

Treatment of Refractory and Relapsed Acute Myelogenous Leukemia With Combination Chemotherapy Plus the Multidrug Resistance Modulator PSC 833 (Valspodar)

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A potential mechanism of chemotherapy resistance in acute myeloid leukemia (AML) is the multidrug resistance (*MDR-1*) gene product P-glycoprotein (P-gp), which is often overexpressed in myeloblasts from refractory or relapsed AML. In a multicenter phase II clinical trial, 37 patients with these poor risk forms of AML were treated with PSC 833 (Valspodar; Novartis Pharmaceutical Corporation, East Hanover, NJ), a potent inhibitor of the *MDR-1* efflux pump, plus mitoxantrone, etoposide, and cytarabine (PSC-MEC). Pharmacokinetic (PK) interactions of etoposide and mitoxantrone with PSC were anticipated, measured in comparison with historical controls without PSC, and showed a 57% decrease in etoposide clearance ($P = .001$) and a 1.8-fold longer beta half-life for mitoxantrone in plasma ($P < .05$). The doses of mitoxantrone and etoposide were substantially reduced to compensate for these interactions and clinical toxicity and in Cohort II were well tolerated at dose levels of 4 mg/m² mitoxantrone, 40 mg/m² etoposide, and 1 g/m² C daily for 5 days. Overall, postchemotherapy marrow hypoplasia was

PATIENTS WITH ACUTE myeloid leukemia (AML) who have relapsed or are refractory to conventional chemotherapy, as well as those whose disease develops after antecedent chemotherapy or prior myeloid stem cell disorders have poorer prognoses and responses to chemotherapy compared with those with de novo AML.¹⁻³ Overexpression of the multidrug resistance (*MDR-1*) gene product P-glycoprotein (P-gp)⁴⁻⁶ is one of the mechanisms associated with poor responses of these forms of AML. A number of adverse prognostic variables such as age, CD34 expression, cytogenetic pattern, or secondary leukemia (because of prior cytotoxic therapy or an antecedent myelodysplastic syndrome) have also been linked to P-gp overexpression.⁷⁻¹⁰ Cells that overexpress *MDR-1* are cross-resistant to several important antileukemia drugs including anthracyclines and epipodophyllotoxins (eg, mitoxantrone and etoposide). Cells with the *MDR* phenotype are characterized by lower intracellular drug accumulation^{11,12} concomitant with reduced sensitivity to these agents.^{7,8,13}

Several agents capable of modulating and decreasing *MDR-1* in vitro, such as quinine, tamoxifen, calcium channel blockers, cyclosporine, and its analogue PSC 833 (PSC; Valspodar; Novartis Pharmaceutical Corporation, East Hanover, NJ) have been used clinically, including evaluation in preliminary studies for treating poor-risk AML.^{6,14-18} The mechanism of *MDR* modulation of cyclosporine (cyclosporin A) differs from its immunosuppressive action.¹⁹ PSC is a more potent inhibitor of P-gp than cyclosporine, without the immunosuppression or renal toxicity of the parent compound.²⁰ We report herein the results of a multicenter phase II trial evaluating PSC, in combination with mitoxantrone, etoposide, and cytarabine (PSC-MEC) for the treatment of AML patients with poor prognostic features. In this trial, we also evaluated the pharmacokinetics of mitoxantrone and etoposide in the presence of

achieved in 33 patients. Twelve patients (32%) achieved complete remission, four achieved partial remission, and 21 failed therapy. The PK observations correlated with enhanced toxicity. The probability of an infectious early death was 36% (4 of 11) in patients with high PK parameters for either drug versus 5% (1 of 20) in those with lower PK parameters ($P = .04$). P-gp function was assessed in 19 patients using rhodamine-123 efflux and its inhibition by PSC. The median percentage of blasts expressing P-gp was increased (49%) for leukemic cells with PSC-inhibitable rhodamine efflux compared with 17% in cases lacking PSC-inhibitable efflux ($P = .004$). PSC-MEC was relatively well tolerated in these patients with poor-risk AML, and had encouraging antileukemic effects. The Eastern Cooperative Oncology Group is currently testing this regimen versus standard MEC chemotherapy in a phase III trial, E2995, in a similar patient population.

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PSC, and the level of AML blastic *MDR-1* expression, using functional as well as flow cytometric analyses.

MATERIALS AND METHODS

Patients. This study was an open-label phase II multicenter trial initiated in June 1995 and closed to accrual in February 1997. The protocol was approved by the Institutional Review Boards and Human Subjects Committees of all participating institutions. Eligibility criteria included patients with a diagnosis of AML by the French-American-British classification,²¹ with the following characteristics: (1) early relapse, ie, 6 months after first complete remission (CR); (2) refractory to chemotherapy, either to initial induction or at first relapse; (3) relapse after autologous or allogeneic bone marrow transplantation (BMT); (4)

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second or greater relapse; (5) secondary AML or AML evolving from myelodysplastic syndromes (MDS) or myeloproliferative disorders (MPD) (not chronic myeloid leukemia). Patients were also required to have a left ventricular ejection fraction of $\geq 50\%$, Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 2, hepatic and renal function tests $\leq 2 \times$ normal, age of 18 to 70 years, and no evidence of active infection or central nervous system leukemia.

