Targeted Plasma Drug Concentration: A New Therapeutic Approach to Relapsed Nonlymphoblastic Leukemia in Children*

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A. Introduction

Chemotherapy produces approximately a 30% long-term disease-free survival in newly diagnosed children with acute nonlymphocytic leukemia (ANLL). Although 75%–80% of children will enter a complete remission (CR), most will relapse with resistant leukemia while receiving postremission chemotherapy.

Since 1976, investigators at St. Jude Children's Research Hospital have conducted a series of clinical trials in an attempt to improve the outcome of therapy for patients with previously untreated ANLL. In AML-76, we tested a cytokinetically based induction and nonmyelosuppressive maintenance therapy. Patients achieving CR were randomized to determine if splenectomy improved outcome. Although 72% of patients achieved a complete remission, the long-term survival was not satisfactory [1]. In 1980 we undertook an intensive chemotherapy trial (AML-80) modeled after the Dana-Farber VAPA study [2, 3]. AML-80 was quite toxic but did increase the median disease-free interval to approximately 18 months. Unfortunately, this approach did not improve long-term disease-free survival [4].

In 1983 we evaluated a new treatment strategy for childhood ANLL which included the introduction of additional drugs, such as etoposide (VP-16), early in therapy to maximize reduction in tumor burden during remission induction. The induction therapy was an intensive five-drug regimen utilizing conventional agents (ara-C, daunorubicin, 6-thioguanine) and newer drugs (VP-16, 5-azacytidine) [5, 6]. Postremission therapy included seven drugs given in sequentially rotated pairs. The objective of the postremission therapy was to expose leukemic cells to as many effective drugs as possible in an attempt to decrease the development of resistance. The induction schema for this protocol (AML-83) is outlined in Fig. 1. Despite this new treatment strategy the probability of event-free survival at 2 years in the AML-83 trial was 33% ± 7%, which was not significantly different from our two previous trials (AML-76 and AML-80) or many other reported trials [1–4, 7–11].

A	VP-16 200 mg/m^2 CI* (IV) d1-3 Ara-C 200 mg/m^2 CI (SQ) d4-8
B	Dauno 50 mg/m^2 (IV) d1,2 Ara-C 200 mg/m^2 CI (SQ) d1-5 6-TG 100 mg/m^2 (PO) d1-5
C	VP-16 250 mg/m^2 (IV) d1,2,3; 6,7 5-AZ 300 mg/m^2 (IV) d4,5

* Continuous infusion

Fig. 1. The induction schema of AML-83 included three combinations (*A*, *B*, and *C*) of antileukemic agents administered at standard dosages. Combination *A* and *B* of AML-83 contained similar drugs to cycle 1 and 2 of AML-R2 but at lower doses

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B. Relapse Trial: AML-R2

Because these therapeutic trials for previously untreated patients failed to improve survival, we designed a completely novel approach to therapy. Our rationale for this new approach was that therapeutic failures may result because leukemic cells are inadequately exposed to effective agents in some patients. Likewise, some patients may develop unnecessary toxicity because of high drug plasma concentration and damage to normal tissues. Previous studies demonstrated marked interpatient variability for the plasma concentration of VP-16 and ara-C when administered in standard doses (per square meter). Therefore, we were interested in determining whether it was possible pharmacokinetically to control this variability by "targeting" the plasma concentration of these drugs to a predetermined level. In this novel approach to therapy, VP-16 and ara-C would not be administered at a standard dose per square meter, but at a dose that would produce a predetermined plasma concentration. This approach is the first step toward "individualizing" chemotherapy and permits evaluation of toxicity and efficacy with a standard systemic exposure to antileukemic agents. Such an approach also guaranteed that we would deliver more intensive therapy to all patients.

