Published in final edited form as:

Pediatr Blood Cancer. 2010 May ; 54(5): 694–702. doi:10.1002/pbc.22366.

Phase I Study of Valspodar (PSC-833) with Mitoxantrone and Etoposide in Refractory and Relapsed Pediatric Acute Leukemia: A Report from the Children's Oncology Group

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Abstract

Background—Valspodar, a non-immunosuppressive analog of cyclosporine, is a potent P-glycoprotein (*MDR1*) inhibitor. As *MDR1*-mediated efflux of chemotherapeutic agents from leukemic blasts may contribute to drug resistance, a phase 1 study of valspodar combined with mitoxantrone and etoposide in pediatric patients with relapsed or refractory leukemias was performed.

Procedure—Patients received a valspodar loading dose (2 mg/kg) followed by a five-day continuous valspodar infusion (8, 10, 12.5 or 15 mg/kg/day) combined with lower than standard doses of mitoxantrone and etoposide. The valspodar dose was escalated using a standard 3 + 3 phase I design.

Results—21 patients were evaluable for toxicity and 20 for response. The maximum tolerated dose (MTD) of valspodar was 12.5 mg/kg/day, combined with 50% dose-reduced mitoxantrone and etoposide. The clearance of mitoxantrone and etoposide was decreased by 64% and 60%, respectively, when combined with valspodar. Dose-limiting toxicities included stomatitis, ataxia, and bone marrow aplasia. Three of 11 patients with acute lymphoblastic leukemia (ALL) had complete responses while no patient with acute myeloid leukemia (AML) had an objective

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Disclosures: The authors have no conflicts of interest to disclose.

response. *In vitro* studies demonstrated P-glycoprotein expression on the blasts of five of 14 patients, although only one had inhibition of rhodamine efflux by valspodar.

Conclusions—While this regimen was tolerable, responses in this heavily pretreated population were limited to a subset of patients with ALL.

Keywords

leukemia; MDR1; multidrug resistance; P-glycoprotein; PSC-833; valspodar

INTRODUCTION

Despite intensive chemotherapy and the selective use of bone marrow transplantation, approximately 50% of children with acute myeloid leukemia (AML) and 20% of those with acute lymphoblastic leukemia (ALL) will die from their disease [1]. For these children, outcomes are poor due in part to resistance to chemotherapeutic drugs [2,3]. The *MDR1* (*ABCB1*) gene encodes P-glycoprotein, an ATPase-dependent multidrug transporter responsible for the efflux of multiple chemotherapeutic agents used in leukemia therapy, including vinca alkaloids, anthracyclines, and epipodophyllotoxins [4,5]. In the hope of improving the efficacy of *MDR1*-substrate chemotherapeutic drugs, pharmacologic agents that block *MDR1*-mediated drug efflux have been developed, including valspodar (PSC-833, Novartis) [6,7].

Valspodar is a non-immunosuppressive analog of cyclosporine (CsA) with more potent P-glycoprotein inhibition and without significant immunosuppression or nephrotoxicity [8]. Toxicities associated with valspodar and concomitant chemotherapy (mitoxantrone, etoposide, cytarabine) in adults with leukemia include stomatitis, cerebellar ataxia, and reversible hyperbilirubinemia, with a maximum tolerated dose (MTD) of 10 mg/kg/day [9]. In the Pediatric Oncology Group (POG) phase II protocol 9222, CsA was added to a five-day induction regimen of mitoxantrone and etoposide in pediatric patients with relapsed or refractory AML with a 50% response rate (complete plus partial) [10]. Based on these results and the correlation of P-glycoprotein expression with outcomes in pediatric leukemias [11,12], we initiated a phase I trial (POG 9423) of valspodar with the reduced-dose mitoxantrone and etoposide regimen from POG 9222 in children with recurrent or refractory leukemia.

METHODS

Patient Eligibility

Patients with relapsed or refractory leukemia < 22 years of age at the time of diagnosis were eligible. Patients with AML were eligible if they met one of the following criteria: (1) duration of first bone marrow remission < 6 months, (2) refractory to standard AML induction, (3) secondary AML or AML evolving from myelodysplastic syndrome, or (4) bone marrow relapse following bone marrow transplantation (BMT). Patients with ALL were eligible if they had: (1) second or subsequent bone marrow relapse or (2) bone marrow relapse following BMT. All patients with prior BMT were required to have had trilineage engraftment and be at least six months from transplant.

