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Dose-finding study of valspodar (PSC 833) with daunorubicin and cytarabine to reverse multidrug resistance in elderly patients with previously untreated acute myeloid leukemia

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Introduction: This trial was designed to determine the maximum tolerated dose of intravenous daunorubicin (DNR) in combination with valspodar and to test the feasibility of P-glycoprotein modulation using valspodar in elderly patients with previously untreated acute myelogenous leukemia receiving standard induction chemotherapy.
Methods: Patients $\geqslant 60$ years of age with previously untreated AML received valspodar (10 mg/kg/24 h by continuous intravenous infusion [CIV] on days 1–4 with a 2-mg/kg loading dose on day 1) in conjunction with two cycles of induction chemotherapy consisting of cytarabine (200 mg/m^2 CIV on days 1–7), and DNR (35 mg/m^2 [cohort 1] or 45 mg/m^2 [cohort 2] on days 1–3, intravenous bolus). Patients were assessed for dose-limiting toxicities (DLT), response rate, event-free and overall survival, and pharmacokinetics of valspodar and DNR.
Results: Valspodar was well tolerated at the lower DNR dose level (ie, 35 mg/m^2) resulting in a 21% rate of DLT and only three toxic deaths. Treatment-related mortality was unacceptably high at the 45 mg/m^2 DNR dose level. The complete response rate was 49% overall and similar in both cohorts. The median overall survival of patients was 333 days in cohort 1 compared to 98 days in cohort 2. At baseline, 70% of assessable patients were P-glycoprotein positive.
Conclusion: Substantial inhibition of P-glycoprotein activity can be achieved in this patient population at clinically tolerable doses of valspodar and DNR. The maximum tolerated dose of DNR was established as 35 mg/m^2. This regimen is being further evaluated in phase III trials.
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Keywords: acute myeloid leukemia; multidrug resistance; reversal of resistance; daunorubicin; valspodar

Introduction

Intrinsic or acquired resistance to chemotherapy remains a major obstacle to achieving long-term survival in patients with acute myeloid leukemia (AML). Standard induction therapy produces complete remission (CR) in 52–72% of patients with *de novo* AML[1–8] depending on a variety of factors, including age of the patient, history of myelodysplastic syndrome (MDS), and cytogenetic and immunophenotypic profile. Lower response rates are observed in older patients[9–13] or in those with secondary AML or a history of MDS.[14] Overall median duration of remission is 8–12 months, and median survival is 9–16 months. Approximately 40% of patients who received intensive induction or consolidation therapy with high-dose cytarabine remain disease free at four years.[8,15,16] However, the benefits of intensive therapy have been confined primarily to younger patients (ie, <55 years of age).

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One hypothesized reason for failure to respond to chemotherapy is the presence of multidrug resistance (MDR), a mechanism of resistance to structurally unrelated cytostatic agents. In AML, MDR is most frequently mediated through enhanced drug efflux by P-glycoprotein (P-gp).[17,18] This 170-kd transmembrane glycoprotein is a member of the adenosine triphosphate-binding cassette (ABC) proteins[19,20] and is encoded by the multidrug resistance gene-1 (*MDR-1*).[21–23] In cancer cells, P-gp is capable of extruding a wide variety of structurally unrelated drugs taken up through passive diffusion, including daunorubicin (DNR), doxorubicin, idarubicin, mitoxantrone, etoposide, and vinca alkaloids that are frequently used for treating AML.[24] P-glycoprotein is also expressed in several normal tissues, such as $CD34^+$ hematopoietic progenitor cells, biliary canaliculi, kidney tubules, and the blood-brain barrier.[18,25]

Since 1989 several studies have reported the *MDR-1* phenotype to be expressed in about 30–50% of AML patients, depending on the technique and the threshold used,[26–29] with a higher percentage in AML patients relapsing after or refractory to chemotherapy.[30] More recent studies by the Southwest Oncology Group (SWOG) indicate that the *MDR-1* gene is expressed with extremely high frequency in elderly AML patients (ie, patients >55 years of age). As many as 80% of elderly patients with *de novo* AML present with a MDR phenotype as a result of P-gp expression; the frequency of P-gp expression is similar in both *de novo* and secondary disease.[31]

The correlation between *MDR-1* gene expression and treatment outcome in AML is well documented.[32] In most studies, *MDR-1* expression was an adverse prognostic factor for achieving a CR.[33–50] In elderly, newly diagnosed patients, the CR rate of strongly P-gp–positive as compared to P-gp–negative patients was recently found to be 31 *vs* 71%.[31] Finally, several recently published large studies have shown that *MDR-1* expression, as well as secondary AML, CD34 positivity of leukemic blasts, unfavorable cytogenetics, and age were each, significantly and independently, associated with lower CR rates in elderly patients.[36,49–52]