Chemotherapy treatment. The chemotherapy regimen is based on a modification of a prior MEC regimen,²² using reduced mitoxantrone and etoposide doses because of anticipated pharmacokinetic (PK) interactions related to coadministration with PSC.^{6,18,23} PSC (Valspodar) was administered as a pretreatment loading dose at 2 mg/kg over 4 hours, with a concomitant continuous infusion at 10 mg/kg/d for 120 hours (5 days). Chemotherapy began immediately after completion of a 4-hour loading dose of PSC. Two dose levels of chemotherapy were used. In cohort I, patients received mitoxantrone 5 mg/m²/d intravenous (IV) bolus, days 1 to 5; etoposide 50 mg/m²/d, days 1 to 5; and cytarabine 1 g/m²/d, days 1 to 5 administered IV as a short infusion over 1 hour. Because of excess toxicity in this cohort as described below, in cohort II, the dose of mitoxantrone was reduced to 4 mg/m² and etoposide to 40 mg/m². A bone marrow aspirate and biopsy was performed between days 8 to 10 (3 to 5 days after completion of chemotherapy) to assess adequacy of marrow hypoplasia. Hematopoietic growth factor support with granulocyte-macrophage colony-stimulating factor (GM-CSF, Sargramostim; Immunex, Seattle, WA) or granulocyte colony-stimulating factor (G-CSF, Neupogen; Amgen, Thousand Oaks, CA) administered daily after marrow hypoplasia was recommended but not required in the protocol. Twenty-nine patients received growth factor support (11 with GM-CSF, 13 with G-CSF). Five patients received both of these drugs as they were crossed over at the individual investigator's discretion. If residual leukemia was present in the marrow at this time, the induction course was repeated for 3 to 5 days depending on residual blastic cellularity (% of blasts relative to the overall cellularity calculated as the product of % cellularity and fraction of blasts). Treatment was administered for 5 days at identical doses as in the first course if the blastic cellularity was 10%, and for 3 days if it was 5% to 10%. A maximum of two induction cycles was permitted to achieve bone marrow hypoplasia (ie, $< 5\%$ blastic cellularity). If disease persisted thereafter, the patient discontinued the treatment protocol and was considered to have failed to respond. Patients who achieved CR were scheduled to receive consolidation treatment with PSC-MEC for one cycle. Toxicity was assessed and graded using the ECOG Leukemia Common Toxicity Criteria.²⁴

Measurement of response. Previously established response criteria were used.²⁵ CR required adequate marrow cellularity with $< 5\%$ blasts documented at the preconsolidation evaluation, no peripheral blasts, an absolute neutrophil count $\geq 1,500/\mu\text{L}$, platelet count $\geq 100,000/\mu\text{L}$, and no evidence of extramedullary leukemia. Partial remission (PR) required all criteria as in CR except that the bone marrow may have contained 5% to 25% blasts, or the bone marrow had $< 5\%$ blasts, in the presence of moderate thrombocytopenia (50 to 100,000/ μL). Failure was defined as leukemia-associated or caused by early death (ie, within 30 days of completion of treatment). Leukemia-related causes of failure were refractory disease (inability to achieve hypoplasia despite two cycles of chemotherapy) and regrowth resistance, a term used to indicate cases in which $> 25\%$ bone marrow blasts were noted on recovery despite adequate marrow hypoplasia having been achieved.²⁶

P-gp expression of leukemic blasts. Leukemic blast cells from bone marrow or peripheral blood were evaluated for P-gp expression and function at the study's central laboratory (Dr Elisabeth Paietta, Albert Einstein Cancer Center, NY). Monoclonal antibody 4E3.16 to a P-gp-specific cell surface peptide epitope (provided by Dr R. Arceci, Children's Hospital, Cincinnati, OH) was tested on mononuclear cells. Cells were incubated with 4E3.16 for 30 minutes at 4°C in the presence of 6% heat-inactivated serum to reduce nonspecific antibody binding,

followed by staining with goat anti-mouse antibody conjugated to fluorescein isothiocyanate. Gating of the leukemic blasts on the flow cytometer was based on linear forward angle light scatter and right angle side scatter and validated using an extensive diagnostic antibody panel.²⁷ The absolute percentages of "P-gp expressing" gated leukemia cells were compared between cases and used in the statistical analysis.^{28,29}