Other eligibility criteria included: (1) recovery from prior therapy; (2) no therapy, with the exception of hydroxyurea, in the 4 weeks prior to enrollment; (3) adequate performance status (Lansky >40% if < 12 years or Karnofsky $\geq$ 50% or ECOG $\leq$ 2); (4) life expectancy > 8 weeks; (5) adequate renal (normal creatinine or glomerular filtration rate for age) and hepatic function [bilirubin < 1.5x normal, alanine aminotransferase (ALT) < 2x normal,

albumin > 3 g/dL]; and (6) cardiac ejection fraction >50% or shortening fraction $\geq 27\%$ with less than 360 mg/m² cumulative prior anthracycline exposure. Exclusion criteria included history of etoposide allergy, pregnancy or breast feeding, use of anticonvulsant medication, uncontrolled infection, and isolated central nervous system or extramedullary relapse.

Local institutional review board approval was obtained by all participating institutions, and all patients or their legal guardians provided written informed consent in accordance with federal and institutional requirements.

Study Design

Dose escalation of valspodar (PSC 833, Novartis Pharmaceutical Corporation, East Hanover, NJ) followed a traditional 3 + 3 phase I design in which cohorts of three patients at a time were treated at a dose level. If no dose limiting toxicity (DLT) was observed, the dose for the next cohort was escalated to the next level. If one of three patients experienced a DLT, three additional patients were enrolled at that dose level. If two of six patients experienced a DLT, then the maximum tolerated dose (MTD) was exceeded. If two of three patients experienced a DLT, then three additional patients were enrolled at the next lower dose level, if six patients had not already been enrolled. The MTD was defined as the dose immediately below the level at which two patients in a cohort of three to six experienced a DLT.

Toxicities were graded according to the National Cancer Institute Common Toxicity Criteria (CTC) version 2.0. Any nonhematologic grade 3 or greater toxicity that was possibly, probably, or definitely attributable to valspodar was considered a DLT with specific exclusions. Due to expected toxicities from mitoxantrone and etoposide, grade 3 nausea, infection, and stomatitis were not considered DLTs. As reversible hyperbilirubinemia was expected with valspodar, grade 3 hyperbilirubinemia that resolved following completion of the valspodar infusion was not considered a DLT. Myelosuppression was not considered a DLT except for prolonged bone marrow aplasia greater than 6 weeks. A daily neurologic examination was required throughout the valspodar infusion and grade 3 cerebellar ataxia (markedly abnormal gait defined as inability to walk without assistance from another person or walker) was considered a DLT. In the event of a single fatal toxicity, study registration would be suspended and the study reviewed with the Phase I Cooperative Agreement Scientific Principal Investigator. Six years following study completion, hard copies of all study documentation for each patient including flow sheets and laboratory values were obtained from the POG archives and retrospectively reviewed to ascertain toxicities which were not reported at the time of the study.

Drug Administration

Each valspodar-ME chemotherapy course lasted five days. On day 1 each patient received etoposide [100 mg/m² intravenously (IV) over 1 hour] followed by mitoxantrone (10 mg/m² IV over 15 minutes) without valspodar. The valspodar loading dose (2 mg/kg/hr IV over 2 hours) was then administered between hours 22 and 24 of the first day of therapy at the same time that a continuous valspodar infusion for 122 hours was initiated at the assigned dose level. This loading dose was administered in an attempt to rapidly achieve a steady-state valspodar level greater than 1 μ M. The valspodar continuous infusion dose for the first cohort was 8 mg/kg/day over 122 hours based on the MTD in adults of 10 mg/kg/day, with subsequent escalations of the infusion dose to 10, 12.5, 15, 17.5 and 20 mg/kg/day over 122 hours. Following the valspodar loading dose, each patient received etoposide and mitoxantrone daily for 4 days (days 2-5) at 50% of the day 1 dose due to known interactions with *MDR1* inhibitors and based on prior experience with cyclosporine plus mitoxantrone

and etoposide [13]. Patients with concurrent CNS leukemia received intrathecal therapy with methotrexate, hydrocortisone, and cytarabine weekly x four doses.

Patient Evaluation

Clinical and laboratory studies prior to treatment included the following: history and physical examination with baseline cerebellar evaluation, performance status, bone marrow aspiration and biopsy, chest x-ray, echocardiogram, complete blood count (CBC), electrolytes, creatinine, and liver function tests. In addition, a bone marrow sample was obtained for P-glycoprotein studies. Evaluations during therapy included weekly physical examinations, twice weekly CBC, electrolytes and creatinine. Bilirubin was monitored daily during the valspodar infusion.