Phase I/II studies of cyclosporine A (CsA), combined with DNR in patients with relapsed/refractory AML or with VAD (vincristine, doxorubicin, dexamethasone) in patients with multiple myeloma, have demonstrated the feasibility of CsA-mediated MDR reversal *in vivo*.[53–55] Valspodar (PSC 833) is an analog of cyclosporine D, which is approximately 5–30-fold more potent than CsA *in vitro*.[56] Unlike CsA, however, valspodar is neither immunosuppressive nor nephrotoxic.[56–58] In fact, unlike CsA, which is a substrate for P-gp and appears to exert its modulatory effects by competitive inhibition,[59,60] valspodar is a high-affinity, noncompetitive inhibitor of P-gp and is not appreciably transported by it.[61] In phase I/II trials, it was established that valspodar blood levels high enough to reverse MDR in resistant cell lines *in vitro* (1000 to 2000 ng/ml)[62–64] could be achieved and were well tolerated. Finally, in hematological malignancies, MDR reversal by valspodar has been demonstrated in patients with VAD-resistant/refractory multiple myeloma.[34,65]

Because P-gp has a physiological role in tissues normally responsible for the transport and excretion of cytotoxic drugs, treatment with P-gp inhibitors delays the elimination of other anticancer drugs. In phase I studies the dose-normalized area under the curve (AUC) increased by 61–74% for doxorubicin and by 84% (mean values) for etoposide when these drugs were given in combination with valspodar.[62,66] This pharmacokinetic interaction therefore requires appropriate dose reduction of the cytotoxic drugs that are P-gp substrates, when used in association with valspodar, to continue to provide effective treatment without increasing toxicity. Herein we report the results of a phase I study to establish the maximum tolerated dose (MTD) of DNR, combined with fixed doses of valspodar and cytarabine, in previously untreated, elderly AML patients.

Methods

The study was an open, multicenter, multinational trial. The study protocol was approved by the Institutional Review Boards of the participating hospitals. All patients gave prior written informed consent.

Patient population

The study population consisted of previously untreated patients $\geqslant 60$ years of age, who had a cytopathologically confirmed diagnosis of primary or secondary AML (M0 to M7) according to the French-American-British (FAB) classification. Patients who had progressed from myelodysplastic syndrome (MDS) were eligible if they had received no previous chemotherapy. Patients with a prior history of polycythemia rubra vera or primary myelofibrosis, or in blast cell crisis of chronic myeloid leukemia, were excluded. Other eligibility criteria included World Health Organization (WHO) performance status $\leqslant 2$, life expectancy $\geqslant 12$ weeks, and suitability for intensive chemotherapy in the opinion of the treating physician. Documented central nervous system involvement, left ventricular ejection fraction of $<50\%$ as measured by cardiac radionuclide-gated blood pool scan, serum creatinine $\geqslant 175$ μmol/l, serum bilirubin $\geqslant 2.5$-fold the institutional upper limit of normal, positive hepatitis B surface antigen or a known history of hepatitis C or human immunodeficiency virus infection, known hypersensitivity to CsA, or prior treatment with cytotoxic agents and/or radiotherapy were all causes for exclusion. All patients received a central venous access catheter and were hospitalized until their granulocytes had recovered to $\geqslant 0.5 \times 10^9$ cells/l.

Schedule of chemotherapy and valspodar

All patients were planned to receive two identical induction cycles, irrespective of the response to the first cycle. In each induction cycle, valspodar (which was supplied in a cremophor EL-based solution and was diluted in saline) was administered via continuous intravenous infusion (CIV) at a dose of 10 mg/kg/24 h on days 1–4; on day 1, the infusion was started concomitantly with a loading dose of 2 mg/kg administered over 2 h. Cytarabine was administered CIV at a dose of 200 mg/m^2/24 h on days 1–7. Daunorubicin was administered as an intravenous (IV) bolus injection on days 1–3: the starting dose (cohort 1) was 35 mg/m^2. This dose was escalated in cohort 2 to 45 mg/m^2. No further dose escalation of DNR was foreseen in the protocol. All patients who were in CR after the induction cycles received a single consolidation course consisting of etoposide at a dose of 80 mg/m^2/day intravenous (IV), mitoxantrone at a dose of 6 mg/m^2/day IV, and cytarabine (1.0 g/m^2) as a 6-h infusion on days 1–4. Valspodar was not administered during the consolidation course.

Determination of maximum tolerated dose and dose-limiting toxicities

The primary objective of the study was to determine the MTD of DNR combined with a fixed dose of valspodar and cytarabine. Two doses of DNR were evaluated: 35 mg/m^2/day (cohort 1) and 45 mg/m^2/day (cohort 2). Dose escalation was allowed if the observed dose-limiting toxicity (DLT) rate in cohort 1 was $<25\%$; dose reduction was necessary if the observed DLT rate was $>40\%$. The decision to escalate (or reduce) the DNR dose in the next cohort was based on the safety profile of the first 16 evaluable patients. However, accrual on the ongoing dose was continued until the next cohort was open, thus allowing for more than 16 patients to be enrolled in each cohort.

Dose-limiting toxicities were defined as: (1) neutropenia (absolute neutrophil count [ANC] $<0.5\times10^9$ cells/l or thrombocytopenia ($<50\times10^9$ platelets/l) in the absence of AML for >8 weeks after the start of treatment cycle 1; (2) death during or after treatment in cycle 1 without recovery of blood cell counts (ANC $\geqslant0.5\times10^9$ cells/l and platelets $\geqslant50\times10^9$/l) or hypoplasia without evidence of persistent AML; and (3) any Common Toxicity Criteria (CTC) grade 3/4 nonhematologic toxicity except nausea, alopecia, cerebellar ataxia, hyperbilirubinemia lasting $\leqslant20$ days, or infection responsive to antibiotic treatment.