P-gp function of leukemic blasts. To test for P-gp function, the uptake and efflux of rhodamine-123 was monitored by flow cytometry in leukemic cells gated according to scatter properties and antigen profile.^{28,29} Cells were prestained with phycoerythrin (PE)-labeled antibody to CD34, or CD33 if the cells did not express CD34, and then incubated with 2 $\mu\text{g/mL}$ rhodamine-123 for 30 minutes at 37°C in the dark. In control experiments, neither the anti-CD34 nor other antibodies used in these double-labeling studies interfered with P-gp function. Subsequently, rhodamine release was measured over a period of 60 minutes at 37°C in the absence and presence of PSC 833 (10 $\mu\text{mol/L}$) or cyclosporine A (15 $\mu\text{mol/L}$). Our preliminary time-course studies of rhodamine release over a range from 30 to 120 minutes at 37°C had established that optimal sensitivity of the assay was achieved at the 60-minute time point. Cell lines used for standardizing P-gp detection and function were the resistant 8226/Dox6 myeloma cells and their sensitive parent line, 8226/s (both provided by Dr W. Dalton, Moffitt Cancer Center, Tampa, FL). To quantitate P-gp function, the relative change of the maximum mean rhodamine fluorescence channel over the 60-minute time period was recorded selectively in the double-stained (rhodamine/PE-antibody) cell population. Measurable rhodamine efflux was defined as a channel shift of $\geq 20\%$. This parameter was based on our experience in de novo AML patients that a mean channel shift of $< 20\%$ was not reversible by PSC 833 or cyclosporine in vitro and was invariably associated with low P-gp expression levels.²⁸⁻³⁰

Pharmacological and PK studies. For PSC levels, samples of venous blood were obtained before the start of the PSC infusion and just before the mitoxantrone doses on days 3 and 5. For mitoxantrone and etoposide, baseline venous blood samples were obtained before mitoxantrone administration on the 5th day of chemotherapy, then at the end of the etoposide infusion (1.1 hours), and 2, 6, 8, and 12 hours after the start of mitoxantrone infusion.

Whole blood PSC concentrations were determined by radioimmunoassay (ANAWA Laboratories, Zürich, Switzerland). Etoposide and mitoxantrone were analyzed by modifications of previously published high performance liquid chromatography methods.³¹⁻³³

Noncompartmental PK analyses of etoposide concentration-time data were performed using the XLPHARM M-IND program (VKPharmacokinetics, Turnhout, Belgium). PK data for the PSC-MEC patients were compared with a historical database of 20 patients treated with etoposide alone in other trials at Stanford University.³¹⁻³² The sampling times for mitoxantrone in this study were not sufficient for characterization of the alpha (distribution) and gamma (terminal elimination) phases but did allow for characterization of the second (beta) elimination phase, $T^{1/2\beta}$. These data were compared with those previously published for mitoxantrone.³⁴

Statistical considerations. One-tailed Wilcoxon rank tests³⁵ were used for statistical analysis of age-related clinical responses and for the comparison of immunophenotype and PSC inhibitable function. *P* values of .05 were considered significant. Remission duration was defined as time to relapse or death from the date of CR. Overall survival was measured from the initiation of induction treatment until death from any cause. Overall survival and remission duration were estimated by the method of Kaplan and Meier.³⁶ PK analyses of etoposide and correlations with patient clinical data were tested for normal distribution using the Wilk-Shapiro statistic.³⁷ Differences in PK parameters for etoposide between the PSC-MEC and historical groups were compared using the Mann-Whitney *U* test with an a priori level of significance of *P* = .05 (two-sided). For mitoxantrone, 95% confidence intervals for

the T-values were calculated for the PSC-MEC group and compared with that of the previously published trial.³⁴ Nonoverlapping 95% confidence intervals indicated a statistical significance at $P < .05$.³⁸

RESULTS

Thirty-nine patients were entered into the trial, of whom 37 patients were evaluable for response. Two patients were ineligible and removed from the study (1 patient was diagnosed with active tuberculosis after protocol entry but before starting treatment and another developed hypotension within an hour of the PSC infusion and did not receive MEC chemotherapy). Pretreatment patient characteristics are shown in Table 1. Disease categories of the 37 evaluable patients entered were early relapse (n = 11), second relapse (n = 5), secondary/post MDS-MPD (n = 10), refractory AML (n = 7), and relapse after BMT (n = 4). The median patient age was 54 years (range, 27 to 70 years).

Chemotherapy responses. Cohort I doses of chemotherapy were used to treat the first 6 patients. All six patients developed marrow hypoplasia. Among these patients three early deaths occurred (ie, within 30 days) because of severe mucositis and infection with prolonged marrow hypoplasia in 5 of the 6 patients. Therefore, for cohort II patients (n = 31), the doses of mitoxantrone and etoposide were reduced 20%, as described in Materials and Methods. Twenty-three patients received one cycle and 14 received two cycles of chemotherapy. Thirty-two of 35 patients (2 were not evaluated for hypoplasia) who had postchemotherapy bone marrow examinations achieved adequate marrow hypoplasia. Twenty-one of the 37 patients achieved marrow hypoplasia with one cycle of treatment (4 of 6 in cohort I, 17 of 31 in cohort II).