Response Assessment

Disease status was evaluated by bone marrow aspirate and biopsy at day 14 of the first cycle and prior to the next cycle. If there was evidence of progressive disease the patient was removed from the study. If the day 14 marrow was hypocellular and definite blasts were absent, no additional therapy was given until count recovery at which time the bone marrow aspirate was repeated to document response status. If the day 14 marrow was hypocellular with <25% leukemic blasts, the patient was eligible for a second course of valspodar-ME once any toxicities had resolved.

Response was defined as: (1) complete remission [CR, M1 marrow (<5% blasts) with peripheral blood absolute neutrophil count (ANC) > 500/ul and platelets (transfusion independent) > 100,000/ul]; (2) partial response [PR, M2 marrow (5-25% blasts) with > 50% reduction in bone marrow blast percentage]; or (3) progressive disease [PD, patients who either had no response by day 14 bone marrow aspirate or whose bone marrow became hypocellular in response to therapy but had leukemia recurrence (M3 marrow; >25% blasts) upon count recovery].

Pharmacokinetic Analyses

Peripheral blood was sampled for pharmacokinetic studies of valspodar, etoposide, and mitoxantrone. For valspodar, 1 mL of blood was obtained in EDTA-containing tubes prior to and at 24, 72, 85, 120, 122, 126, and 144 hours following the start of the infusion. Samples were kept frozen at less than -20°C until processing. Valspodar concentrations were determined by radioimmunoassay (ANAWA Laboratories, Zürich, Switzerland) by Novartis Pharmaceuticals Corporation (East Hanover, NJ) as previously described [14]. The lower limit of quantification was 75 ng/mL.

Etoposide and mitoxantrone concentrations were determined with and without concomitant valspodar. Blood (2.5 to 5 mL) was collected in heparinized tubes prior to the chemotherapy infusion and at 0, 0.5, 5, 12, and 21 hours after the infusion on days 1 (without valspodar) and 4 (with valspodar) of cycle 1. Mitoxantrone plasma samples were stabilized with the addition of 0.3 ml of 5% ascorbate and stored at -80°C prior to analysis. Total plasma mitoxantrone was analyzed as previously described using a reverse-phase HPLC method using ametantrone (a gift provided by Wyeth-Ayerst Research, Philadelphia, PA, USA) as an internal standard [15]. Total plasma etoposide was analyzed by reverse-phase HPLC using a previously described method [15,16].

Pharmacokinetic Modeling

A linear two-compartment model with the intravenous infusion modeled as a zero order input was used to characterize mitoxantrone and etoposide pharmacokinetics. A hierarchical model was used to account for inter-individual and intra-individual variability [17]. Inter-

individual variability in CL (clearance from the central compartment), V1 (volume of the central compartment), and V2 (volume in the peripheral compartment) were modeled assuming a lognormal distribution. Intra-individual residual variability was described using a proportional component.

To examine and quantify the drug interactions with valspodar (VAL) we modeled the change in clearance of mitoxantrone or etoposide as follows: $CL_{\text{Mitox/etop}} = CL_0 + (CL_1 - CL_0) * VAL / (VAL + EC_{50})$ where CL_0 is the baseline clearance, CL_1 is the asymptotic value of CL for large concentrations of valspodar (when $VAL \rightarrow \infty$), and EC_{50} is the concentration of valspodar when the clearance of the chemotherapy agent is 50% of the baseline clearance (CL_0). Model selection was based on a comparison of the $-2 \log$ likelihood and visual inspection of goodness of fit diagnostic plots. Variances are expressed as percent coefficients of variation (%CV). All data analysis was carried out using NONMEM V (GloboMax LLC, Hanover, MD) [18]. Graphical output was generated with S-Plus [19].

P-glycoprotein expression—At enrollment, bone marrow (or peripheral blood if blast concentration was greater than 15%) was obtained for P-glycoprotein cell surface expression and function. Samples were diluted in an equal volume of RPMI-1640 medium and transported at room temperature by overnight courier to the central *MDR1*/drug-resistance reference laboratory (Y.R.; Wayne State University, Detroit, MI). Mononuclear cells were separated by centrifugation on Ficoll-Hypaque gradients and resuspended in RPMI-1640 medium containing 20% dialyzed fetal bovine serum and insulin, transferrin, and selenite. Surface expression of P-glycoprotein was measured by flow cytometry at room temperature using the phycoerythrin-conjugated monoclonal antibody 4E3 [20,21]. Staining intensity was evaluated by comparing 4E3-PE binding with that of its matched isotype control with the Kolmogorov-Smirnov statistic, expressed as a D-value ranging from 0 (identical distribution histograms) to 1.0 (no overlap in distribution histograms) [22]. A D-value of ≥ 0.15 is considered positive. P-glycoprotein function was measured by rhodamine123 (R123) efflux in the presence or absence of CsA or valspodar, as previously described [23]. Cell lines with and without P-glycoprotein expression (CEM, S226, CEM-VBL, S226-Dox) were used as controls. R123 efflux was quantified as the log-shift of the peak of the fluorescence curve with the addition of CsA or valspodar. A shift of 0.5 log or greater was considered evidence of inhibition of R123 efflux.