Response criteria and treatment outcome

Complete remission was defined as: (1) a normal marrow cellularity (ie, $>20\%$) with $<5\%$ blasts and no Auer rods; (2) no evidence of extramedullary leukemia; and (3) recovery of peripheral blood neutrophils to at least 1500 cells/μl and platelets to at least 100 000/μl (all persisting for at least 28 days). Treatment failure was defined as: (1) absolute drug resistance with failure to achieve a reduction of the leukemic infiltration in the marrow that would meet the criteria of CR, or persistent leukemia in the peripheral blood, or persistent extramedullary disease; (2) regeneration failure with persistent hypoplasia for more than 40 days after completion of induction therapy; or (3) aplastic death: death during hypoplasia without signs of persistent leukemia. The overall survival was calculated from start of treatment until the date of death. The frequencies of excessive toxicities, infections, and hemorrhages were evaluated separately.

Pharmacokinetic analyses

Measurements of valspodar blood levels were performed to determine that the concentration of valspodar was within the range of maximal *in vitro* P-gp inhibition. Blood samples (2 ml) were drawn before starting valspodar infusion on days 1–4. The samples were stored in EDTA-coated tubes at -20°C. The valspodar concentration in whole blood was determined using a radioimmunoassay (RIA) as previously published.[67]

Samples for measurement of plasma concentrations of DNR and the hydroxylated metabolite DNR-ol were taken on day 3 of cycle 1, just before, just after, and at 4, 24, and 48 h after DNR administration. Blood samples (5 ml) were drawn into heparinized tubes and centrifuged as soon as possible, and the plasma was immediately transferred into uncoated polypropylene tubes and deep frozen at -20°C. Plasma levels of DNR and DNR-ol were evaluated by high-performance liquid chromatography as previously described.[68] Plasma AUC of DNR and DNR-ol up to the last measurable sampling time point (AUC_{last}) was calculated by the log-linear trapezoidal method. The terminal half-life was assessed by linear regression to the log-concentration-time plot and AUC from time zero to infinity was determined by the formula $AUC_{inf}=AUC_{last}+C\ (last)/(0.693/t_{½})$. The AUC_{inf} was corrected for carry-over from the two previous doses by $AUC_{inf,corr}=AUC_{inf}-C(0h)/0.693/t_{½})$. This correction of AUC allowed for a comparison to literature values.

Determination of P-gp expression and function

At diagnosis, and after informed consent was given, bone marrow aspirates were obtained from the sternum or the posterior iliac crest. Marrow samples (3 ml) were collected into 10-ml tubes containing 5000 International units (IU) of heparin in 2 ml of DMEM (Gibco BRL, Paisley, UK), cooled to between 0°C and 4°C, and shipped on ice overnight to the central laboratory. Mononuclear bone marrow cells were collected by centrifugation over Lymphoprep (Nycomed, Oslo, Norway), and suspended at a concentration of 4×10^9 cells/ml in DMEM supplemented with 10% fetal calf serum and gentamycin.

For measurement of P-gp expression, cells were incubated at room temperature with a monoclonal antibody against P-gp, either MRK16 (Kamiya Biomedical Company, Tukwila, USA) at a concentration of 10 μg/ml or UIC-2 (Immunotech, Marseille, France) at a concentration of 12.5 μg/ml, or a matching aspecific isotype control antibody mouse IgG2a (Sigma) at a concentration of 10 μg/ml. Cell-bound antibodies were detected by isothiocyanate (FITC)-labeled rabbit antimouse immunoglobulin antibodies (DAKO, Glostrup, Denmark). Cells were incubated with 7-aminoactinomycin (7-AAD, Sigma) to exclude nonviable cells. Fluorescence was measured using a FACScalibur (Becton and Dickinson, San Jose, USA). The results are expressed as the ratio of cell-associated fluorescence of cells incubated with the anti–P-gp antibody to the mean of cell-associated fluorescence of cells incubated with the matching aspecific control antibody.[40]

For measurement of P-gp function, cells were incubated for 1 h at 37°C at 5% CO_2 in the absence or presence of 2 μM valspodar. After this incubation, 2 ng/ml rhodamin-123 (Rho) obtained from Sigma (St Louis, USA) was added to the cells. A sample was taken at time 0 to correct for background fluorescence and after 75 min to measure intracellular Rho accumulation. Cells were incubated with 7-AAD to exclude nonviable cells. Fluorescence was measured using a FACScalibur. Results are given as the ratio of the mean intracellular Rho fluorescence of cells exposed to valspodar to the mean intracellular fluorescence of cells not exposed.