Overall, 12 patients (32%) achieved a CR and four achieved a PR. The treatment failed in twenty-one patients (Table 2). The responses and failures were evenly distributed between disease categories and occurred within both cohorts (Tables 2 and 3).

Table 1. Clinical Characteristics of Poor-Risk AML Patients in This Study

Characteristic	No. of Patients
Age: median 54; range, 27 to 70 years old	
Gender: male = 23, female = 14	
De novo AML patients	27
Early relapse	11
Refractory AML	7
Second or greater relapse	5
Relapse post-BMT	4
Duration of 1st remission <6 months*	20
Prior salvage therapy	15
Prior salvage responses	6
Unfavorable cytogenetics†	11
Antecedent hematologic disease‡	10
Unfavorable cytogenetics†	7
Total no. of patients	37

*Duration of first remission, including six patients with prior salvage efforts.

†Abnormalities of chromosome 5 and/or 7, or complex (≥3 abnormalities).

‡Refers to AML developing after myelodysplastic syndrome or myeloproliferative disorders (excluding chronic myeloid leukemia).

Table 2. Response of Poor-Risk AML Patients to Induction Therapy With PSC-MEC

Patients	CR (%)	PR	Failed
Cohort I* n = 6	1 (17%)	1	4
Cohort II† n = 31	11 (35%)	3	17
Total n = 37	12 (32%)	4	21

*Cohort I patients were treated with mitoxantrone 5 mg/m²/d, etoposide 50 mg/m²/d, Ara-C 1 g/m²/d plus PSC for 5 days.

†Cohort II patients received 20% lower doses of mitoxantrone (4 mg/m²/d) and etoposide (40 mg/m²/d), with similar cytarabine and PSC doses as for cohort I for 5 days.

Three of 13 patients (23%) over 60 years old achieved CRs compared with 9 of 24 patients (38%) ≤60 years old. In the group of 6 patients in cohort I there was only one CR whereas in cohort II, 11 of 31 patients (36%) achieved CRs. The median time to document achievement of CR after starting chemotherapy was 52 days (range, 20 to 77 days). For patients treated with one cycle of chemotherapy this was slightly shorter (51 days; range, 20 to 77 days; n = 7) compared with those who required two cycles of treatment (59 days; range, 51 to 72 days; n = 5).

Nine patients in CR received consolidative therapy, including six with consolidation chemotherapy (four with PSC-MEC, one with fludarabine plus cytarabine, and one high-dose cytarabine). Overall, five patients have undergone BMT, four in CR (two allogeneic, two autologous) and one in PR from a matched unrelated donor. The median remission duration for patients achieving CR was 6 months (range, 0.7 to 13 months) with a median survival of 8.3 months (range, 5 to 18+ months). The overall median survival was 6.0 months (range, 0 to 18+ months).

Twenty-one patients failed to respond to therapy, as summarized in Table 4. Twelve patients died during the hypoplastic period, eight patients had leukemic regrowth resistance, and one patient had leukemia that was refractory to treatment.

Toxicity. Grade 3 or 4 mucositis occurred in 3 of 6 (50%) patients in cohort I and in only 6 of 31 (19%) patients in cohort II. Other significant toxicities included transient mental status changes and transient peripheral neuropathy in 1 patient each. No ataxia was reported. Transient hyperbilirubinemia was an anticipated side effect of PSC therapy, as previously reported^{6,39}; bilirubin levels of 1.5 mg/dL occurred in 34 of 37 (92%) patients in cycle 1 and in 12 of 14 (89%) patients in cycle 2. The median peak serum bilirubin levels and peak times to reach this level after starting each course of chemotherapy were similar for those requiring one or two courses of induction

Table 3. Relationship of Leukemia Responses to Patient Subgroups

	Total	CR	PR	Failed	
				Refractory/Regrowth Resistance	Early Death
Early relapse	11	2	1	4	4
≥Second relapse	5	2	1	1	1
Secondary AML	10	4	2	1	3
Refractory AML	7	3	0	2	2
Relapse post-BMT	4	1	0	1	2
Total patients	37	12	4	9	12

Table 4. Causes of Treatment Failure for PSC-MEC Induction Therapy in Poor-Risk AML Patients in This Study

	Cohort I	Cohort II	Total
Death during hypoplasia			
Infection	3	6	9
Arrhythmia	0	1*	1
Myocardial infarction	0	1†	1
Hemorrhage	0	1‡	1
Leukemia-associated			
Regrowth resistance	1	7	8
Refractory	0	1	1
Total	4	17	21

*Fatal arrhythmia in the setting of severe hypokalemia in a patient receiving amphotericin infusion.

†Fatal acute myocardial infarction in the setting of a severe hemolytic anemia.