RESULTS

Patients

Twenty-three patients were enrolled on POG 9421 between November 1997 and June 2001. One patient was ineligible due to pre-existing mucositis requiring intravenous narcotics at the time of enrollment. Table I shows the demographic and clinical characteristics for the 22 eligible patients. One patient (#21) was initially enrolled but developed hyperbilirubinemia from cholelithiasis and biliary obstruction prior to starting treatment; he did not receive the study drug and was not evaluable for toxicity or response. One patient (#13) died at day 17 due to infection and was therefore not evaluable for response. Thus, 21 patients were evaluable for toxicity and 20 for response.

Dose Escalation and Toxicities

Table II shows the grade 3 and 4 toxicities at the administered valspodar dose levels. Twenty-one patients were evaluable for toxicity. No dose-limiting toxicities were initially reported for the three patients treated at dose level 1 (8 mg/kg/day). However, after subsequent patients had been treated at dose level 2 (10 mg/kg/day), it was discovered that the first patient at dose level 1 had developed grade 4 stomatitis in the setting of Down

syndrome (DS). Given the known sensitivity of patients with DS to cytotoxic chemotherapy, and the fact the subsequent non-DS patients treated at dose levels 1 and 2 had not had significant stomatitis, this was not deemed to be a DLT. While the study was not formally amended to exclude patients with DS, no further patients with DS were enrolled.

Five eligible patients were treated at dose level 2 (10 mg/kg/day), without a reported DLT. This cohort was expanded to more than three patients due to the occurrence of significant stomatitis in one patient, who was subsequently found to be ineligible due to presence of pre-existing grade 3 stomatitis from prior cytotoxic chemotherapy at the time of enrollment. At dose level 3 (12.5 mg/kg/day), one patient experienced a DLT (grade 3 cerebellar toxicity) and the cohort was expanded to six patients. The fourth patient treated at dose level 3 developed bacterial sepsis while neutropenic at day 17 and died. The study was temporarily suspended for review, and it was decided that this event was not considered related to valspodar but rather to expected myelosuppression from the conventional chemotherapy and therefore was not considered a DLT in the absence of prolonged marrow aplasia. At dose level 4 (15 mg/kg/day), three of seven patients experienced grade 4 stomatitis, one with culture-positive herpes simplex virus infection. One patient at this dose level had prolonged aplasia with <10% bone marrow cellularity for 7 weeks at which point the bone marrow recovered with > 25% blasts. One patient also had grade 4 hypokalemia. Therefore, for this regimen, the maximum tolerated dose of valspodar in combination with mitoxantrone and etoposide (at 50% dosing) was 12.5 mg/kg/day (dose level 3).

Cerebellar toxicity, manifested by ataxia or dizziness, was reported in five patients (one grade 3, two grade 2, two grade 1). Valspodar concentrations were measured in four of these patients (#3,11,13,19); in each case the median valspodar concentration over the course of the continuous infusion and the valspodar concentration at hour 85 (mid-infusion) were in the uppermost tertile, regardless of assigned dose level (Figure 1). Symptoms resolved following completion of the valspodar infusion in four of five patients, including the patient with grade 3 toxicity. The patient with persistent symptoms initially reported mild dizziness (grade 1) on day 4 of the valspodar infusion, which resolved within 48 hours. However, 20 days later she developed dizziness with movement diagnosed as vertigo after full neurologic evaluation. Symptoms improved with meclizine.

As anticipated, all patients experienced profound myelosuppression and required hematologic support with packed red blood cell and platelet transfusions. All patients experienced febrile neutropenia and were hospitalized for broad-spectrum antimicrobial therapy. Infections were common, including varicella zoster (n=1), sinusitis (n=1), central line cellulitis (n=1), pneumonia (n=3), and bacteremia (n=5). Only one fungal infection was reported (*Candida sp.* pneumonia). Transient hyperbilirubinemia (median maximum 2 mg/dl, range 0.8-6.6 mg/dl) that resolved after completion of valspodar infusion occurred in 16 of 21 patients. Four patients also had valspodar infusion-related transaminase elevations (one grade 1, two grade 2, one grade 3), which self-resolved.

