Mitoxantrone, Etoposide, and Cytarabine plus Cyclosporine for Patients with Relapsed or Refractory Acute Myeloid Leukemia

An Eastern Cooperative Oncology Group Pilot Study

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BACKGROUND. One potential mechanism of drug resistance to chemotherapy is the overexpression of multidrug resistance (MDR) genes coding for P-glycoprotein (P-gp), which leads to reduced intracellular retention of chemotherapy. This study tested the efficacy and toxicity of mitoxantrone, etoposide, and intermediate dose cytarabine (MEC) with cyclosporine (CSP) as an MDR modulator in patients with recurrent and refractory acute myeloid leukemia, and also correlated P-gp expression in leukemia cells with response.

METHODS. Thirty-eight eligible patients who were in first recurrence after < 6 months of complete remission (CR) (11 patients), refractory to initial induction therapy or to one attempt at reinduction after recurrence (18 patients), in second recurrence (4 patients), or in recurrence after either allogeneic or autologous bone marrow transplantation (5 patients) received either MEC alone (13 patients) or MEC-CSP (25 patients). CSP was given as a loading dose of 6 mg/kg for 2 hours intravenously (i.v.) starting 2 hours before the first dose of etoposide, followed by a continuous i.v. infusion of 18 mg/kg/day for 98 hours.

RESULTS. Three of the 13 patients (23%) who received MEC achieved CR, as did 6 of the 25 patients (24%) who received MEC-CSP. The median remission duration for all patients who achieved CR was 149 days (range, 26–466 days), 91 days (range, 81–172 days) for the 3 patients who received MEC, and 189.5 days (range, 26–466 days) for the patients treated with MEC-CSP. The median survival for the patients treated with MEC and MEC-CSP was 104 and 72 days, respectively.

CONCLUSIONS. No significant association was found between P-gp expression and response. No apparent benefit in the CR rate, remission duration, or survival was observed with the addition of CSP to MEC. ***Cancer* 1999;85:358–67.**

KEYWORDS: acute myeloid leukemia, cyclosporine, multidrug resistance, P-glycoprotein expression.

Complete remission (CR) currently is achievable in approximately 60-80% of adults with previously untreated acute myeloid leukemia (AML) using chemotherapy that includes an anthracycline and cytarabine.[1-3] However, despite intensive consolidation chemotherapy only 30-40% of patients who achieved CR remain alive and disease free 5 years later, with the majority of treatment failures attributable to recurrent leukemia.[4-7] Chemotherapy for patients with recurrent disease generally is unsatisfactory. Although second remissions can be induced with single agents or combinations of agents used during initial remission induction, the median duration of sec-

ond remissions usually is brief.[8-10] Although a number of studies have reported high second CR rates and even prolonged survival with high dose cytarabine alone or in combination with an anthracycline, asparaginase, or amsacrine, in general the duration of such remissions is short.[11-15]

Drug resistance is an important cause of failure of chemotherapy.[16,17] Recently, our understanding of multidrug resistance (MDR) at the cellular and molecular levels has improved.[18,19] The majority of cellular models of drug resistance selected in vitro by anthracyclines or vinca alkaloids display an MDR phenotype related to overexpression of the *MDR*-1 gene coding for P-glycoprotein (P-gp). The *MDR*-1 P-glycoprotein functions as a transmembrane efflux pump, which leads to reduced intracellular retention of chemotherapeutic drugs because of a membrane efflux pump.[20] The drugs involved in MDR include anthracyclines (doxorubicin, daunorubicin, and idarubicin), vinca alkaloids (vincristine, vinblastine, and vinorelbine), and the epipodophyllotoxins (etoposide and teniposide). Several drugs including cyclosporine, the potent immunosuppressive agent in wide use for organ transplantation, are capable of reversing MDR in vitro.[21-29] Several new agents have shown effectiveness in recurrent and refractory AML, including mitoxantrone[30-35] and the epipodophyllotoxins.[36] There appears to be a significant increase in the response rate when etoposide is used in combination with an anthracycline and cytarabine.[37-40]

The combination of mitoxantrone and etoposide has yielded encouraging results in patients with recurrent and refractory AML.[37,41] High dose as well as conventional dose cytarabine also has been combined with mitoxantrone successfully in patients with recurrent and refractory AML.[38,40] Furthermore, several studies have demonstrated the effectiveness of a combination of intermediate dose mitoxantrone, etoposide, and cytarabine.[42-44] Response rates ranging from 35-87% were reported among patients in first recurrence, those with refractory disease, and those in second recurrence after allogeneic bone marrow transplantation (BMT).