The drug-sensitive cell line RMPI 8226 S and the drug-resistant variant 8226 D6, kindly provided by Dr W Dalton (Lee Moffit Cancer Centre, Tampa, USA), were used as controls in each experiment. Based on all control tests run to measure P-gp function, the mean $\pm$ s.d. ratio of Rho fluorescence of the 8226 S was 0.90 ± 0.04 and of the 8226 D6 13.80 ± 7.48. For P-gp expression, the mean MRK16/ IgG2a ratio or UIC-2/IgG2a ratio of the negative controls, of ie the 8226 S cell line, was 1.25 ± 0.29 or 1.24 ± 0.26 respectively; the mean fluorescence ratio of the positive controls, ie the 8226 D6 cell line, with MRK16 or UIC-2 were 34.05 ± 4.00 and 36.18 ± 4.00 respectively. Patient bone marrow samples were considered positive for P-gp function when the ratio of Rho fluorescence was >1.05, this value representing a significantly higher ratio than that observed with 8226 S ($P<0.05$, 2-sided *t*-test). Patient samples were considered positive for P-gp expression if the ratio of P-gp fluorescence was >1.50 ($P<0.05$, 2-sided *t*-test).

Statistical methods

The association between response to treatment and P-gp expression was described by Kendall's correlation coefficient. This coefficient was also used to assess a possible correlation between the AUC of DNR and the occurrence of a DLT. Overall survival distributions were analysed descriptively by cohort using the Kaplan-Meier estimation method, and median survival times were derived.

Results

From March 1996 to March 1999, 39 patients with *de novo* AML were accrued into the study at 15 centers in five countries. The median age was 67 years (range, 60–83 years); other baseline characteristics are summarized in Table 1. The majority of patients (80%) had newly diagnosed AML, and 20% of patients had a prior MDS. The most common subtype was FAB M2 disease (33%), and 41% of patients had M4 disease or higher. The majority of patients (74%) had WHO performance status $\leqslant 1$. Among 34 patients with viable bone marrow samples at baseline who were evaluable for P-gp expression and function, 24 (70%) patients were P-gp positive by either immunohistochemical reactivity with the MRK16 antibody or by measurement of P-gp activity as measured by the Rho retention assay. Ten evaluable patients were clearly P-gp negative at baseline by both assays.

The first 19 patients were treated with 35 mg/m^2 DNR (cohort 1), and in the next 20 patients, the dose of DNR was escalated to 45 mg/m^2 (cohort 2). A total of 27 (69%) patients discontinued during the study, 16 due to death, three due to adverse events, seven due to treatment failure, and one due to inability to tolerate further treatment.

Table 1 Demographics and baseline characteristics of patients

	Number of patients (%)		
	Cohort 1 (n = 19)	*Cohort 2 (n = 20)*	*Total (n = 39)*
Median age, year (range)	67 (60–73)	66 (60–83)	67 (60–83)
Gender, male/female	13/6	10/10	23/16
WHO performance index			
0	8 (42)	4 (20)	12 (31)
1	6 (32)	11 (55)	17 (44)
2	5 (26)	5 (25)	10 (26)
AML FAB			
M0	0 (0)	1 (5)	1 (3)
M1	2 (11)	5 (25)	7 (18)
M2	9 (47)	4 (20)	13 (33)
M4	5 (26)	2 (10)	7 (18)
M5A	2 (11)	2 (10)	4 (10)
M5B	1 (5)	3 (15)	4 (10)
M6	0 (0)	1 (5)	1 (3)
unknown	0 (0)	2 (10)	2 (5)
De novo AML	14 (74)	17 (85)	31 (80)
Prior MDS	5 (26)	3 (15)	8 (20)
WBC $>20\times10^6$/L	5 (26)	10 (50)	15 (38)
Karyotype*			
NN	11 (58)	10 (50)	21 (54)
Unfavorable	9 (47)	8 (40)	17 (44)
Not done	0 (0)	1 (5)	1 (3)
P-gp expression or function†	14 (74)	10 (50)	24 (70)

FAB = French-American-British Classification; MDS = myelodysplastic syndrome; WBC = white blood cell count; P-gp = P glycoprotein. *NN:46(xy) or 46(xx); Unfavorable: all abnormal except t(8,21),t(15,17),inv(16). †Positive for either MRK16 staining or P-gp activity measured by rhodamine intracellular retention assay.

Safety

The incidence of DLT was <25% in cohort 1, therefore, the planned DNR dose escalation was carried out. In cohort 2, a high rate of DLTs and toxic deaths suggested that 35 mg/m^2 DNR was the MTD in combination with valspodar.

In cohort 1, four of 19 (21%) patients experienced a nonhematologic protocol-specified DLT during the first induction cycle (Table 2): CTC grade 3 mucositis, grade 3 hypotension, cardiac arrhythmia, and myocardial infarction (one patient each). None of these was fatal. The myocardial infarction occurred in a patient with pre-existing active coronary heart disease. There were seven deaths in cohort 1 (six deaths during cycle 1 and one death during cycle 2); six deaths occurred within 30 days of the start of treatment. Three patients died while in aplasia, and three patients died with leukemic re-growth. The cause of death was one of the following: gastrointestinal bleeding, septic shock, pneumonia, pulmonary embolism, or concomitant bronchopneumopathy. Ten patients were treated on the second induction cycle: one of these patients died with persistent leukemia before day 30 of the second cycle. One patient developed grade 3 mucositis during cycle 2.