Pharmacokinetic Analysis

Valspodar concentrations were measured in 16 of 20 patients and are summarized in Figure 1 by assigned dose level. Ten of 16 patient had the majority of required samples obtained while six had adequate samples submitted at only hour 85 (mid-infusion) and hour 144 (24 hours following the completion of the infusion). Valspodar concentrations were highly variable and did not correlate with assigned dose level; higher levels did correlate with cerebellar symptoms. At the mid-infusion sampling time (hour 85), measured valspodar levels ranged from 1,240 ng/mL to 11,500 ng/mL (median 2,590 ng/mL). Thus, at some point during the infusion, all patients had valspodar levels greater than those which achieve *MDR1* reversal *in vitro* (1,000 ng/mL) [24]. Median valspodar concentrations for each

patient over the course of the infusion ranged from 1,130 ng/mL to 7,825 ng/mL (overall median 2,030 ng/mL).

Valspodar, mitoxantrone, and etoposide levels were concurrently measured in 14 patients; levels were fit to the 2-compartment structural model as described in the methods section. The population pharmacokinetic parameters for mitoxantrone and etoposide are summarized in Table III. The population estimate of the day 1 mitoxantrone clearance (pre-valsopodar) was 16.4 L/h, which decreased by 64% to 5.88 L/h on day 4 in the presence of valsopodar. The population estimate of the day 1 etoposide clearance (pre-valsopodar) was 1.52 L/h with a 60% decrease to 0.61 L/h with concomitant valsopodar. Because the achieved valsopodar concentrations exceeded the estimated EC_{50} by greater than 2 to 3-fold in the majority of patients (mean BSA 1.3 m²), the CL_1 values were used to estimate the area under the curve (AUC) of mitoxantrone and etoposide. During valsopodar treatment, the estimated mean AUC value for mitoxantrone increased 33% (1.11 versus 0.79 mg*h/L) and for etoposide, 23% (106 versus 85.5 mg*h/L). Thus, the empiric 50% dose reduction of chemotherapy during valsopodar infusion maintained exposure in the typical patient.

Treatment Response

Twenty patients were evaluable for response. One patient with pre-B ALL who died of *Citrobacter freundii* sepsis at day 17 was not evaluable for response. Bone marrow biopsy at autopsy was hypocellular with no evidence of blasts. Three patients, one with T-ALL and two with pre-B ALL, experienced complete remission (CR). Both pre-B ALL patients entered the study in their third bone marrow relapse. One was treated at dose level 2 with median valsopodar level of 1,240 ng/mL (Figure 1, #9) and the other received two courses of valsopodar-ME at dose level 3 with median valsopodar level in the first course of 2,405 ng/mL (Figure 1, #15); both proceeded to bone marrow transplant. The T-ALL patient was treated at dose level 1, but had the highest measured median valsopodar level (7,825 ng/mL) of any patient for whom levels were obtained (Figure 1, #3). Fifteen patients had progressive disease (9 of 11 AML, 5 of 7 pre-B ALL, and one of one biphenotypic leukemia). One patient with therapy-related AML ten years after successful treatment for juvenile myelomonocytic leukemia (JMML) received two cycles of valsopodar-ME and had ANC recovery > 500/uL. Bone marrow biopsy at that time showed only 20% cellularity with 3% blasts consistent with a partial response. The patient was taken off study to pursue matched unrelated bone marrow transplantation.

P-glycoprotein expression—P-glycoprotein surface expression and function were measured in 14 patients (Table IV). Three patients had D-values and histograms consistent with p-glycoprotein expression on the majority of the blast population, while two had expression on a subpopulation of blasts, and nine had no evidence of p-glycoprotein expression. Rhodamine efflux studies demonstrated blockade of efflux in four samples. Patient #4 had evidence of efflux blockade with CsA but no P-glycoprotein expression by flow cytometry with the 4E3 antibody, suggesting the presence of a non-*MDR1* transporter. The three other patients with evidence of efflux blockade (#5, #7, #9) had P-glycoprotein expression on the majority of blasts. However, only one (#9) had significant efflux blockade with valsopodar. This patient did achieve a CR following one cycle of valsopodar-ME.