‡Fatal subdural hematoma while thrombocytopenic.

treatment: median 2.6 mg% (range, 1.5 to 27.7 mg%) on median day 5.5 (range, 2 to 23 days). The hyperbilirubinemia was transient in 27 of the 33 patients in whom follow-up values were available, and resolved in a median of 10 days (range, 4 to 19 days). The 6 patients with persistent hyperbilirubinemia had contributing factors in addition to PSC administration, such as infections, sepsis, and hepatitis. Clinical responses were not related to the bilirubin level or time course of bilirubin elevations.

One patient developed a possible allergic reaction to PSC (hypotension within 1 hour of the PSC infusion despite premedications) and was taken off study before receiving chemotherapy. Twelve deaths occurred on study, 3 of 6 in cohort I and 9 of 31 in cohort II (Table 4). Nine of the 12 deaths were caused by infection (cohort I, 3 of 6 patients; cohort II, 6 of 31 patients) and were unrelated to the number of induction cycles. However, the infectious deaths correlated with PK parameters as discussed below. One death was caused by an arrhythmia secondary to severe hypokalemia, one myocardial infarction occurred in the setting of a hemolytic anemia, and one patient died of a subdural hemorrhage related to thrombocytopenia.

Results of P-gp and CD34 analyses. P-gp expression/function and CD34 expression were evaluated in all patients for whom adequate bone marrow sample quality and volume were obtained. Detailed data are presented in Table 5 and a collated summary in Table 6. In 19 patients, rhodamine efflux was measured as an index of P-gp function, and is listed as the % mean channel shift in Table 5. Rhodamine efflux was detected in 15 cases. Ten of these cases had relatively increased P-gp expression (27% to 96% of the leukemic blasts stained with the 4E3.16 antibody) (Tables 5 and 6). In 8 of these 15 patients, the efflux of rhodamine was inhibitable by PSC with the extent of inhibition of function ranging from 45% to 100%. In these 8 patients the median proportion of blasts expressing P-gp was increased, 49% (range, 27% to 96%) compared with 17% (range, 1% to 40%) in the 7 patients with PSC noninhibitable P-gp function, $P = .004$ (Table 6). Figure 1 shows P-gp expression and the corresponding rhodamine-123 efflux activity in two representative patients. CD34 expression was also higher in the PSC-inhibitable versus PSC noninhibitable blasts (me-

Table 5. P-gp Expression and Function in Leukemic Blasts From 19 Patients in Whom P-gp Expression, Rhodamine Accumulation, and Inhibition of Rhodamine Efflux by PSC 833 Were Measured

Patient No.	% P-gp-Positive Cells*	% Mean Channel Shift†	% CD34-Positive Cells	Response
PSC 833 Inhibitable P-gp Function				
3	53	30	98	F
8	40	34	97	F
9	54	47	94	PR
16	35	40	97	CR
19	27	43	45	F
20	45	29	95	CR
22	77	47	16	F
34	96	90	88	F
PSC 833 Noninhibitable P-gp Function				
2	6	42	82	F
10	16	33	4	F
18	1	46	86	F
21	17	69	51	F
29	40	35	25	F
30	20	70	85	F
39	37	38	27	F
No P-gp Function				
1	2	0	2	PR
4	7	0	6	CR
5	13	0	28	F
7	3	0	73	F

Abbreviation: F, failure.

*% 4E3 positive blast cells.

†The relative rhodamine-123 mean fluorescence channel shift from baseline (maximal dye uptake and retention) that occurred during a 1-hour incubation of cells at 37°C (to induce dye efflux) was expressed as percent of maximal dye retention (100%).

dian 95% v 51%, $P = .02$), Table 6. In 4 patients no measurable rhodamine efflux function was noted, associated with low 4E3.16 and CD34 expression on the blasts (Table 5). No patient had appreciable P-gp expression without rhodamine efflux. Also, none of the patients with CD33-positive/CD34-negative blast cells showed classical PSC 833 inhibitable rhodamine efflux activity.

No correlation was shown between P-gp expression and

Table 6. MDR-1 Analysis of Blasts From AML Patients Obtained Before PSC-MEC Induction Chemotherapy

No. of Patients	P-gp Function	Leukemic Blast Immunophenotype	
		P-gp+ (%) Median (range)	CD34+ (%) Median (range)
8	PSC 833 inhibitable	49 (27-96)*	95 (16-98)†
7	PSC 833 noninhibitable	17 (1-40)	51 (4-86)
15	Total	37 (1-96)	85 (4-98)

Leukemic blasts were evaluated for MDR-1 immunophenotype using the 4E3 monoclonal anti-P-gp antibody. MDR-1 function was evaluated using the rhodamine-123 efflux assay and its inhibitability by PSC, as defined in Materials and Methods.

* $P = .004$, PSC 833 inhibitable versus PSC 833 noninhibitable.