In cohort 2, six of 20 (30%) patients experienced a protocol-specified DLT during the first cycle (Table 2). Three patients developed grade 3 mucositis, one patient each had a fatal cardiac arrhythmia and cardiac arrest, and one patient had a grade 3 rash. In addition, one patient developed during cycle 1 a grade 4 hyperbilirubinemia that persisted >20 days and thus fulfilled the protocol criteria for a DLT, yet was deemed secondary to persisting AML documented at day 22. A total of five patients died during cycle 1, four in the aplastic phase before day 30 of the cycle from either cerebral hemorrhage, pulmonary hemorrhage, septic shock, or rapid disease progression. Twelve patients in cohort 2 received a second induction cycle: five of these patients died either from sepsis ($n=3$), pneumonia ($n=1$), or cerebral hemorrhage ($n=1$). Four of the patients who died during the second induction cycle had achieved CR after the first cycle, the fifth patient had persistent leukemia and died on day 37. One patient had grade 4 hyperbilirubinemia, and four patients had grade 3 mucositis following cytotoxic treatment in cycle 2.

Table 2 Protocol-specified dose-limiting toxicities occurring in cycle 1

Number of patients	*Times of onset (Day)*	*Description*
Cohort 1		
1	3	Grade 3 cardiac arrhythmia
1	2	Grade 4 myocardial infarction
1	2	Grade 3 hypotension
1	24	Grade 3 stomatitis
Cohort 2		
3	4, 9, 10	Grade 3 mucositis
1	4	Cardiac arrhythmia (death)
1	31	Cardiac arrest (death)
1	4	Grade 3 skin rash

Fever, nausea, hypokalemia, and peripheral edema and were the most frequently observed nonhematologic toxicities in both cohorts (Table 3). The DLT of valspodar is cerebellar ataxia, which is transient and fully reversible. In this trial, 10 (26%) patients experiencing grade 1/2 ataxia, and three (8%) patients experienced grade 3 ataxia or cerebellar syndrome, which usually occurred between days 3 and 4 of valspodar administration. No patient experienced grade 4 ataxia. One patient had a dose reduction of valspodar to 7.5 mg/kg/day as a result of grade 3 cerebellar syndrome.

The incidence of hematologic toxicity was as expected with conventional anthracycline-based remission induction schedules in elderly patients. Only 46% of patients were assessable for neutrophil recovery. The median time to neutrophil recovery (ANC $\geqslant 0.5\times10^9$ cells/l) in assessable patients was approximately 31 days (range, 21–48 days) and was similar in both cohorts following cycles 1, 2, and 3. During cycle 1, all assessable patients recovered by day 56, the maximum number of days allowed per protocol. Similarly, 92% of patients developed grade 4 thrombocytopenia; however, recovery was complete by day 56 in all assessable patients. The incidence of grade 3/4 bleeding events was higher in cohort 2 (20%) than in cohort 1 (5%).

Response and survival

Eight of 19 (42%) patients in cohort 1 and 11 of 20 (55%) patients in cohort 2 achieved a CR for an overall response rate of 49% (Table 4). The majority of responding patients achieved a CR after 1 cycle of therapy. There were 13 treatment failures as a result of absolute or relative resistance: eight in cohort 1, and five in cohort 2. In cohort 1, early death (ie, within 30 days of the beginning of chemotherapy of either induction cycle) occurred in seven patients, compared with 10 patients in cohort 2, four of whom were in CR after cycle 1.

Table 3 Proportion of patients with the most common CTC grade 1/2 and grade 3/4 nonhematologic toxicities

	Cohort 1 (n=19; 30 cycles)		*Cohort 2 (n=20; 28 cycles)*	
Toxicity	*Grade 1/2 (%)*	*Grade 3/4 (%)*	*Grade 1/2 (%)*	*Grade 3/4 (%)*
Fever	89	11	60	15
Nausea	58	0	55	5
Edema	53	5	35	0
Skin	26	0	0	5
Mucositis	27	5	25	25
Hyperbilirubinemia	6	5	0	25
Hypokalemia	53	0	55	5
Arrhythmia	25	5	20	5
Sepsis	21	16	15	20
Ataxia	21	5	30	5
Headache	37	0	20	0
Hemorrhage	15	5	25	20
Hypertension	21	11	15	0

The median overall survival was 333 days in cohort 1 compared with 98 days in cohort 2 (Figure 1). The majority of patients died of progressive disease; however, the incidence of toxic deaths, primarily as a result of sepsis, was greater in cohort 2. The 6-, 9-, and 12-month survival rates were consistently higher in cohort 1 compared with cohort 2.

