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VP 16-213 and Cytosine-arabinoside Combination Chemotherapy for Refractory Acute Lymphoblastic Leukemia in Adults

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Abstract—*Fifteen adult patients with refractory or relapsing acute lymphoblastic leukemia (ALL) received a 5-day remission induction regimen consisting of VP 16-213 (60 mg/m² every 12 hr) and cytosine-arabinoside (100 mg/m² every 12 hr) up to a maximum of three courses. The overall response rate was 60% (9/15), four patients (27%) achieving CR and five (33%) attaining PR. Responders were maintained with monthly courses of the same combination until progressive disease developed. The median duration of response was 4.5 months (2–12+ months). The regimen was relatively well tolerated. The major toxicity was hematologic. Non-hematologic toxicities included mild nausea and vomiting (11/15) and total alopecia (15/15). The results obtained with this combination are encouraging, in view of the poor prognosis associated with refractory or relapsing ALL in adults.*

INTRODUCTION

DESPITE modern treatment modalities, a relative high fraction (about 30%) of adult patients with acute lymphoblastic leukemia (ALL) fail to enter complete remission. In addition, approximately one-half of remitters relapse during the first 2 yr of maintenance therapy [1, 2]. Since the introduction of effective central nervous system (CNS) prophylaxis, the first site of relapse is reported to be bone marrow in about 80% of cases [1, 2]. Second-line combination regimens including active antileukemic agents not previously used are therefore necessary to overcome drug resistance in patients with refractory and relapsed ALL. The epipodophyllotoxins VM 26 and VP 16-213 have been established to have single-agent activity against acute lymphocytic and non-lymphocytic leukemias [3, 4]. Preclinical studies showing a synergism between cytosine-arabinoside (ara-C) and both the podophyllotoxin derivatives VM 26 and VP 16-213 [5] formed the rationale for using combined VM 26 and ara-C in treatment of refractory childhood lymphocytic leukemia. This combination appeared to be effective in patients either unresponsive to initial induction therapy or with advanced leukemia in relapse [6, 7]. In addition, the association of the second podophyllum compound VP 16-213 with ara-C plus an anthracycline proved to be an active induction regimen for adult acute myeloid leukemia [8–10]. Based on these observations we have evaluated a combination of VP 16-213 and ara-C as second-line regimen in 15 adult patients with relapsed or resistant ALL. This treatment was designed to be given twice daily for 5 days with VP 16-213 in a 60-min i.v. infusion and ara-C by rapid i.v. injection. The effectiveness of ara-C given by bolus injection in combination with an epipodophyllotoxin was established in previous studies on childhood ALL [6, 7]. The present report indicates that this regimen is effective and warrants further study.

MATERIALS AND METHODS

Between March 1982 and February 1983, 15 adult patients with refractory ALL were treated with a combination of VP 16-213 and ara-C in 5-day courses to induce remission. All patients had received prior extensive therapy, as shown in Table 1. Four cases were the induction failures of a group of 16 consecutive ALL treated during the period of the study with a protocol including vincristine, daunorubicin, cytoxan and prednisone. The remaining 11 cases were all the patients with

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*Table 1. Patient characteristics and response to therapy**

	Patient No.	Age/sex	All type	Risk assessment at diagnosis†	Previous induction therapy	Previous remissions (duration, months)	Previous therapy in relapse	Response to VP 16-213 and ara-C	Response duration (months)
Induction failures	1	20/F	L_2, TdT+, E+	high	VDCP	0	–	NR	–
	2	26/M	L_2, TdT+, E+	high	VDCP	0	–	NR	–
	3	16/M	L_2, TdT+, E–	high	VDCP	0	–	NR	–
	4	35/M	L_2, TdT+, E–	high	VDCP	0	–	PR	4
Patients in relapse	5	22/F	L_2, TdT+, E–	standard	VDP	1 (8)	A,V,C,ara-C,P	CR	6
	6	30/F	L_1, TdT+, E–	standard	VDP	1 (5)	A,V,C,ara-C,P,MTX	CR	4.5
	7	18/M	L_1, TdT+, E–	standard	VDP	1 (51)	A,V,C,ara-C,P	PR	2
	8	13/F	L_2, TdT+, E–	standard	VDP	1 (17)	–	NR	–
	9	15/F	L_2, TdT+, E–	high	VDCP	1 (14)	–	PR	2
	10	43/M	L_2, TdT+, E+	high	VDCP	1 (19)	–	PR	4
	11	17/M	L_2, TdT+, E–	high	VDCP	1 (3)	–	NR	–
	12	18/M	L_2, TdT+, E–	standard	VDCP	1 (31)	–	CR	12+
	13	19/M	L_1, TdT+, E–	standard	VDP	2 (36, 29)	–	CR	4.5
	14	46/M	L_2, TdT+, E–	standard	VDP	1 (18)	–	PR	4.5
	15	15/M	L_2, TdT+, E+	high	VDCP	1 (3)	–	NR	–