† $P = .02$, PSC 833 inhibitable versus PSC 833 noninhibitable.

PATIENT #1

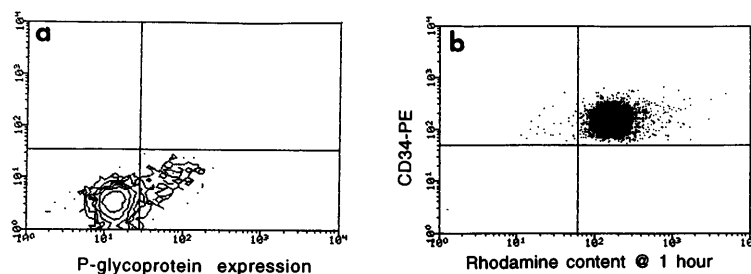
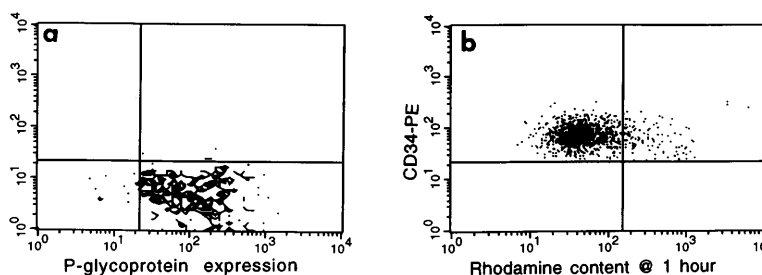


Fig 1. P-gp expression and the corresponding rhodamine-123 efflux activity. The two left flow cytometer contour plots (a) reflect P-gp expression as measured by staining of blast cells with antibody 4E3.16. Cells in the lower right quadrant are positive for 4E3.16, whereas cells in the left lower quadrant are negative for 4E3.16 because they lack fluorescence above background. The two right flow cytometer cytograms (b) show cellular rhodamine-123 fluorescence after 1 hour of cell incubation at 37°C in CD34-positive blast cells. A shift of rhodamine-123 fluorescence from the right upper to the left upper quadrant reflects cellular efflux of rhodamine-123. Patient #1 showed P-gp expression in a small subpopulation of blast cells and lacked measurable rhodamine-123 efflux activity despite CD34 expression. In patient #2, P-gp was detected on >90% of blast cells and these blast cells showed marked rhodamine-123 efflux activity, which was inhibitable by PSC 833 (data not shown).

PATIENT #2



response to therapy in these small groups of patients. In the patients with PSC-inhibitable rhodamine efflux there were 2 CRs, 1 PR, and 5 failures, whereas all 7 patients with noninhibitable efflux failed therapy. Two responses were observed in the 4 patients with no rhodamine efflux (1 CR and 1 PR).

In addition to the 19 patients for whom sufficient material was available for P-gp functional studies (Table 5), 7 patients had flow cytometric measurements of P-gp and 10 for CD34 expression. Among these 26 patients having P-gp expression studies, 14 (54%) had $\geq 20\%$ blasts positive for P-gp, 4 (15%) had 10% to 19% positive blasts, and 8 (31%) had $\leq 10\%$ positive blasts. The median % of CD34-positive cells in the 29 patients tested was 76% (range, 2% to 98%), and 25 of these 29 patients had >20% CD34-positive blast cells.

PK data. Whole blood PSC levels >1,000 ng/mL, which correspond to serum PSC levels of 1,500 to 2,000 ng/mL, were achieved in all 33 patients. These levels of PSC are known to substantially inhibit P-gp *in vitro*.⁴⁰ Median whole blood PSC levels were 3,070 ng/mL on the 5th day of infusion (range, 1,390 to 10,460 ng/mL).

PK variables of etoposide during 30 courses of PSC-MEC in 23 patients were significantly different from etoposide without PSC in a historical control group.^{31,32} These data show a 57% decrease in clearance of etoposide, from 1.17 to 0.50 L/h/m² ($P = .001$). During 30 PSC-MEC courses in 28 patients, the mean $T^{1/2\beta}$ for mitoxantrone was 3.6 hours (95% confidence intervals 3.3 to 4.0 hours), which is 1.8-fold longer than previously reported for mitoxantrone in the absence of PSC (mean, 2.03 hours; 95% confidence intervals 1.4 to 2.7 hours; $P < .05$).^{34,41} PK data for patients in cohort I ($n = 3$) showed a

1.4-fold higher mean mitoxantrone area under the curve (AUC) and a 1.6-fold higher mean etoposide AUC when compared with cohort II ($n = 28$). PK parameters correlated with toxicity. Early deaths caused by infections occurred in 9 patients. Patients with PK studies, 4 of 11 (36%) who had high AUC values (etoposide >80 $\mu\text{g} \times \text{h/mL}$ and mitoxantrone >160 $\text{ng} \times \text{h/mL}$) versus 1 of 20 (5%) with lower AUCs, had infectious early deaths, ($P = .004$). The mean plasma AUC for mitoxantrone in patients with infectious early death was 253 $\text{ng} \times \text{h/mL}$ compared with 105 $\text{ng} \times \text{h/mL}$ for the remaining patients ($P = .031$). The mean etoposide level in this latter group was 96 $\mu\text{g} \times \text{h/mL}$ compared with 60 $\mu\text{g} \times \text{h/mL}$, $P = .66$. No correlation was observed between PK values and remission rates.