P-gp Expression

Among 34 patients evaluable for P-gp expression and function, 24 (70%) patients were positive for either P-gp expression or function (ie, bone marrow blasts were either positive for P-gp expression by flow cytometry using the MRK16 antibody or demonstrated P-gp activity by the Rho retention assay). Of these 24 P-gp–positive patients, 11 (46%) achieved a CR (Table 5). In 10 of these 24 patients, MRK16 reactivity and P-gp functional assays were discordant (ie, four patients were $MRK16^+/Rho^-$ and six patients were $MRK16^-/Rho^+$). Ten patients were negative for P-gp expression and function: of these, six (60%) achieved a CR. The CR rate was not significantly different in P-gp–positive compared with P-gp–negative patients. Furthermore, analysis based on Kendall's correlation coefficient demonstrated no correlation between response to treatment and P-gp expression or function. Among the 13 patients with absolute resistance (ie, treatment failures), three patients were P-gp positive, three patients were P-gp negative, and four were $MRK16^+/Rho^-$.

Pharmacokinetics

Repeated daily measurements of blood concentrations of valspodar were taken in 34 patients. One patient, however, was excluded from the pharmacodynamic analysis because he only received one-tenth of the planned valspodar dose. Daily blood concentrations of valspodar are shown in Figure 2. The median whole blood concentration during the continuous infusion of valspodar was approximately 2200 ng/ml (range, 778–

Table 4 Clinical outcomes

	Cohort 1 (n = *19*)	*Cohort 2* (n = *20*)	*Total* (n = *39*)
Complete remission	8 (42)	11 (55)	19 (49)
Treatment failure	8 (42)	5 (25)	13 (33)
Alive in CR after cycle 2	9 (47)	6 (30)	15 (38)
Total deaths during study	7 (37)	10 (50)	17 (43)
Early deaths in CR during cycle 2	0 (0)	4 (20)	4 (10)
Death while in aplasia	3 (16)	4 (20)	7 (18)
Death from persistent leukemia	4 (21)	2 (10)	6 (15)

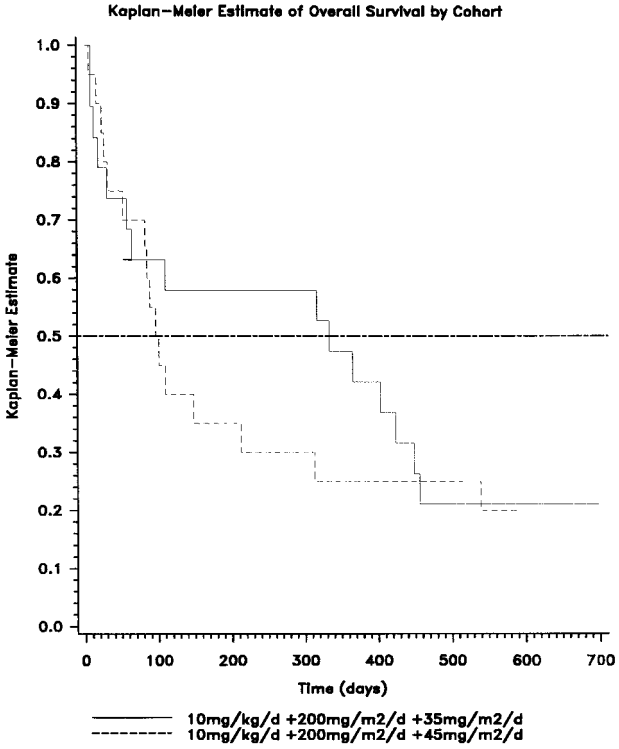

Figure 1 Kaplan-Meier estimate of overall survival time by cohort for patients treated with daunorubicin, cytarabine, and valspodar.

Table 5 P-glycoprotein expression and function and clinical response in relation to P-gp expression

	No. Patients	*CR*	*No CR*
P-glycoprotein positive ($MRK16^+$ or Rho^+)	24	11	13
$MRK16^+$, Rho^+	12	8	4
$MRK16^+$, Rho^-	4	1	3
$MRK16^-$, Rho^+	6	1	5
$MRK16^+$, Rho not assessable	2	1	1
P-glycoprotein negative ($MRK16^-$ and Rho^-)	10	6	4
Unassessable	5	2	3
Total	39	19	20

$MRK16^+$ indicates positive for staining with the MRK16 antibody; Rho^+ indicates a positive P-gp functional rhodamine 123 cellular retention assay.

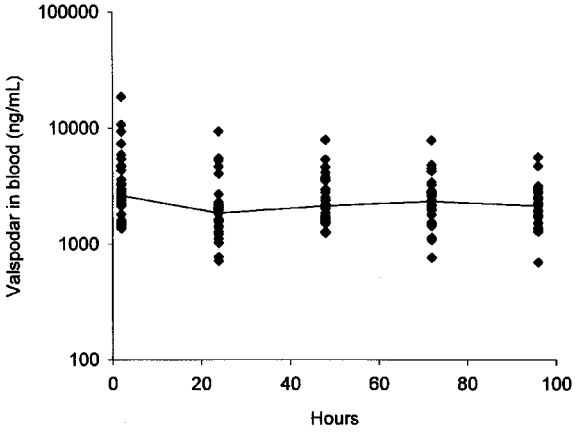

Figure 2 Daily valspodar blood concentrations during and immediately after the period of valspodar administration.

9394 ng/ml). With very few exceptions, all patients showed valspodar blood levels >1000 ng/ml at all time points. The four patients showing grade 2/3 cerebellar toxicity had mean peak valspodar blood concentrations similar to those with grade 1 or no cerebellar toxicity (4680±1316 ng/ml *vs* 5254±3733 ng/ml, $n=26$).