***Abbreviations:** TdT, terminal deoxynucleotidyl transferase; E+, E rosette-positive ALL; V, vincristine; D, daunorubicin; C, cyclophosphamide; P, prednisone; A, asparaginase; ara-C, cytosine-arabinoside; MTX, methotrexate.

†'High risk' designates patients with one or more of the following initial features: WBC count $>35 \times 10^9$/l, tumoral presentation, CNS leukemia, E rosette-positive lymphoblasts.

ALL in relapse observed in the same period at our institution. Among them, eight had relapsed while receiving multiple-drug maintenance and reinforcement chemotherapy including 6-mercaptopurine, methotrexate vincristine, daunorubicin and prednisone, and three patients (cases 5–7) were ALL relapses unresponsive to reinduction attempts with conventional-dose ara-C in other drug combinations, just prior to entering the study. Attempts to re-induce remission with conventional agents such as vincristine, anthracyclines, prednisone ± L-asparaginase were not made since all the patients in relapse had been recently exposed to vincristine, daunorubicin and prednisone during the maintenance–reinforcement period. Three of them had also been unresponsive to L-asparaginase in the first reinduction attempt. The median pretreatment WBC count was 2.9×10^9/l (range $1.1–24 \times 10^9$/l). As concerns marrow status of the 15 patients entering the trial, the median percentage of blastic cells was 85% (range 45–98%). Only three cases showed hypoplastic bone marrow (cases 2, 5 and 7). All patients in relapse had received CNS prophylaxis consisting of cranial irradiation and intrathecal methotrexate as part of their initial treatment. No patients showed CNS leukemia at study entry nor had they received previous therapy for CNS disease. None of the 15 patients was eligible for bone marrow transplantation.

The regimen consisted of 5-day courses of VP 16-213, 60 mg/m^2 every 12 hr by 60-min intravenous infusion, and ara-C, 100 mg/m^2 every 12 hr by intravenous bolus injection. A maximum of three cycles of induction therapy were given. Induction courses were repeated every 3 weeks at the same doses as allowed by hematological toxicity. Complete blood counts, and differential and platelet counts were monitored three times per week. A bone marrow examination was performed 2 weeks after completing each induction course. If the marrow was markedly hypocellular, therapy was delayed until the bone marrow had regenerated. The treatment design did not call for drug escalation. Patients whose disease progressed after two courses of treatment were considered chemotherapy failures and were removed from the study. An intensive supportive care, including platelet and red cell transfusions, broad-spectrum antibiotics and antifungal agents, was provided as required.

Complete remission (CR) was defined as normal blood count, normal cerebrospinal fluid and a marrow with active hematopoiesis and less than 5% blast cells (M-1 marrow). Partial remission (PR) was defined as attainment of a normocellular marrow containing from 6 to 25% leukemic cells, with normal maturation of other cell lines (M-2 marrow). Duration of response was measured from the time of M-1 or M-2 marrow to relapse. Patients who achieved CR or PR were given monthly courses of the same combination until progressive disease developed on bone marrow examination.

RESULTS

All patients completed at least two courses of therapy and were fully evaluable for response. The overall response rate to the VP 16-213 and ara-C regimen was 60% (9/15): four patients (27%) achieved CR and five (33%) attained PR. Three additional patients (cases 2, 8 and 11) experienced an objective improvement but did not meet the criteria for PR. Three patients (cases 1, 3 and 15) regenerated with a predominance of leukemic cells after completion of the second induction course and two of them (cases 1 and 15, presenting with T-ALL features) developed CNS disease at the same time. These three patients were then removed from the study. Among responders, one patient (case 12) achieved CR after one course and eight patients required two cycles to attain M-1 of M-2 marrow. The third course improved response from M-2 to M-1 marrow in case 6. The response to the combined VP 16-213 and ara-C therapy according to patient characteristics and treatment histories is summarized in Table 1. Regarding the influence of marrow status on response to chemotherapy, neither bone marrow cellularity nor percentage of blasts at study entry were significantly associated with the achievement of CR. A partial response was observed in one of the four patients who entered the study after failing the initial induction therapy. Of the 11 patients who were treated in relapse four achieved a CR and four a PR. Most important, three of the nine responders had failed reinduction with ara-C in other drug combinations immediately before study entry.