The current study was designed to test the efficacy and toxicities of the three-drug combination of mitoxantrone, etoposide, and intermediate dose cytarabine (MEC) with cyclosporine (CSP) as an MDR modulator in patients with recurrent and refractory AML. We also sought to correlate MDR expression in leukemic cells with response.

MATERIALS AND METHODS

Patient Eligibility

Patients with recurrent or refractory AML fulfilling the following eligibility criteria were eligible for this Eastern Cooperative Oncology Group (ECOG) trial: 1) morphologic proof of AML established by central pathology review and classified according to the French-American-British classification[45]; 2) qualify as one of the following: in first recurrence after $\leq$ 6 months of CR, refractory to initial induction therapy or to one attempt at reinduction after recurrent, in second recurrence (as of May 1992), or in recurrence after either allogeneic or autologous BMT; 3) age 15-65 years; 4) no history of recent myocardial infarction (within 3 months), significant congestive heart failure, or cardiac arrhythmia; 5) normal ejection fraction by multigated angiogram scan; 6) no prior therapy with mitoxantrone or etoposide; 7) ECOG performance status of 0-2; and 8) obtainment of written informed consent. Prior high dose cytarabine $\geq$ 6 months previously or prior conventional dose cytarabine was permissible. Patients whose disease recurred $>$ 6 months from their initial CR were excluded.

Treatment Protocol

Induction and Consolidation Chemotherapy

Two groups of patients were accrued sequentially, one without and one with CSP. The first group without CSP received mitoxantrone, 10 mg/m^2/day, by continuos intravenous (i.v.) infusion over 15 minutes for 5 days, etoposide, 100 mg/m^2/day, i.v. over 1 hour daily for 5 days, and cytarabine, 1 g/m^2/day, i.v. over 1 hour daily for 5 days. All other patients initially received induction chemotherapy with mitoxantrone, 6 mg/m^2/day, by i.v. infusion over 15 minutes daily for 5 days, etoposide, 80 mg/m^2/day, i.v. over 1 hour daily for 5 days, and cytarabine at a dose of 1 g/m^2/day i.v. over 1 hour daily for 5 days (MEC). The doses for all drugs were calculated using the patient's actual weight.

After the initial cohort of 13 patients without CSP were treated, all other patients were given the chemotherapy described earlier with the addition of cyclosporine (MEC-CSP) at a dose of 6 mg/kg i.v. over 2 hours followed by 18 mg/kg/day by continuous i.v. for 98 hours. In this group, the doses of the MDR-related chemotherapy agents, mitoxantrone and etoposide, were reduced because of the previously described drug interactions of MDR drugs with CSP.[25-29,46]

Seven to 10 days after completion of the first cycle of therapy, a bone marrow aspiration and biopsy were performed to determine whether bone marrow aplasia had been achieved. Patients received a second cycle of therapy in identical doses 14-21 days after initiation of the first course of therapy if either circulating blasts persisted, or if the bone marrow aspirate and biopsy were not markedly hypocellular or aplastic and there were $>$ 5% blasts.

Patients were allowed 1 or 2 cycles of induction chemotherapy to achieve CR and then received 1 cycle of consolidation chemotherapy with the same regimen given in induction, either MEC or MEC-CSP, within 6 weeks. During treatment all patients were examined daily and had a daily complete blood count (CBC); during disease remission physical examination and CBC both were performed monthly.