DISCUSSION

This study showed encouraging antileukemic effects and acceptable tolerance of the PSC-MEC regimen in patients with poor prognosis AML. Recently, Estey et al⁴² have proposed placing poor-risk AML patients in four categories that correlated their responses to conventional treatment with presenting clinical features. These patients were categorized as being in one of four prognostic risk groups, based on their background treatment history. Group 1 had expected CR rates of approximately 70% (patients with a first CR lasting >2 years being treated with conventional therapy); Group 2 had expected CR rates of approximately 40% (patients with a first CR duration of 1 to 2 years being treated with conventional treatment); Group 3 patients had expected CR rates of approximately 10% to 20% (patients with a first CR lasting <1 year, or with no initial CR, who were receiving their initial salvage attempt with conven-

tional treatment); and Group 4 patients had expected CR rates of <1% (patients with an initial CR <1 year, or with no initial CR, who were receiving a second or subsequent salvage regimen, having not responded to a first salvage attempt with conventional drugs). Virtually all of our patients (26 of 27 patients with de novo AML) are categorized as being in the very poor prognostic risk Groups 3 and 4 (Table 1). The remaining 10 patients in our study had poor-risk features related to their having developed secondary AML after an antecedent hematologic disease (Table 1).

Despite these poor-risk features, antileukemic effectiveness of PSC-MEC was shown, with 12 CRs (32%) being achieved among the 37 patients treated (Table 2). Responses occurred in all subgroups of patients entered in the study (Table 3). The overall early mortality rate of 30% (12 of 37) is comparable with that occurring in patients receiving other salvage protocols.⁴³⁻⁴⁵ Most of the deaths (9 of 12) were secondary to infections during the period of hypoplasia (Table 4). This complication was unrelated to whether the patient received one or two cycles of PSC-MEC induction therapy but correlated with the described PK parameters.

The median time to achieve a CR was relatively long (52 days). This slow hematopoietic recovery (despite use of GM-CSF or G-CSF in 29 patients) was likely multifactorial, ie, related to the specific drug regimen itself, plus to the patients' prior therapy, relatively elderly ages and secondary types of AML, all of which may contribute to prolonged postchemotherapy cytopenias.⁴³⁻⁴⁷ These data indicate the need for some delay during the period of recovery before determining response, as well as the need for prolonged postinduction supportive care in patients receiving this treatment regimen.

For these poor-risk patients, an important therapeutic aim of such a salvage regimen was to enable the option of BMT, which was performed in 5 patients. After achievement of CR, 9 of the 12 patients received consolidative therapy, including 4 with BMT. The median remission and survival duration of patients achieving a CR were 6 and 8.3 months, respectively. The median overall survival for all the 37 patients was 6.0 months. These values are comparable with prior studies with similar patients.^{22,48}

PSC is 2- to 10-fold more potent than its parent compound cyclosporine for modulating MDR *in vitro* and *in vivo* in animal models.^{19,20} In this study, target blood levels of PSC capable of *in vitro* P-gp modulation were achieved in all patients and were maintained for the 5 days of administration of this drug. This finding, plus the tolerability of the drug, indicated effective dosing of PSC with this regimen. Anticipating substantial PK interaction between PSC and the MDR-modulated agents used for treatment of leukemia, the doses of mitoxantrone and etoposide used for cohort I were significantly reduced compared with the doses used for these drugs without PSC.^{6,18,22,23} These doses were also lower than those previously used for MEC in combination with cyclosporine.⁴⁸ Despite reduction of the mitoxantrone and etoposide doses, the initial six patients treated (cohort I) experienced substantial hematologic and gastrointestinal toxicity. Therefore, for cohort II, the doses of mitoxantrone and etoposide were further reduced by 20%. With these lower doses of mitoxantrone and etoposide, adequate marrow hypoplasia was achieved and the drugs were relatively well tolerated,

even in elderly patients. Transient reversible hyperbilirubinemia attributable to PSC blockade of bilirubin excretion^{16,39} was observed in most patients.