The major toxicity was hematologic. Twelve of the 15 patients developed myelosuppression after each induction course. The median neutrophil nadir during induction courses was 0.1×10^9/l (range $0–0.4 \times 10^9$/l). The median time to nadir was 14 days (range 11–18 days) from the start of each course, and by day 21 recovery was seen in the majority of cases. Ten episodes of sustained fever requiring antibiotics occurred in six patients (cases 2, 4, 5, 7, 11 and 14) during the WBC nadirs. In case 5 *E. coli* was isolated from biological samples. Thrombocytopenia less than 50×10^9/l occurred in all patients but was not a major problem since we transfused all patients with a platelet count $<20 \times 10^9$/l. No patient died as a result of the induction therapy. Drug-induced non-hematologic toxicity was acceptable (mild

nausea and vomiting in 11 cases, total alopecia in all patients). No patient showed hypotension with a 60-min infusion of VP 16-213. Liver function abnormalities were not seen. No patient developed evidence of cardiac disorders.

The responders received a continuation treatment with the same regimen every 4 weeks until progression of disease occurred. The median duration of response was 4.5 months (range 2–12+ months). The maintenance regimen was reasonably well tolerated and caused predictable and reversible myelosuppression, so it was used in most instances on an outpatient basis. On a total of 25 courses of VP 16-213 and ara-C given as continuation treatment, neutropenia ($<0.5 \times 10^9/l$) occurred by day 14 at every course and recovery was generally complete by week 4. During granulocytopenia all patients received trimethoprim-sulfamethoxazole plus amphotericin B as prophylaxis against bacterial and fungal infections. Nevertheless, five episodes of hyperpyrexia related to neutropenia occurred in 3 patients (cases 4, 5 and 7). One patient (case 5) expired secondary to *E. coli* septicemia while still in remission. Mild anemia not requiring transfusions and asymptomatic thrombocytopenia ($30–150 \times 10^9/l$) were common.

DISCUSSION

Refractory and relapsed adult ALL has a poor prognosis. Several second-line regimens involving new combinations of drugs or investigational agents have been evaluated to overcome drug resistance. These reinduction protocols for adult ALL include combinations of vincristine, adriamycin, prednisolone and L-asparaginase [11]; sequential L-asparaginase and methotrexate [12]; high-dose ara-C [13]; and amsacrine [14]. Response rates from 25 to 69% were obtained but remissions were disappointingly short, with a median duration usually not exceeding 4 months. Response duration was shown to be related to the disease status at study entry. In fact, the best results were seen in patients who relapsed while off therapy after a long initial CR [11]. A biweekly administration of the podophyllum compound VM 26 given as a 30- to 45-min i.v. infusion with ara-C by rapid i.v. injection was reported by Rivera *et al.* [6, 7] to be effective in refractory childhood ALL. This combination reinduced remission in 10/33 children with advanced disease and in 9/14 ALL who failed to respond to initial induction therapy. Prolonged remission durations were obtained in this latter group.

Our study indicates that the combination of the second podophyllum compound VP 16-213 with ara-C, both given 12-hourly in 5-day courses, can induce objective response in a considerable proportion of adult patients with resistant ALL. At this dose and schedule drug-induced non-hematologic toxicity was acceptable. Of the 15 patients treated, four (27%) achieved CR and five (33%) had PR, resulting in an overall response rate of 60%. The median duration of response was 4.5 months. These results are encouraging when one considers the particularly difficult group of patients who entered the study. In fact, of the 15 patients treated, seven were induction or reinduction failures and entered this study acutely ill because of bone marrow failure due to persistent disease and intensive prior treatment. The remaining eight relapsed early during maintenance and reinforcement chemotherapy, comprising monthly pulses of vincristine, cyclophosphamide and prednisone (± daunorubicin every 3 months). Indeed, all of these features are known to characterize a category of patients with very short life expectancy. Most important, three of the nine patients who responded had been resistant to prior ara-C in other drug combinations. This fact seems to indicate that the addition of VP 16-213 may overcome clinical resistance to ara-C given in conventional doses. Whether the use of more intensive dosages with drug escalation and of different schedules (e.g. continuous ara-C infusion) will improve these results without producing intolerable non-hematologic toxicity remains to be determined.