DISCUSSION

We found that the second-generation P-glycoprotein modulator valsopodar can be administered in combination with 50% dose-reduced mitoxantrone and etoposide in children with relapsed leukemia with moderate toxicity. Adequate valsopodar concentrations for P-glycoprotein inhibition (>1,000 ng/mL) were achieved in all patients. The MTD of

valspodar for this regimen is 12.5 mg/kg/day continuous intravenous infusion, with stomatitis as the most common DLT. Cerebellar symptoms such as dizziness and ataxia were generally mild and reversible and were not associated with administered dose, but rather with higher serum valspodar concentrations. Retrospective chart review identified additional toxicities not reported during the study, including grade 3 hypertension, grade 3 and 4 hypokalemia, and grade 3 hyperglycemia. The relationship of these toxicities to valspodar is difficult to ascertain due to concurrent infection, pain, and treatment with multiple supportive care medications and parenteral nutrition. All of these toxicities were controlled or corrected with medications or with electrolyte supplementation.

Pharmacokinetic studies of mitoxantrone and etoposide demonstrated that the 50% dose reduction was necessary because clearance of both agents was reduced by approximately 60% in the presence of valspodar. This may be due to inhibition of both the cytochrome P450 system (3A4) and biliary excretion. The utility of second-generation P-glycoprotein inhibitors, including valspodar, is limited by these drug interactions. Third-generation inhibitors [tariquidar (XR-9576, Xenova) and zosuquidar (LY-335979, Kanisa Pharmaceuticals)] have high P-glycoprotein affinity and unlike valspodar, do not affect cytochrome P450 (3A4) at clinically relevant concentrations. Clinical trials of these drugs in adults have demonstrated only modest alterations in the pharmacokinetics of coadministered chemotherapy drugs [25-27]. However, dosing, efficacy, and pharmacokinetic interactions between chemotherapeutic drugs and these *MDR1* modulators in children remain to be determined.

Response with this regimen appears unrelated to valspodar. The patient with T-ALL had a short-lived CR and the highest measured valspodar concentrations. However, her blasts had no detectable P-glycoprotein expression or inhibition of rhodamine efflux (Table IV, patient #3), suggesting that her response was unrelated to valspodar inhibition of P-glycoprotein. Each of three pre-B ALL patients with CR (2) or hypocellular marrow without blasts at the time of death from sepsis (1) had previously achieved CR twice and each time had relapsed after being off therapy at least one year. In contrast, the five patients with pre-B ALL who failed to achieve remission with valspodar-ME therapy either were refractory to all prior therapies (2) or had relapsed while on therapy (3). Therefore, the responders likely had more chemotherapy-sensitive disease and response was unrelated to P-glycoprotein inhibition.

Of the 14 patients with cells available for P-glycoprotein expression and efflux analysis, five had P-glycoprotein expression on at least a subset of blasts. Of these five, only one had evidence of blockade of rhodamine efflux with valspodar. This patient did have a CR with this regimen. Interestingly, three samples had evidence of rhodamine efflux blockade with CsA but not with valspodar, raising the possibility that drug efflux in these samples is occurring via alternative transporters, which are blocked by CsA but not valspodar. Preclinical data has demonstrated that CsA modulates the *MRP-1*, *BCRP*, and *LRP* transporters in addition to *MDR1* P-glycoprotein, while valspodar modulation is limited to *MDR1* [28]. As different transporters may be clinically relevant in different patients with leukemia, a more broad-spectrum inhibitor may be more effective.

Despite the theoretical advantages of multiple-transporter inhibition with CsA, the results of POG 9421, in which P-glycoprotein modulation with CsA during consolidation chemotherapy did not improve outcomes in children with *de novo* AML, suggest that there is little future role for P-glycoprotein inhibitors in *de novo* pediatric leukemia [29,30]. The results of this study suggest that P-glycoprotein inhibition may only be clinically effective in patients with relapsed disease and high P-glycoprotein expression in combination with specific therapeutic agents, such as gemtuzumab ozogamicin (Mylotarg, Wyeth Pharmaceuticals), that are targeted to the leukemic cell and substrates of P-glycoprotein-

mediated drug efflux [31]. In adults, P-glycoprotein drug efflux activity predicts both *in vitro* resistance and clinical response to gemtuzumab ozogamicin monotherapy even when adjusting for CD33 expression [32-34]. In a phase I study of gemtuzumab ozogamicin monotherapy in relapsed and refractory pediatric AML patients, the mean *MDR1*-mediated drug efflux was significantly lower in the 30% of patients who achieved CR compared with nonresponders [35].

Modulation of drug resistance in pediatric leukemia requires a multifaceted approach, as demonstrated by this cohort of children with multiply relapsed or refractory leukemia. Only a subset of patients had detectable P-glycoprotein expression on their blasts, and in some cases, expression was heterogeneous, indicating that mechanisms other than P-glycoprotein-mediated drug efflux significantly contribute to clinical drug resistance. For children with high-risk or relapsed leukemias, improved understanding of the biology of resistance mechanisms and novel targeted strategies will be needed to impact clinical outcomes.