Our PK analyses showed a 57% decrease in clearance of etoposide compared with historical controls without PSC.^{31,32} For mitoxantrone, the mean $T_{1/2\beta}$ was 1.8-fold longer than previously in the literature for patients treated in the absence of PSC.^{34,41} These findings for mitoxantrone are likely an underestimation of the effect of PSC on mitoxantrone PKs, because our sampling times did not allow determination of the characteristically long terminal gamma elimination phase for mitoxantrone.³⁴ Our observations are in concert with the previous observations of the effect of PSC on etoposide or mitoxantrone PKs.^{40,49} These PK interactions of these drugs are also consistent with clinical, laboratory, and animal model observations,^{50,51} and corroborate the need for dose reduction of mitoxantrone and etoposide when they are combined with PSC. Correlative evaluation further suggests that early deaths were PK related, particularly in patients with high mitoxantrone AUCs. These PK data have been separately reported in detail.³²

The expression of P-gp in de novo AML blast cells is generally lower than that occurring at relapse or in patients whose disease was refractory to chemotherapy or post-MDS/MPD.^{11,13,52,53} The predictive value of P-gp as an independent marker for treatment failure in AML is controversial, with most^{7,54,55} but not all^{17,48} studies showing that P-gp expression is correlated with a poor prognosis. Prior studies have suggested the importance of measuring the efflux of rhodamine to assess the function of P-gp and its potential blockade by modulators.^{28,29,54,56} In the report herein, the median level of P-gp expression (using the monoclonal antibody 4E3.16) was higher in blasts from patients with PSC-inhibitable rhodamine efflux than in those whose blasts lacked this effect (49% v 17%, $P = .004$; Table 6). It should be noted that no consensus has been reached in the First MDR Detection Methods Workshop.⁵⁷ There is currently no published agreement as to the extent of rhodamine-123 efflux which is predictive for clinically relevant P-gp function.

In this study, all 10 patients whose leukemia cells were positive for P-gp with 27% to 96% staining by 4E3.16 also showed efflux of rhodamine. In addition, there were 5 patients with low P-gp expression and high rhodamine efflux, indicating the possibility of another rhodamine transporter in these leukemias. Rhodamine efflux in these 5 cases was not inhibitable by PSC. It has previously been shown that drug efflux mechanisms unrelated to P-gp exist in a subgroup of AML patients.⁵⁸ Among the 10 leukemias expressing high levels of P-gp, two showed noninhibitable efflux, suggesting the coexpression of one of these non-P-gp rhodamine transporters. The expression level of P-gp in patients with noninhibitable P-gp function was significantly lower than that found on blast cells from patients with inhibitable function. Correlation between P-gp expression and response to treatment in this study could not be made in this relatively small group of patients. However, none of the 7 patients with noninhibitable rhodamine efflux achieved a remission.

A recent preliminary report of a randomized study using quinine as an MDR modulator plus intensive chemotherapy for AML post-MDS and high-risk MDS patients indicated that

quinine increased the CR rate and disease-free survival in patients considered to be P-gp positive, but not in those who were P-gp negative.⁵⁹ Other recent preliminary investigations using PSC plus chemotherapy for relapsed and refractory AML have also shown encouraging results.^{60,61} In contrast, a recent study not showing a benefit of PSC plus chemotherapy had a differing experimental design and marked regimen-related toxicity, which may have contributed to the relatively poor clinical outcomes of those patients.⁴⁹

There is increasing evidence that mechanisms in addition to P-gp contribute to the MDR resistance phenotype in human malignancies.⁶²⁻⁶⁷ These include the MDR-related protein,^{68,69} the lung-resistance protein,⁶⁷ and the transporter of antigenic peptides.⁷⁰ These mechanisms, like P-gp, may cause increased efflux of drugs (and surrogate markers like rhodamine) and/or intracellular redistribution of drugs. The *bcl-2* family of proto-oncogenes, which are critical regulators of apoptosis, may also play a role in drug resistance in leukemias.⁷¹ Altered topoisomerase II expression may contribute to resistance to anthracyclines, etoposide, and mitoxantrone in leukemias.⁷² These other mechanisms of resistance may limit the efficacy of P-gp modulators either by their coexpression at the outset of therapy or by their emergence after the elimination of P-gp-positive tumor cells. Development of multifunctional MDR modulators or combined blockade of more than one resistance mechanism may be necessary to effectively circumvent MDR in hematologic malignancies.

Prevention of the emergence of drug resistance has been shown in preclinical experiments in which drug-sensitive cancer cells are treated with MDR-related cytotoxins together with P-gp modulators.⁷³⁻⁷⁵ The suppression of *MDR1* expression in these models suggests that MDR modulation could be considered early in the course of the disease, before the emergence of either P-gp or other mechanisms of drug resistance.

The study reported herein suggests that MDR-modulating agents such as PSC plus chemotherapy are potentially useful for treating poor-risk AML patients. However, the determination of the relative contribution of PSC to clinical responses in this setting will require phase III randomized trials comparing this regimen with equitoxic control regimens without an MDR-modulator. Such a study of PSC-MEC versus MEC chemotherapy (E2995), based on this phase II trial, has recently been activated within the ECOG in a similar subgroup of patients.

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