We piloted our targeted plasma drug concentration therapy on relapsed patients in the AML-R2 protocol. This protocol included antileukemic agents which patients received previously, but were known to be highly effective. The schema for the relapsed trial is shown in Fig. 2. The background data used for standardizing plasma concentrations was based on the previous plasma concentrations of VP-16 and ara-C measured in our AML-83 trial (Fig. 1). When VP-16 and ara-C are administered as a standard dose/m^2 per day there is a wide variability in plasma concentrations among patients. For example, there was a four- to five-fold variability in the plasma concentration of

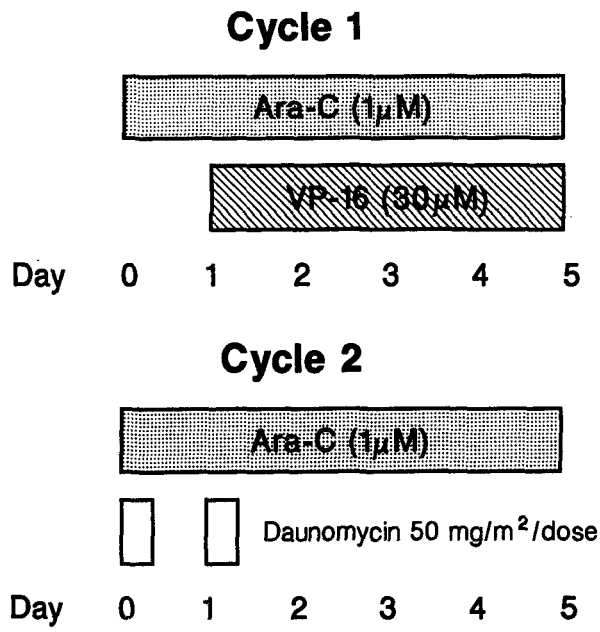

Fig. 2. Schema of AML-R2 which included targeted plasma concentrations of VP-16 and ara-C in cycle 1 and a targeted plasma concentration of ara-C with daunorubicin in cycle 2

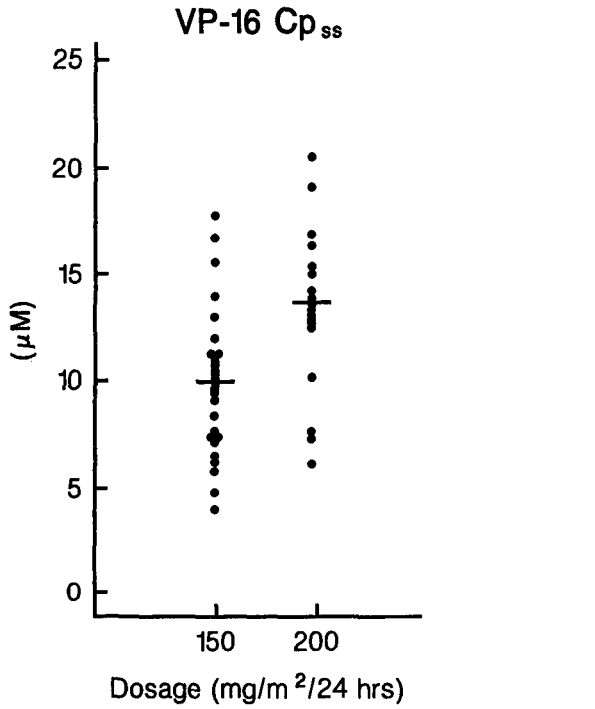

Fig. 3. The variability in the plasma steady-state concentrations of VP-16. Individual patients are marked and the median value is shown. All patients received VP-16 administered in a standard fashion at 150 or 200 mg/m^2 per dose

VP-16 when administered based on standard per square meter dosage (Fig. 3). A similar four-fold variability in the plasma concentrations of ara-C was also seen on AML-83 (data not shown). We selected

the maximum plasma concentrations of VP-16 and of ara-C which patients tolerated on AML-83 and used these as our planned target concentrations. If we could increase (maximize) the plasma concentration for all patients we would increase total exposure and standardize therapy.