Acknowledgments

This study was supported in part by NIH grants: R01 52168 (B.I.S.), M01 RR 00070 (General Clinical Research Center, Stanford University School of Medicine; N.J.L. - Clinical Associate Physician Award), U10 CA98543 (COG Chair's Grant) and U10 CA98413 (COG Statistics and Data Center Grant). A complete listing of grant support for research conducted by the Children's Cancer Group and Pediatric Oncology Group before initiation of the COG grant in 2003 is available online at <http://www.childrensoncologygroup.org/admin/grantinfo.htm>. The work was in part supported by an endowment to Robert J. Arceci for the King Fahd Chair in Pediatric Oncology.

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FIGURE 1.

Individual patient valspodar serum concentrations were measured during (hours 24-122) and after (hours 126-144) the valspodar continuous infusion in 16 patients (2 patients at dose level 1, 5 patients at dose level 2, 6 patients at dose level 3, 3 patients at dose level 4). Hour 85 is the midpoint of the continuous infusion. The median valspodar concentration over the course of the infusion was determined for each patient. Conc = concentration.

TABLE I

Characteristics of eligible patients (n = 22)

Patient ID	Valspodar dose level	Diagnosis	Age (years)	Gender	Prior induction attempts	Clinical history	Response
1	1	DS-AML	2.3	Male	1	On-therapy relapse after CR	PD
2	1	AML	15.8	Male	1	Off-therapy relapse	PD
3	1	T-cell ALL (post B-precursor ALL)	18.2	Female	3	Third bone marrow relapse	CR
4	2	AML	5.2	Male	1	Refractory to induction	PD
6	2	Biphenotypic	6.9	Female	3	On-therapy relapse after CR	PD
7	2	B-precursor ALL	20.3	Male	5	Initial disease refractory to chemotherapy, relapsed post-BMT	PD
8	2	AML	11.2	Male	2	Refractory to induction	PD
9	2	B-precursor ALL	13.2	Female	3	Second off-therapy relapse; refractory to reinduction	CR
10	3	AML	10	Male	1	On-therapy relapse after CR	PD
11	3	AML	17.6	Male	1	Off-therapy relapse	PD
12	3	AML	6.7	Male	1	Refractory to induction	PD
13	3	B-precursor ALL	8.5	Male	3	Second off-therapy relapse; s/p HSCT	Not evaluable; death at day 17 from sepsis
14	3	AML	9.3	Female	3	Second off-therapy relapse; s/p HSCT	PD
15	3	B-precursor ALL	9.4	Male	2	Second off-therapy relapse; s/p HSCT	CR
16	4	B-precursor ALL	11.1	Male	2	Refractory to induction	PD
17	4	AML	10.9	Male	1	Off-therapy relapse; s/p HSCT	PD
18	4	AML	12.6	Male	4	Refractory to induction	PR
19	4	B-precursor ALL	7.4	Female	2	On-therapy relapse after CR	PD
20	4	B-precursor ALL	4.6	Male	6	On-therapy relapse followed by HSCT; relapse after HSCT, refractory to reinduction	PD
21	4	AML	3.7	Male	1	On-therapy relapse after CR	Not

Patient ID	Valspodar dose level	Diagnosis	Age (years)	Gender	Prior induction attempts	Clinical history	Response
22	4	tAML (post JMML)	13	Male	1	N/A	PR
23	4	B-precursor ALL	4.3	Male	2	On-therapy relapse after CR; refractory to reinduction	PD

Abbreviations: AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; CR, complete response; DS, Down syndrome; HSCT, hematopoietic stem cell transplant; JMML, juvenile myelomonocytic leukemia; PR, partial response; PD, progressive disease; tAML, therapy-related AML

TABLE II

Valspodar dose levels and non-bilirubin, non-hematologic grade 3 or 4 toxicity

Dose Level	Valspodar Dose (mg/kg/day)	Number of Patients Treated at Dose Level	Number of Patients with Grade 3 or 4 Toxicity in First Course	Specific Toxicity	Toxicity Grade
1	8	3	1	Stomatitis [§]	Grade 4
2	10	5	1	Stomatitis	Grade 3
3	12.5	6	1	Cerebellar	Grade 3*
			1	Infection	Grade 5 (death)
			1	Stomatitis	Grade 3
			1	Hyperglycemia	Grade 3 [#]
			2	Hypokalemia	Grade 3 [#]
4	15	7	3	Stomatitis	Grade 4*
			1	Bone marrow aplasia > 6 weeks	Grade 4*
			1	Hypokalemia	Grade 4*
			2	Hypertension	Grade 3 [#]