Mean plasma concentrations of DNR and DNR-ol were nearly the same in cohort 1 and cohort 2 despite a 29% dose increase in cohort 2. The mean plasma 48-h AUCs for DNR was 2.26±1.67 μM·h in cohort 1 ($n=15$) compared with 2.02±1.00 μM·h in cohort 2 ($n=16$). The mean plasma 48-h AUC for the hydroxyl metabolite DNR-ol was 17.7±6.4 μM·h in cohort 1 *vs* 19.9±6.2 μM·h in cohort 2. When extrapolated to infinity and corrected for carry-over from previous doses the mean AUCs for DNR in cohorts 1 and 2 were 2.57±1.85 and 2.29±1.13 μM·h, respectively. The terminal plasma half-life of DNR was 51.3±25.9 h in cohort 1 and 68.6±47.7 h in cohort 2, thus considerably longer than the 15–47 h reported in the literature when DNR is given without valspodar.[69–74]

No significant correlation was found between the plasma AUC of DNR and DLT. Kendall's correlation coefficient was 0.12. Further, no significant correlation between the observed response to treatment and the DNR AUC was apparent (Figure 3).

The study design did not allow for a comparison of the anthracycline PK with and without valspodar. During the first 4 h after administration, the pharmacokinetic profile of DNR in combination with valspodar in this trial is comparable to that observed with the 45-mg/m^2 dose of DNR alone in elderly AML patients in a previous trial[75] (Figure 4) (ie, peak levels were comparable). However, due to the prolonged terminal half-life, DNR persisted in plasma for a longer period of time and hence resulted in an increase in AUC_{inf} when compared to literature values for DNR given without valspodar.[68,70–74] For doses of 45–75 mg/m^2/d, mean AUCs in the range of 0.57–1.97 μM·h have been reported. The AUC_{inf} values of the present study were higher than all the reported values and it is estimated that a DNR dose of 90–120 mg/m^2/d given without valspodar would be needed in order to achieve similar AUCs.

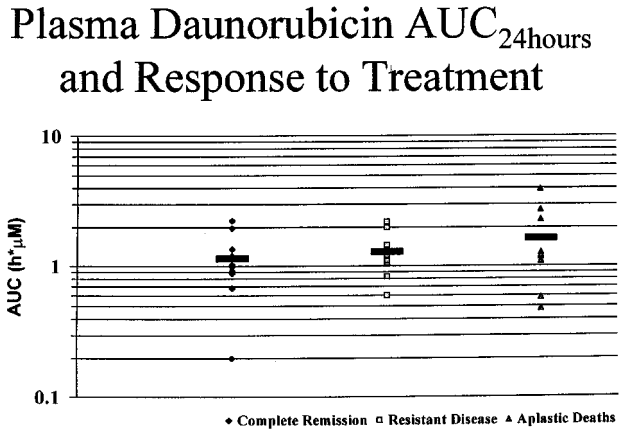

Figure 3 Relationship between plasma daunorubicin $AUC_{48\ h}$ and patient response to treatment. No difference was observed between cohorts.

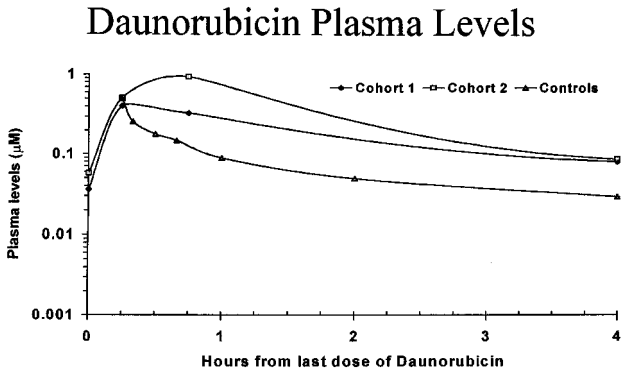

Figure 4 Pharmacokinetic profile of daunorubicin in combination with valspodar. No difference was observed between cohorts.

Discussion

Resistance to combination chemotherapy is a major obstacle to improving remission rates and survival in AML. Unlike many other tumors, in which MDR is often acquired during therapy, many untreated AML patients exhibit P-gp–related MDR at diagnosis. Several independent studies have found that P-gp expression is an unfavorable prognostic factor for response and/or survival. Reversal of the MDR phenotype by blockade of P-gp offers a possible route to overcome MDR. Several trials have attempted to demonstrate MDR reversal in AML patients using a P-gp modulator. However, most of these trials have been inconclusive because they failed to achieve blood levels of the P-gp modulator high enough to reverse drug resistance and/or they included patients whose tumor cells possessed other mechanisms of resistance in addition to P-gp expression. Therefore, previously untreated, elderly AML patients in whom only P-gp expression has been shown to be predictive of clinical outcome[50] provide a good model for investigating the clinical utility of P-gp modulators.