In the first cycle of AML-R2 therapy (Fig. 2) the ara-C is administered as a continuous subcutaneous infusion for 5 days. The dose is adjusted within 12 h to achieve a plasma concentration of 1 μM. This dose adjustment is based on the plasma concentration determined by high-pressure liquid chromatography (HPLC) from samples obtained 1 and 6 h after the start of the ara-C infusion. A 4-day continuous intravenous infusion of VP-16 is started 24 h after starting ara-C and is administered to achieve a plasma concentration of 30 μM within 12 h based on the plasma concentrations assayed 1 and 6 h after the start of the VP-16 infusion. Cycle 2 of AML-R2 consists of ara-C, again administered to achieve a 1 μM plasma concentration in combination with daunorubicin 50 mg/m^2 per dose $\times$ 2 doses, given with the start of ara-C and 24 h later (Fig. 2).

All patients enrolled on this trial had previously received VP-16, ara-C, and daunorubicin at standard doses during initial induction therapy. Most relapsed while receiving the AML-83 regimen (Fig. 1), which contained almost identical induction therapy as AML-R2, but with much lower drug dosages of VP-16 and ara-C. Furthermore, with the targeted plasma concentrations on the AML-R2 relapse study all patients received the same total systemic exposure to VP-16 and ara-C despite being given different dosages.

Responses were evaluated by standard criteria. A CR was defined as a cellular marrow aspirate with $<5\%$ blasts cells, normal hemograms, and performance status for >1 month. Partial remission (PR) was an absence of peripheral blasts, $<25\%$ marrow blasts, and recovery from all toxicity. Toxicity was evaluated by the National Cancer Institute (NCI) common toxicity criteria.

C. Results

Nineteen children with relapsed ANLL were enrolled in the AML-R2 protocol (nine females and ten males). The median age at enrollment was 10.5 years (range 8 months to 17.1 years). Six patients had myeloblastic leukemic subtypes (FAB-M1 or M2), 1 patient had progranulocytic leukemia, and 12 patients had myelomonocytic or monocytic leukemic subtypes (FAB M4 or M5) (Table 1).

In cycle 1 of AML-R2 the median ara-C dose was 550 mg/m^2 per day (range 412–750 mg/m^2 per day). The median VP-16 dose was 500 mg/m^2 per day (range 350–750 mg/m^2 per day) (Table 2). The dose of ara-C administered in AML-R2 was approximately 2.5 times higher than on AML-83. Similarly the dose of VP-16 in AML-R2 was 2.5-fold higher than on AML-83. The "targeted" ara-C concentration of 1 μM on AML-R2 was readily achieved (Table 3). AML-R2 subjects also achieved the "targeted" VP-16 concentration of 30 μM with measured concentration of 32.44 ± 5.0 μM (median, $\pm$SD) (Table 3).

The therapeutic results of the AML-R2 protocol are encouraging. The overall complete response rate was 10 of 19 patients (53%) for patients who had previously received VP-16, ara-C, and daunorubicin therapy. There were 17 patients enrolled on this relapse trial (AML-R2) that relapsed after (or on) AML-83. Of the three patients enrolled on AML-R2 that achieved a complete remission after combination A of AML-83 with VP-16 and ara-C, all three also achieved a second complete remission after cycle 1 of AML-R2 containing the same two drugs. More interestingly, however, are the results of the 14 patients who were previously treated on AML-83 but had residual disease after combination A. Five of these 14 patients achieved a second complete remission using higher

No. patients	19
Sex	9 female/10 male
Age (median)	10.5 years
(range)	8 months to 17.1 years
FAB classification	
M1	4
M2	2
M3	1
M4	7
M5	5

Table 1. AML-R2 patient characteristics

AML-R2 cycle	Drug	Dose administered ($mg/m^2/day$)	
		Median	Range
1	Ara-C	550	412–750
1	VP-16	500	350–700
2	Ara-C	600	350–750

Table 2. AML-R2 drug doses administered

Table 3. Comparison of doses administered and plasma concentrations achieved on AML-83 and AML-R2