Cyclosporine Administration

Cyclosporine was given 2 hours before the first dose of etoposide. An initial loading dose of 6 mg/kg of CSP for 2 hours i.v. was given followed by continuous i.v. of 18 mg/kg/day for 98 hours (total of 100 hours of CSP).[25] The 100-hour time of infusion was chosen to reduce the risk of nephrotoxicity from CSP anticipating that levels of CSP would remain high for several hours after the infusion ended. The goal was to achieve a steady-state serum CSP level $>$ 2.5 mM (3000 ng/mL by nonspecific immunoassay). If the initial CSP dose was tolerated without nephrotoxicity or other evidence of Grade 3 or Grade 4 CSP toxicity (except Grade 3 nausea, hypertension, or hyperbilirubinemia), the CSP level did not exceed 3.0 μM (3600 ng/mL), and the patient required a second induction course, the subsequent dose of CSP then could be escalated to 7 mg/kg loading and 21 mg/kg/day infusion.

Dose Modifications

The doses of mitoxantrone, etoposide, and cytarabine were not modified for serum bilirubin concentration during the initial induction phase. Modification of drug dosage in the second induction cycle was made as follows: if the bilirubin concentration was $<$ 1.5 mg/dL, full doses of MEC were given; if the bilirubin concentration was between 1.5-3.0 mg/dL, 50% of the doses of MEC were given; if the bilirubin concentration was $>$ 3.0 mg/dL, 25% of the doses of MEC were given. The initial CSP dose for the second induction course or consolidation based on the bilirubin concentration on Day 1 was as follows: if the bilirubin concentration was $<$ 1.5 mg/dL, a full dose of CSP was given; if the bilirubin concentration was $\geq$ 1.5 mg/dL and $<$ 2.0 mg/dL, the CSP loading dose was 6 mg/kg and the CSP daily infusion dose was 12 mg/kg/day. If the bilirubin concentration was $\geq$ 2.0 mg/dL and $<$ 3.0 mg/dL, the CSP loading dose was 4 mg/kg and the CSP daily infusion dose was 8 mg/kg/day; if the bilirubin concentration was $\geq$ 3.0 mg/dL, the CSP loading dose was 4 mg/kg and the CSP daily infusion dose was 4 mg/kg/day. If the creatinine clearance (Crcl) was $<$ 40 mL/minute but was $>$ 20 mL/minute at the time the patient was due for retreatment, the CSP dose was reduced by 25%. CSP was not to be administered if the Crcl fell to $<$ 20 mL/minute. The CSP dose also was adjusted for increases in serum creatinine on Days 2 and 3 of treatment. If the Crcl was $\geq$ 2.0 mg/dL and $<$ 2.5 mg/dL, the infusion dose was reduced by 50%. If the Crcl was $>$ 2.5 mg/dL, CSP was withdrawn.

The CSP dose was adjusted to maintain a plasma level of 2.5-4.0 mM (3000-4800 ng/mL), according to the plasma CSP levels drawn at 14, 26, 38, 50, and 74 hours after the initiation of treatment. If the plasma CSP was $>$ 6000 ng/mL, the infusion was discontinued until the next level was known. If the plasma CSP was between 4800-6000 ng/mL, the dose was reduced by 25% until the next level was known. If the plasma CSP was between 3000-4800 ng/mL (2.5-4 mM), the dose was not modified. If the plasma CSP was $<$ 3000 ng/mL, the dose was increased by 25%.

Supportive Care

Hematopoietic growth factors were not given during induction. If the bone marrow biopsy after induction was free of leukemia, hematopoietic growth factors were permitted during the consolidation cycle. The growth factor was to begin on Day 11 of the consolidation cycle and continue until the granulocyte count was $\geq$ 500/μL for 3 consecutive days.

Response Criteria

Response criteria used were based on those of the National Cancer Institute-Sponsored Workshop (with a minor modification of the required neutrophil count).[47] CR required all of the following: 1) normal peripheral blood counts including a neutrophil count $\geq$ 1000/μL, and a platelet count $\geq$ 100,000/μL without leukemic blasts in the peripheral blood; 2) a bone marrow biopsy that was at least 20% cellular with maturation of all cell lines, $\leq$ 5% blasts, and no Auer rods; and 3) no evidence of extramedullary leukemia. Partial remission (PR) required that all the criteria for CR be satisfied except that the bone marrow could contain $>$ 5% but $<$ 25% blasts. If all other criteria for CR were met, a value $\leq$ 5% blasts with Auer rods or abnormal morphology was considered a PR.