In conclusion, our study suggests that this salvage therapy is effective in refractory adult ALL. We feel that this protocol might have greater activity when used earlier in the course of the disease. This combination could play a potential role in preventing drug resistance when integrated into the primary treatment of adult patients with ALL.

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REFERENCES

1. Lister TA, Whitehouse JMA, Beard MEJ *et al.* Combination chemotherapy for acute lymphoblastic leukemia in adults. *Br Med J* 1978, **1**, 199–203.
2. Lazzarino M, Morra E, Alessandrino EP *et al.* Adult acute lymphoblastic leukemia. Response to therapy according to presenting features in 62 patients. *Eur J Cancer Clin Oncol* 1982, **18**, 813–819.

3. Rivera G, Avery T, Pratt C. 4'-Demethylepipodophyllotoxin 9-(4,6-*o*-2-thenylidene-α-D-glucopyranoside) (NSC-122819; VM 26) and 4'-demethylepipodophyllotoxin-9-(4,6-*o*-ethylidene-α-D-glucopyranoside) (NSC-141540; VP 16-213) in childhood cancer: preliminary observations. *Cancer Chemother Rep* 1975, **59**, 743–749.
4. Cavalli F, Sonntag R, Brunner KW. Epipodophyllotoxin VP 16-213 in acute non-lymphoblastic leukemia. *Br Med J* 1975, **4**, 227.
5. Rivera G, Avery T, Roberts D. Response of L1210 to combinations of cytosine-arabinoside and VM 26 or VP 16-213. *Eur J Cancer* 1975, **11**, 639–647.
6. Rivera G, Aur R, Dahl GV, Pratt CB, Wood A, Avery TL. Combined VM 26 and cytosine-arabinoside in treatment of refractory childhood lymphocytic leukemia. *Cancer* 1980, **45**, 1284–1288.
7. Rivera G, Dahl CV, Bowman P, Avery TL, Wood A, Aur R. VM 26 and cytosine-arabinoside combination chemotherapy for initial induction failures in childhood lymphocytic leukemia. *Cancer* 1980, **46**, 1727–1730.
8. Bernasconi C, Lazzarino M, Morra E. The use of an epipodophyllotoxin derivative (VP 16-213) in the treatment of acute monocytic and myelomonocytic leukemias. In: Stacher A, Höcker P eds. *Erkrankungen der Myelopoese.* München, Urban & Schwarzenberg, 1976, 189–191.
9. Dubovsky D, Kernoff L, Jacobs P. Rapid remission induction in adult acute nonlymphoblastic leukaemia. *Eur J Cancer* 1978, **14**, 1179–1183.
10. Bernasconi C, Lazzarino M, Morra E *et al.* Etoposide (VP 16-213) in the treatment of acute monocytic and myelomonocytic leukemias. *Chemioterapia* 1982, **1**, 181–185.
11. Woodruff RK, Lister TA, Paxton AM, Whitehouse JMA, Malpas JS. Combination chemotherapy for hematological relapse in adult acute lymphoblastic leukaemia (ALL). *Am J Hematol* 1978, **4**, 173–177.
12. Yap BS, McCredie KB, Keating MJ, Bodey GP, Freireich EJ. Asparaginase and methotrexate combination chemotherapy in relapsed acute lymphoblastic leukemia in adults. *Cancer Treat Rep* 1981, **65**, 83–87.
13. Early AP, Preisler HD, Higby DJ, Brecher M, Browman G, McBride JA. High-dose cytosine arabinoside: clinical response to therapy in acute leukemia. *Med Pediatr Oncol* 1982, **1**, 239–250.
14. Winton EF, Hearn EB, Vogler WR, Johnson L, Logan T, Raney M. Amsacrine in refractory adult acute leukemia. A pilot study of the Southeastern Cancer Study Group. *Cancer Treat Rep* 1983, **67**, 977–980.