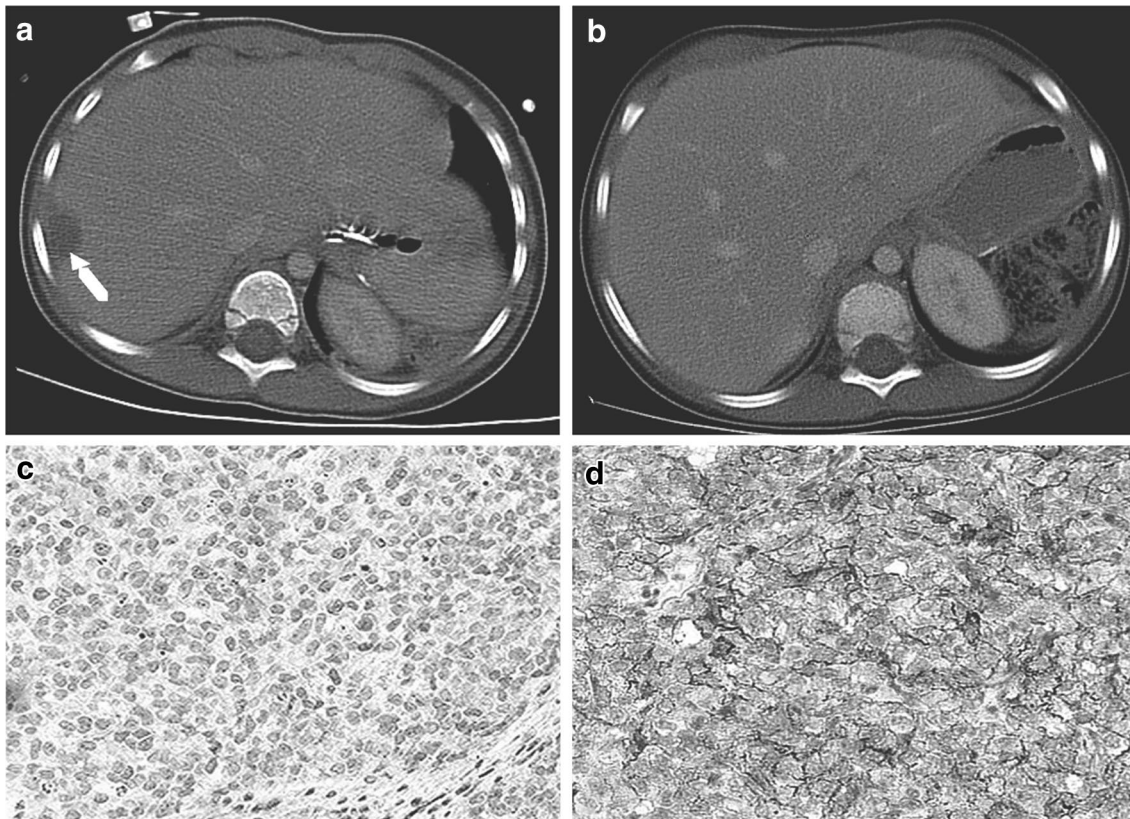
$C_{max}$  maximum concentration,  $AUC_{0-48}$  measured area under the plasma concentration  $\times$  time curve from 0 to 48 h, CL clearance



**Fig. 2** Rhodamine assay using flow cytometry to measure the intracellular rhodamine fluorescence in CD56+ staining cells. *Dot plots* of a patient's cells at **a** baseline, **b** 24 h after systemic administration of 2 mg/kg of tariquidar, and **c** control (patient's blood exposed ex vivo to 3 μM tariquidar for 1 h) are presented. CD56+ cells are in the *upper portion* of the graph (positive in FL2), and the intracellular rhodamine content is plotted on the FL1 axis. A histogram **d** of the mean fluorescent intensity of intracellular rhodamine (FL1) is presented at

**a** baseline, **b** 24 h after systemic administration of tariquidar, and **c** control. For each patient, percent inhibition as a function of tariquidar dose is plotted and a maximum effect model of drug effect is fit to the data (**e**). Percent inhibition of Pgp in the rhodamine assay is dose dependent. At the 2 mg/kg dose level, the median percent inhibition was 77 %. The maximum effect model predicts maximal inhibition of 80 %





**Fig. 3** Subject 009, a 2-year-old female with recurrent pancreatoblastoma, had recurrent tumor in liver (abdominal CT baseline, **a**), and a complete radiographic and clinical response was confirmed after 16 cycles of tariquidar in combination with vinorelbine (**b**). **c**

Immunohistochemistry staining for Pgp at initial diagnosis and at relapse prior to enrollment on this study (**d**) in this subject. This subject completed 31 cycles of therapy without cumulative toxicity and remains free of disease more than 5 year after completion of therapy

in combination with chemotherapy exceeded  $2 \mu\text{M}$ . Overall, the clearance of docetaxel and vinorelbine was reduced compared to previously published results (Table 3).

Assessment of Pgp function using rhodamine retention in CD56+ lymphocytes was completed prior to and 24 h after the first dose of tariquidar in all patients. Data from five patients were excluded due to inadequate recovery of live lymphocytes. The median (range) percent inhibition of rhodamine efflux was 22.5 (range 12.2–35.5), 54 (range 47–61), 63.5 (range 52–74), and 77 (range 66.2–86) at the control, 1, 1.5, and 2 mg/kg dose levels, respectively. A dose-dependent inhibition in rhodamine efflux was observed (Fig. 2). The maximum effect model fit to the data predicts a plateau of 80 % inhibition of Pgp function in CD56+ lymphocytes 24 h after administration of 2 mg/kg of tariquidar.