Recurrence after CR was defined as either 1) the reappearance of blasts in the peripheral blood or 2) the presence of $>$ 5% blasts in the bone marrow aspirate and biopsy not attributable to another cause such as bone marrow regeneration.

CSP Levels

CSP levels were measured at individual institutions. Of the 21 patients for whom CSP levels were available, the level was measured by Tdx immunoassay in 15

patients and high performance liquid chromatography in 6 patients.

P-gp Expression

Detection of P-gp on gated blast cells by flow cytometry was performed using two antibodies that recognize cell surface epitopes of P-gp: 4E3.16 (provided by Dr. Arceci, Cincinnati, OH) and MRK 16 (purchased from Kamiya Biomedical Company, Thousand Oaks, CA). Cell surface antibody binding was evaluated by flow cytometry using a FACScan and the Lysys II software program (Bectin Dickinson, Mountain View, CA). Because all patients in this study were tested centrally in ECOG's Immunophenotyping Laboratory, sample preparation and analysis were uniform and reagents were standardized with respect to antibody source, epitope specificity, and avidity.[48,49] Negative and positive cell populations were differentiated by setting quadrants so that $< 2\%$ of the cells stained with the isotype control were positive. Data are expressed as the percentage of blast cells that stained for a given antibody with a fluorescence intensity $> 98\%$ of the negative isotype control. The data presented were generated with 4E3.16. Data using MRK 16 were not different.

Statistical Considerations

For the MEC-CSP group, a two-stage design by Simon[50] was used to allow for an early termination if this treatment demonstrated no beneficial effects with respect to CR. Significance for predicting response by P-gp expression (%) was tested using logistic regression.[51] Based on the P-gp expression data, *MDR*-1 gene status was defined as positive (P-gp $\geq 36\%$) and negative (P-gp $< 36\%$). Associations between response and treatment and between response and *MDR*-1 gene status were assessed by a Fisher's exact test.[52] Remission duration was calculated from the date of CR until recurrence or censored on the last day known in CR. Remission durations were described by treatment. Survival was measured from date of study entry until death or last follow-up. The Kaplan-Meier method[52] was used to estimate survival curves and the significance of the difference between curves was tested using a log rank test.[53]

RESULTS

Forty-nine patients with recurrent and refractory AML were accrued between January 1992 and September 1995. Analysis was performed based on available data as of February 14, 1997. Thirty-eight of these 49 patients were eligible. Of the 11 patients who were ineligible, 3 patients canceled after registering because 1 refused therapy, 1 was registered in duplicate, 1 was in disease remission, and 2 had acute lymphoblastic leukemia after central review. Six other patients were ineligible because of the following: one was in first recurrence > 6 months from CR, one had an ECOG performance status of 3, one had received prior therapy with mitoxantrone and etoposide, one was receiving an antifungal medication at the time of study entry, and two had no pathology material available for central review. Therefore, 38 patients were available for analysis.

There were 19 females and 19 males. The median age of the patients was 46.5 years (range, 19-65 years) (Table 1). Thirteen patients were treated with MEC alone and 25 patients were treated with MEC-CSP. Of the 13 patients treated with MEC alone, 4 were in first recurrent, 8 had refractory disease, and 1 was in second recurrence. Among the 25 patients treated with MEC-CSP, 7 were in first recurrence, 10 had refractory disease, 5 developed disease recurrence after BMT, and 3 were in second recurrence.

Remission Induction

Three of the 13 patients (23%) receiving MEC achieved CR (95% confidence interval [95%CI], 5.0-53.9%) and 10 had no response. Six of the 25 patients (24%) receiving MEC-CSP achieved CR (95% CI, 9.5-45.2%), and 19 had no response. The 95% CI for MEC-CSP was adjusted for the two-stage design. There was no significant difference in the CR rates mentioned earlier for the two groups. Two of the three patients achieving CR with MEC alone had refractory disease and one patient was in first recurrence (Table 2). Of the six patients who achieved CR with MEC-CSP, three had refractory disease, one had disease recurrence after a BMT, and two were in first recurrence. CRs were achieved with one cycle of induction therapy in all cases. Induction failures were classified according to the classification of Priesler.[54] Of the ten patients failing to achieve CR with MEC, one was a treatment-related death and nine were cases of disease-related death. Of the 19 patients failing to achieve CR with MEC-CSP, 3 were treatment-related deaths all attributable to infection, 10 were cases of disease-related death, 2 were neither treatment-related nor disease-related deaths, and 3 did not have available data. A total of 29 patients failed to achieve CR. Of those 29 patients, 11 were age > 50 years.