Protocol Phase of therapy	AML-83 Combination A	AML-R2 Cycle #1
Ara-C dose	200 $mg/m^2/day \times 5$	550 $mg/m^2/day \times 5$ (median)
Ara-C plasma concentration median (range)	0.1 μm (<0.1–0.28 μ*M*)	1.08 μ*M* (0.5–1.7μ*M*)
VP-16 dose	200 $mg/m^2/day \times 3$	500 $mg/m^2/day \times 4$ (median)
VP-16 plasma concentration median (range)	13.97 μ*M* (4–19.5 μ*M*)	32.44 μ*M* (25.7–43.9 μ*M*)

Toxicity	Cycle	Incidence
Sepsis	Cycle #1 and 2	6 Bacterial (32%) 3 Fungal (16%)
Fever	Cycle 1	100%
	Cycle 2	80%
Mucositis[a]	Cycle 1	90%
	Cycle 2	40%
Hepatotoxicity[a]	Cycle 1	26%
	Cycle 2	33%
Days hospitalized	Cycle 1	17.6 (5–35)
	Cycle 2	15.5 (7–39)

[a] Grade 3 or 4 by NCI common toxicity criteria

Table 4. AML-R2 toxicity

doses of VP-16 and ara-C on the AML-R2 study.

The AML-R2 protocol is extremely toxic producing prolonged pancytopenia and mucositis. One patient died from bacterial sepsis during drug-induced aplasia. Six patients had documented bacterial sepsis and three had documented fungal sepsis. In cycle 1, 100% of the patients had fever with neutropenia and were hospitalized for antibiotics, while in cycle 2, 80% required hospitalization for fever with neutropenia. Mucositis occurred predictably after cycle 1 in 90% of patients; in cycle 2 mucositis was much less of a problem and grade 3 or 4 mucositis occurred in approximately 40% of the patients (Table 4).

The incidence of abnormal liver enzymes was 26% during cycle 1 and 33% during cycle 2; alternations were usually mild and reversible. Cycle 1 resulted in skin toxicity with diffuse erythemia in 90% of the patients. No patient developed CNS toxicity on the AML-R2 protocol. The average hospitalization for cycle 1 was 17.6 days (range of 5–35 days) and for cycle 2 was 15.5 days (range of 7–39 days).

D. Discussion

The AML-R2 protocol was designed to determine if it is possible to "target" the plasma concentration and standardize the total systemic exposure for VP-16 and ara-C among all patients. In addition to achieving this objective, the protocol demonstrated that it was possible to increase the dose of VP-16 and ara-C by over two-fold. However, the dose was increased far less than the total systemic exposure. For ara-C the total systemic exposure on AML-R2 was two to ten times greater than on AML-83. Likewise, the VP-16 total systemic exposure was three to eight times greater on AML-R2 than on AML-83 (the VP-16 infusion was 1 day longer on AML-R2).

The AML-R2 protocol demonstrated the feasibility of adjusting drug dosage to achieve a target plasma concentration within 8–12 h of starting each agent. The approach of standardizing plasma concentration yielded a more uniform total drug exposure for all patients. This higher total exposure resulted in severe but very predictable toxicity with encouraging results.

The results of AML-R2 suggest a plasma concentration: response relationship for VP-16 and ara-C. Patients who achieved their initial CR with lower doses of VP-16 and ara-C on AML-83 achieved a second CR on AML-R2. More surprisingly, of the 14 patients who had residual disease on AML-83 after receiving VP-16 and ara-C in combination A, 5 achieved a second complete response after receiving higher dosages of the same drugs on cycle 1 of AML-R2. Unfortunately, the small number of patients treated on AML-R2 does not permit meaningful statistical analysis but our results support the hypothesis that the antileukemic effects of VP-16 and ara-C might be improved by increasing the total exposure to these agents. Our preliminary conclusion from the AML-R2 protocol is that more therapy may prove to be better for children with ANLL. This novel approach to antileukemic therapy will be used in our new front-line trial.

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