Six patients completed paired  $^{99\text{m}}\text{Tc}$ -sestamibi scans, and two patients had tumors that were evaluable by  $^{99\text{m}}\text{Tc}$ -sestamibi scintigraphy. In six patients, the median (range) percent change in  $^{99\text{m}}\text{Tc}$ -sestamibi accumulation in lung was 15 % (3–22 %) and liver was 162 % (68–305 %). For patients with tumor evaluable by  $^{99\text{m}}\text{Tc}$ -sestamibi scintigraphy, the percent change in tumor was 140 % in the

hepatic metastasis ( $n = 1$ ), 18 % in soft tissue metastatic sites ( $n = 2$ ), and 28 % (14–40 %) in pulmonary nodules ( $n = 5$ ). The enhanced uptake of tracer in the hepatic metastasis after tariquidar may in part be related to the increased uptake in normal liver tissue anterior and posterior to the metastatic lesion and may not reflect the actual change in the tumor uptake and retention.

Tumor Pgp expression determined by immunohistochemical staining using JSB-1 is presented in Table 1. Archival tumor specimens were available from 18 subjects. There was no association between Pgp-positive immunohistochemical staining of tumor and radiographic response.

## Response

A 2-year-old with pancreatoblastoma and recurrent hepatic metastasis achieved a complete radiographic response by Response Evaluation Criteria in Solid Tumors (RECIST) after 16 cycles of tariquidar in combination with vinorelbine (Fig. 3). She continued on protocol therapy for 31 cycles and remains in continuous complete remission more than 5 years after completing therapy. This subject's tumor had the highest tumor expression of Pgp (Table 1). Two patients

experienced partial responses. A 14-year-old female with rhabdomyosarcoma with metastatic recurrence to her breast experienced a partial response after four cycles of doxorubicin in combination with tariquidar. She declined further protocol therapy and received local radiation therapy to achieve a complete response. She refused adjuvant chemotherapy, her disease recurred, and she died of complications of recurrent rhabdomyosarcoma. A 7-year-old male with metastatic adrenocortical carcinoma experienced a partial response during two cycles of therapy with tariquidar in combination with doxorubicin. Due to his cumulative dose of doxorubicin, his therapy was switched to docetaxel in combination with tariquidar and after seven additional cycles was removed from protocol therapy due to progressive disease.

## Discussion

In children and adolescents, toxicities related to tariquidar were mild and included transient hypotension, taste disturbance, and nausea. No dose-limiting toxicities were observed when tariquidar was administered alone. The pharmacokinetics of tariquidar were highly variable. At all dose levels, the  $C_{\max}$  of tariquidar exceeded the concentration required for maximal inhibition of Pgp in CD56+ lymphocytes in prior studies. The inhibition of rhodamine efflux from CD56+ lymphocytes 24 h after systemic administration of tariquidar was dose dependent. At the 2 mg/kg dose level, the median percent inhibition was 77 %. Using a maximum effect model of drug effect fit to the data, we predict maximal inhibition of rhodamine efflux is 80 %. Therefore, based on this surrogate assay, increasing the dose of tariquidar above 2 mg/kg is unlikely to increase Pgp inhibition. Using functional imaging to assess Pgp function, we observed that  $^{99m}\text{Tc}$ -sestamibi accumulation in the liver, which expresses Pgp, and in some tumors increased after tariquidar administration. Whereas 2 mg/kg was well tolerated and biologically active by our surrogate biomarker endpoint, we conclude that the recommended dose of tariquidar in children is 2 mg/kg IV over 30 min.

In children, the clearance of docetaxel and vinorelbine administered with tariquidar was less than previously reported. Reduced total body clearance resulting in increased drug exposure (AUC) could contribute to the unexpected toxicity of docetaxel and vinorelbine when administered in combination with tariquidar. Of the nine participants who received tariquidar in combination with docetaxel, three (33 %) experienced grade 3 thrombocytopenia (platelet count  $\geq 10,000$ ,  $< 50,000/\mu\text{L}$ ). In a prior dose finding study of single-agent docetaxel (150–235 mg/m<sup>2</sup>) in children and adolescents with refractory solid tumors, platelet counts less than 50,000/ $\mu\text{L}$  were not observed [8, 31]. In addition, gastrointestinal toxicity (grade 3 nausea,

vomiting, and constipation) associated with vinorelbine in combination with tariquidar may be more prevalent than previously reported for vinorelbine alone [19].

A limitation of the functional imaging studies performed in this trial was the use of planar imaging. The results confounded by activity overlying and underlying foci of disease, and by attenuation of photons by overlying and underlying normal tissues decreases sensitivity, particularly for low-intensity lesions. Thus, the percent change in sestamibi uptake in the liver metastases in this study likely reflects increased uptake in overlying and underlying normal liver tissue. These limitations could be significantly mitigated through the use of SPECT CT or  $^{94m}\text{Tc}$ -sestamibi PET CT. The limitations of immunohistochemical staining for Pgp included the use of archival tissue and the very high expression of Pgp of our positive control cells, MCF7 ADR. Therefore, we may have underestimated the Pgp expression in tumors in children and adolescents participating in this study.

In this trial, we identified a tolerable and biologically active dose of a specific Pgp inhibitor, tariquidar, in children and adolescents. We did not identify an association between tumor Pgp expression by immunohistochemistry and clinical response. The prognostic significance of Pgp in childhood cancers remains controversial, and the availability of tariquidar for additional clinical trials is limited. However, this trial demonstrates that modulators of resistance can be evaluated in combination with chemotherapy and that pharmacokinetic and pharmacodynamic correlates can be useful in establishing the optimal dose.

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**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no conflict of interest.

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