Remission Duration

The median remission duration among patients achieving CR was 149 days (range, 26-466 days). Of the 3 patients achieving a CR with MEC, the median remission duration was 91 days (range, 81-172 days). Of the 6 patients achieving CR with MEC-CSP, the me-

TABLE 1
Patient Characteristics (n = 38)

	MEC (n = 13)	MEC-CSP (n = 25)
Age (yrs), median (range)	47 (22–65)	46 (19–65)
Gender		
Male	6 (46.2%)	13 (52.0%)
Female	7 (53.8%)	12 (48.0%)
ECOG performance status		
0	2 (15.4%)	7 (28.0%)
1	6 (46.2%)	16 (64.0%)
2	5 (38.4%)	2 (8.0%)
FAB classification		
M1	2 (15.4%)	5 (20.0%)
M2	7 (53.9%)	7 (28.0%)
M3	0 (0%)	1 (4.0%)
M4	1 (7.7%)	1 (4.0%)
M5	1 (7.7%)	6 (24.0%)
Other[a]	2 (15.4%)	5 (20.0%)
Recurrence status		
1st recurrence	4 (30.8%)	7 (28.0%)
Refractory	8 (61.5%)	10 (40.0%)
Recurrence after BMT	0	5 (20.0%)
2nd recurrence	1 (7.7%)	3 (12.0%)
Duration of first CR (days) median (range)	91 (81–172) (n = 3)	189.5 (26–466) (n = 6)
Leukocyte count at diagnosis (per μL), median (range)	5.6 (0.4–55.7)	5.6 (0.6–179.4)
Hemoglobin (g/dL), median (range)	9.3 (5.9–19.1)	9.9 (6.8–13)
Platelet count ($\times 10^3$ per mL), median (range)	48.0 (20–139)	58 (10–428)
Peripheral blast cell count (%), median (range)	17.5 (0–83)	6.0 (0–93)
Bone marrow blast cell count (%), median (range)	50.0 (7–153)	50.5 (0–95)

MEC: mitoxantrone, etoposide, and cytarabine; MEC-CSP: cyclosporine, mitoxantrone, etoposide, and cytarabine; ECOG: Eastern Cooperative Oncology Group; FAB: French–American–British; BMT: bone marrow transplantation; CR: complete remission.

[a] Two other patients were in the mitoxantrone, etoposide, and cytarabine arm with recurrent acute myeloid leukemia. Five other patients were in the cyclosporine plus mitoxantrone, etoposide, and cytarabine arm; two patients with acute myeloid leukemia had a disease recurrence, one patient had acute myeloid leukemia that was either in recurrence or refractory, one patient had acute myeloid leukemia with trilineage dysplasia, and one patient was nondiagnostic.

dian remission duration was 189.5 days (range, 26-466 days). A summary of the nine patients achieving CR is presented in Table 2.

Survival

The median survival for the patients treated with MEC was 104 days (95% CI, 69-206 days) and the median survival for the patients given MEC-CSP was 72 days (95% CI, 33-199 days). At last follow-up, all patients except one who were treated with MEC-CSP had died. At last follow-up, the duration of follow-up for the 1 surviving patient was 986 days. There was no significant difference in survival between patients treated with MEC and those who received MEC-CSP.