[§] Occurred in patient with Down syndrome

* Dose-limiting toxicity

[#] noted during retrospective chart review after study completion, not reported during therapy

TABLE III

Population pharmacokinetic parameters for mitoxantrone and etoposide in presence and absence of valspodar

MITOXANTRONE				
Parameter	Estimate	% CV ^a	Interindividual variability estimate ^b	% CV ^c
CL ₀ (L/h)	16.4	17.9	48.1	69
CL ₁ (L/h)	5.88	30.8		
V1 (L)	23.2	19.3	64.4	68.5
V2 (L)	102	19.9	-	-
Q (L/h)	8.13	23.7	-	-
EC _{50mitox} (mg/L)	0.525	121	-	-
Residual error ^d	60.6%	17.8	-	-

ETOPOSIDE				
PK parameter	Estimate	% CV ^a	Interindividual variability estimate ^b	% CV ^c
CL ₀ (L/h)	1.52	0.36	61.56	5.83
CL ₁ (L/h)	0.611	0.37		
V1 (L)	3.58	0.18	98.5	4.56
V2 (L)	3.82	0.35	-	-
Q (L/h)	2.38	0.428	-	-
EC _{50eto} (mg/L)	0.271	0.53	-	-
Residual error ^d	32.1%	0.43	-	-

V1 = volume of the central compartment, V2 = volume in the peripheral compartment, Q = inter-compartmental clearance, CL = clearance from the central compartment, CL₀ is the baseline clearance and CL₁ is the asymptotic value of CL for large concentrations of valspodar (when valspodar $\rightarrow \infty$), EC_{50mitox} = the concentration of valspodar required to reduce mitoxantrone clearance by 50%, EC_{50eto} = the concentration of valspodar required to reduce etoposide clearance by 50%.

^a Relative standard error of estimate (SE estimate/estimate) expressed as a percentage

^b Estimates of variability, expressed as % ($100 \times \sqrt{\text{variance}}$)

^c Percent square root of the relative standard error of the coefficient of variation ($100 \sqrt{SE_{\text{estimate}} / \text{estimate}}$)

^d Residual intrasubject variability

TABLE IV

P-glycoprotein (*MDR1*) surface expression and function in patient leukemic blasts

Patient ID	Diagnosis	Response	<i>MDR1</i> P-glycoprotein expression		Rhodamine Efflux (log shift)		
			D-value	Interpretation [^]	CsA	Valsopodar	Interpretation [#]
#2	AML	PD	0.04	Negative	0.3	0.1	No inhibition
#3	T-cell ALL	CR	0.08	Negative	0	0	No inhibition
#4	AML	PD	0.12	Negative	0.7	0.3	Inhibition with CsA
#5	t-AML	PD	0.47	Entire population with expression	1	0.1	Inhibition with CsA
#6	Biphenotypic leukemia	PD	0.35	Subpopulation with expression	0.1	0	No inhibition
#7	Pre-B ALL	PD	0.65	Entire population with expression	0.7	0.4	Inhibition with CsA
#9	Pre-B ALL	CR	0.26	Entire population with expression	0.5	0.9	Inhibition with valsopodar >CsA
#10	AML	PD	0.32	Subpopulation with expression	0	0	No inhibition
#11	AML	PD	0.03	Negative	0.2	0.1	No inhibition
#17	AML	PD	0.02	Negative	0	ND	No inhibition
#18	AML	PD	0.09	Negative	0.2	ND	No inhibition
#19	Pre-B ALL	PD	0.13	Negative	0.2	ND	No inhibition
#20	Pre-B ALL	PD	0.14	Negative	0.4	ND	No inhibition
#22	t-AML	PR	0.05	Negative	0.3	ND	No inhibition

Abbreviations: AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; t-AML, therapy-related AML; CR, complete response; PD, progressive disease; PR, partial response; CsA, cyclosporine A; ND, not done

[^] P-glycoprotein expression interpretation based on combination of D-value (≥ 0.15 considered positive) as well as visual inspection of flow cytometry data to determine if expression present on entire blast population or a subset of blasts

[#] Rhodamine efflux interpretation based on quantification of shift in peak of fluorescence intensity curve on log scale. Shift of ≥ 0.5 log considered evidence of inhibition of rhodamine efflux from blasts.