Because inhibition of P-pg alters the PK of concurrently administered cytostatic agents that are substrates for P-gp, including DNR, the dose of these chemotherapy agents must be reduced to avoid dose-limiting toxicity. The primary goal of this study was to establish the optimal dose of DNR when administered concomitantly with a dose of valspodar that can fully reverse MDR. Cytarabine is not transported by P-gp; therefore, the dose of this drug was left unchanged. The dose of DNR used in protocols for elderly AML patients has varied greatly; however, 45 mg/m^2 is a fairly typical standard dose.[12,76,77] In the present study a reduced dose of 35 mg/m^2 was chosen as the starting

dose based on the schedule used in a large cooperative group study conducted by the European Organization for the Research and Treatment of Cancer, Leukemia Cooperative Group (EORTC-LCG) and the Dutch Hematology-Oncology Cooperative Group (HOVON).[77] In that study, the median time to hematologic recovery was 25 days in responding patients. The median time to recovery observed in the present study (ie, 31 days) was slightly longer compared with the previous experience with this regimen. The longer hematologic recovery time may have been due to the effect of valspodar on undifferentiated $CD34^+$ hematopoietic cells, which express functional P-gp in the cell membrane, thus making them more sensitive to the cytotoxic effects of DNR. However, these data have to be interpreted with caution because only 46% of patients were assessable for ANC recovery.

The results of this trial established 35 mg/m^2 DNR as the MTD when administered concurrently with 10 mg/kg/day valspodar. This dose of valspodar yielded blood concentrations that have been shown to effectively reverse MDR (ie, median blood level $\geqslant$2000 ng/ml)[62–64] over four days and was associated with a low incidence of grade 3/4 toxicities. Severe ataxia and hyperbilirubinemia occurred in $\leqslant$10% of patients. In the absence of a dose relationship, the specific contributions of valspodar and chemotherapy to the occurrence of cardiac arrhythmias remain unclear. In general, the toxicity profile observed when valspodar was added to 35 mg/m^2 DNR did not differ from that normally observed in elderly AML patients treated with an anthracycline-based induction chemotherapy regimen. Although the toxicity profiles of the two cohorts did not differ dramatically, cohort 1 had a consistently more favorable profile than cohort 2. In cohort 1 (35 mg/m^2 DNR), 10 of 19 (53%) patients completed a second induction cycle, and a total of seven deaths occurred over 30 cycles, mostly due to disease progression. Only three deaths in cohort 1 were a result of fatal infections or hemorrhage precipitated by hematologic toxicity. In contrast, at the higher dose level of DNR (ie, 45 mg/m^2), 10 deaths occurred over 31 cycles, and seven deaths could be attributed to treatment-related toxicity, including the death of four patients who had achieved a CR after the first cycle. This increase in treatment-related mortality at the higher DNR dose level may have been related to increased DNR exposure as a result of the interaction with valspodar. Furthermore, there was a higher incidence of grade 3/4 mucositis and bleeding events in cohort 2.

The CR rates were 42% in cohort 1 and 55% in cohort 2, and the majority (14 of 19) of patients entered CR after only one treatment cycle, which may be an important benefit for elderly patients. Although the number of patients included in this study is relatively small, the overall survival analysis clearly shows an advantage for the patients treated with the lower dose of DNR. Most encouraging was the observation that the CR rate did not differ in P-gp–positive compared with P-gp–negative patients, suggesting that valspodar effectively reversed MDR in the group of P-gp–positive patients who would ordinarily be expected to have a substantially lower CR rate compared with comparable patients with a P-gp–negative phenotype. Interestingly, four patients had negative functional tests although P-gp was present in the cell membrane as demonstrated by MRK16 staining. Nevertheless, only one of these patients achieved CR, suggesting the possible involvement of other functional mechanisms of drug resistance. Despite the relatively small number of patients included in this study, these results suggest that the adverse impact of P-gp on treatment outcome in patients with P-gp–positive AML blasts may be overcome by treating these patients with induction chemotherapy plus valspodar.

No relationship was found between the plasma concentration of the DNR or DNR-ol and the response to treatment. This is in agreement with the previous observation that no plasma DNR or DNR-ol PK parameter is predictive of the clinical outcome of therapy in *de novo* AML and ALL.[75,78] Galletis *et al*. found that intracellular DNR concentrations may be a better predictor for clinical outcome.[78]

Although the dose of DNR was increased by 29% in cohort 2, there was no difference in the AUC between cohorts. The AUC of DNR in this study was consistent with a theoretical DNR dose of 90–120 mg/m^2 without valspodar, but this high AUC did not translate into increased toxicity. The interpretation of these findings is unclear, but it is difficult to draw definitive conclusions because of the large inter-patient variability in the DNR AUC.

In conclusion, the results of this study suggest that substantial inhibition of P-gp can be achieved *in vivo* at clinically tolerable doses of both valspodar and DNR. The feasibility of administering valspodar to elderly patients with untreated AML at a safe and well-tolerated dose of DNR has been established. The higher than expected response rate of P-gp–positive patients suggests that valspodar, at the dose used in this study, may reverse MDR. However, phase III randomized trials are needed to determine if this approach will ultimately benefit patients. The 35-mg/m^2 dose of DNR has been recommended for further study. A series of randomized phase III trials of valspodar in different subsets of AML are ongoing, and the results will be available in the near future.

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