P-gp Expression

The percentage of gated blasts expressing P-gp as recognized by reactivity with antibody 4E3.16 ranged from 0-99% (median, 5.5%) (Table 3). This is based on 28 patients with P-gp expression data, 9 of whom were treated with MEC and 19 of whom were treated with MEC-CSP. The threshold P-gp expression that predicts for clinical resistance still is unresolved. In an ECOG database analysis of $>$ 700 patients with de novo AML, P-gp expression by leukemic blasts per se did not predict for response.[56] However, in combination with CD34 expression, a threshold level of 36.5% P-gp positive blast cells identified a subpopulation of patients with a significantly inferior prognosis. Because functional P-gp studies were not performed in the current study, we relied on this 36% prognostic cutoff level for P-gp to define high and low P-gp expression. Using this 36.5% threshold in the current analysis, blasts from 8 patients showed a high P-gp expression (3 among the patients treated with MEC and 5 among the patients treated with MEC-CSP).

There was no difference in outcome between the three patients treated with MEC and the five patients treated with MEC-CSP.

Correlation between P-gp Expression and Response

No significant association was found between MDR gene expression and response in patients treated with or without CSP (Table 3) (Fig. 1).

Toxicity

Hematologic Toxicity

Hematologic toxicity was manifested by profound cytopenias in all patients (Table 4).

Extramedullary Toxicity

Toxicity data are summarized in Table 4. Treatment with MEC or MEC-CSP was well tolerated. Six patients (2 of the 13 treated with MEC [15%] and 4 of the 25 treated with MEC-CSP [16%]) died of infection. The infections included gram-positive sepsis in two patients, gram-negative sepsis in two patients, fungal pneumonia in one patient, and cerebral *Aspergillus* in one patient. Grade 4 liver toxicity, which occurred in 4 of the 13 patients treated with MEC (31%) compared with 13 of the 25 patients treated with MEC-CSP (52%), primarily was due to transient reversible hyperbilirubinemia. Grade 4 stomatitis was not observed among patients treated with MEC, but occurred in 6 patients (24%) treated with MEC-CSP. One patient had

TABLE 2
Summary of Patients Achieving CR

Patient	Therapy	Age (yrs)	Gender	Status	CR duration (days)
1	MEC	27	M	Refractory	81
2	MEC	62	M	Refractory	91
3	MEC	22	F	1st recurrence	172
4	MEC-CSP	52	F	1st recurrence	237
5	MEC-CSP	38	F	Recurrence after BMT	466
6	MEC-CSP	61	M	Refractory	149
7	MEC-CSP	19	M	1st recurrence	101
8	MEC-CSP	44	M	Refractory	26
9	MEC-CSP	37	F	Refractory	230

CR: complete remission; MEC: mitoxantrone, etoposide, and cytarabine; M: male; F: female; MEC-CSP: cyclosporine, mitoxantrone, etoposide, and cytarabine; BMT: bone marrow transplantation.

cytarabine therapy withdrawn after 2 days because of cerebellar toxicity, but continued to receive mitoxantrone and etoposide.

CSP Pharmacokinetics

Of the 25 patients who received MEC-CSP, 21 had CSP levels available. Eighteen of these 21 patients (86%) received full dose CSP whereas 3 patients (14%) required a dose reduction primarily because of hyperbilirubinemia. Seventeen of these 18 patients (94%) achieved steady state CSP levels $\geq$ a target level of 3000 ng/mL. Nine patients received increased doses of CSP during induction.

DISCUSSION

Our hypothesis in this study was that high expression of *MDR*-1 in the cells from patients with recurrent and refractory AML results in clinical resistance to certain drugs and the administration of CSP with such agents may reverse this resistance. Recent reports of the expression of MDR genes in patients with AML and an anecdotal report of the reversal of clinical resistance by CSP provides additional rationale for this approach.[23,24,27,57,58] This study was designed specifically to answer several questions. First, what is the CR rate and CR duration of MEC in patients with recurrent and refractory AML? Second, can CSP be added safely to this regimen? Third, does the addition of CSP, as an MDR modulator, improve the CR rate, theoretically by reversing MDR resistance? Fourth, is there a correlation between MDR expression in leukemic cells and response?

The CR rate of 23% achieved with MEC alone appears less favorable than that in previous reports. Amadori et al. and Archimbaud et al. reported significantly higher response rates both in patients in first recurrence and those with refractory disease.[42,43,44] However, patients in the current study had a particularly poor prognosis in that they were required to have disease recurrence occurring $<$ 6 months from their initial CR, and patients who had developed disease recurrence after a BMT were included. Although the same poor prognosis patients were included in the study by Amadori et al.,[42] the median age of the patients in the latter report was 24 years compared with 46.5 years in the current study. In the study by Amadori et al., the response rate among patients age $>$ 50 years was significantly less than patients age $<$ 50 years (29% vs. 76%, respectively). Patients in the study by Amadori et al.[42] received approximately 20% more chemotherapy because the same doses were administered for 6 days rather than 5 days as in the current study. In the 1991 study by Archimbaud et al., the overall response rate was higher (61%) than that reported in the current study despite a similar median age, but the highest response rates (81%) were achieved in patients who had developed disease recurrence after $\geq$ 6 months of CR, who represented the largest cohort of patients.[43] A long term follow-up study in a larger group of patients by these investigators confirmed the high CR rates in patients whose disease recurred after a long first CR.[44]

The current study showed that CSP can be added to MEC chemotherapy with acceptable toxicity. The induction mortality rate of 17% among the patients treated with MEC-CSP was attributable solely to infection, is no different than that among patients treated with MEC alone, and is acceptable in this population. The only other serious extramedullary toxicities in patients treated with MEC-CSP included Grade 4 stomatitis in 6 patients (24%) and transient reversible hyperbilirubinemia (59%). The latter most likely was due to inhibition of an as-yet unidentified non-P-gp hepatic transporter of bilirubin by CSP. There was no

TABLE 3
Correlation of MDR Gene Expression in Leukemic Cells with Response

Patient	MEC/MEC-CSP	P-gp expression	Response
1	MEC	5%	NR
2	MEC	30%	NR
3	MEC	3%	NR
4	MEC	NA	CR
5	MEC	8%	NR
6	MEC	NA	CR
7	MEC	NA	NR
8	MEC	48%	NR
9	MEC	3%	NR
10	MEC	66%	NR
11	MEC	NA	NR
12	MEC	3%	NR
13	MEC	92%	CR
14	MEC-CSP	15%	CR
15	MEC-CSP	3%	NR
16	MEC-CSP	20%	CR
17	MEC-CSP	43%	NR
18	MEC-CSP	NA	NR
19	MEC-CSP	4%	NR
20	MEC-CSP	54%	NR
21	MEC-CSP	5%	CR
22	MEC-CSP	5%	CR
23	MEC-CSP	5%	NR
24	MEC-CSP	NA	CR
25	MEC-CSP	0%	NR
26	MEC-CSP	33%	CR
27	MEC-CSP	4%	NR
28	MEC-CSP	1%	NR
39	MEC-CSP	6%	NR
30	MEC-CSP	4%	NR
31	MEC-CSP	37%	NR
32	MEC-CSP	NA	NR
33	MEC-CSP	NA	NR
34	MEC-CSP	NA	NR
35	MEC-CSP	3%	NR
36	MEC-CSP	85%	NR
37	MEC-CSP	NA	NR
38	MEC-CSP	99%	NR

MDR: multidrug resistant; MEC: mitoxantrone, etoposide, and cytarabine; MEC-CSP: cyclosporine, mitoxantrone, etoposide, and cytarabine; P-gp: P-glycoprotein; NR: no response; NA: not available; CR: complete remission.

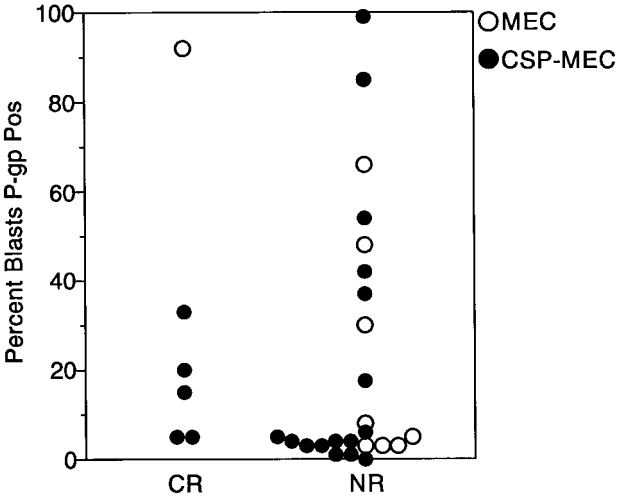

FIGURE 1. Response of patients according to P-glycoprotein (P-gp) expression of the leukemic cells determined using the antibody 4E3.16. Three additional patients achieved a complete remission (CR), but had no P-gp data available. Pos: positive; MEC: mitoxantrone, etoposide, and cytarabine; CSP-MEC: cyclosporine, mitoxantrone, etoposide, and cytarabine; NR: no response.

TABLE 4
Hematologic and Nonhematologic (Grade 4 and 5) Toxicity

	MEC (n = 13)		MEC-CSP (n = 25)	
	Grade		Grade	
Toxicity	4	5	4	5
Leukopenia	13	0	22	0
Anemia	0	0	6	0
Thrombocytopenia	13	0	23	0
Hemorrhage	0	0	1	0
Infection	1	2	5	4
Hepatic	4	0	13	0
Genitourinary	0	0	1	0
Stomatitis	0	0	6	0
Pulmonary	0	0	1	0
Skin	0	0	1	0
Neurologic	0	0	1	0
Hyperglycemia	0	0	1	0
Pain	0	0	1	0

MEC: mitoxantrone, etoposide, and cytarabine; MEC-CSP: cyclosporine plus mitoxantrone, etoposide, and cytarabine.

other evidence of liver toxicity, such as transaminase elevation, and the hyperbilirubinemia rapidly resolved.

Although this was not a randomized study, patient characteristics were similar and therefore we compared the two groups with respect to CR rate, CR duration, and survival. There was no apparent benefit in the CR rate, CR duration, or survival with the addition of CSP to MEC in this study of patients with advanced AML. Several factors may explain the lack of benefit. It is possible that CSP was not potent enough as an MDR modulator. CSP[59] and other first-generation MDR modulators such as dexverapamil[60] and quinidine[61] have been shown to have modest or no clinical benefit in other studies. More potent MDR modulators, such as the CSP analog PSC 833, may be required to demonstrate a significant improvement in response.[62-64] Although the CSP levels necessary to overcome resistance were attained, it is evident in retrospect that these levels most likely are not associated with complete inhibition of P-gp in patients because of the effect of protein binding sequestering the

modulator.[65] It is apparent that the clinical potential of MDR modulators often is limited due to high plasma binding.[66] However, as these authors demonstrated, modulators vary considerably in the degree of protein binding and bioavailability of P-gp inhibition. With the exception of PSC-833, all MDR modulators show an insufficient or suboptimal modulation of P-gp under serum conditions and concentrations achievable in vivo, including verapamil, dexnigludipine-HCL, CSP, and the protein kinase C inhibition CGP 41251.

No definite correlation between P-gp expression and response could be made in the current study. It must be recognized that the number of patients treated and tested for P-gp was too small to draw any definitive conclusions regarding an association between P-gp and response. The threshold level of 36.5% for defining high P-gp expression is higher than commonly used ranges of arbitrary cutoff points (1-50%, but most commonly 20%). The cutoff point used in the current study was derived by computer-assisted real time transcription analysis.[56] Although it does not represent a prognostically significant cutoff point per se, it is the optimal cutoff point for this antigen, and when analyzed in combination with CD34 becomes a prognostically significant cutoff point. Given that the cutoff point used in the current study is higher than what usually is used to define P-gp "positivity," it appears that the incidence of "P-gp positivity" in our patient population was particularly low. We[67] and others[68] previously have shown that high CD34 expression in AML correlates with P-gp function; therefore, in the combined CD34/P-gp analysis, CD34 is a surrogate marker for P-gp function. The blasts from only 8 patients (21%) examined in the current study were above this threshold. It is possible that mechanisms of resistance other than increased P-gp expression may be important, including the expression of multidrug resistance-associated protein and[69,70] lung resistance-associated protein,[71] or the enhanced activity of glutathione-S-transferase protein.[72]

Clinical studies testing potentially more potent MDR modulators, such as the CSP analog PSC-833, currently are underway.[55,73] Larger, prospective randomized studies comparing the outcome of patients treated with or without potent P-gp reversing agents are needed to determine the true value of MDR modulators.

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