Cancer Chemother Pharmacol (1987) 19: 69–74

Cancer Chemotherapy and Pharmacology

Abrogation of the prognostic significance of low leukemic cell retention of cytosine arabinoside triphosphate by intensification of therapy and by alteration in the dose and schedule of administration of cytosine arabinoside*

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Summary. The ability of leukemic cells to phosphorylate cytosine arabinoside (araC) and retain the triphosphate form of the drug (araCTP) is strongly predictive of remission duration for patients with acute nonlymphocytic leukemia who are treated with araC-based maintenance therapy. An increase in the intensity of therapy improves the overall median duration of remission, the increased intensity of therapy being especially beneficial for patients whose leukemic cells do not retain araCTP. This alteration in therapy reduces the prognostic significance of leukemic cell araCTP retention. Further, it seems that the use of high-dose araC as intensive consolidation therapy and the administration of conventional-dose araC by continuous infusion make it possible to further reduce or even abrogate the adverse prognostic significance of low leukemic cell retention of araCTP.

Introduction

Cytosine arabinoside (araC) is the most important chemotherapeutic agent in the treatment of patients with acute nonlymphocytic leukemia (ANLL) [7, 14, 15, 19]. To be effective, araC must be phosphorylated to the triphosphate form (araCTP) [2, 5, 16]. Since 1976 we have been engaged in studies directed at determining the relationship between leukemic cell metabolism of araC and the response of ANLL to chemotherapy. Our first study demonstrated that remission duration was correlated with the ability of leukemic cells to activate araC and retain ara CTP [17]. The follow-up study confirmed this observation and suggested that the relationship between araCTP retention and remission duration was related to the duration of therapeutic intracellular araCTP levels during consolidation/maintenance therapy [9, 11]. In the present study we demonstrate that the adverse prognostic significance of low leukemic cell araCTP retention can be overcome by intensive consolidation therapy, especially therapy in which araC is given in very high doses.

* This work was supported by grants CA 41285, CA 21071. The authors would also like to thank Dr George Royer and the Upjohn Company for partial support for the studies described here

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Methods

Treatment protocols. Between 1975 and 1983 three successive protocols were used to treat patients with newly diagnosed ANLL [1]. All three had similar remission induction phases consisting of a 7- or 10-day continuous infusion of cytosine arabinoside together with adriamycin administration on days 1, 2, 3. The complete remission rates were similar, ranging from 66% to 70%. The treatment protocols differed, however, in the therapy administered to patients whose leukemia entered complete remission.

Protocol 950501, which was used between 1975 and 1977, provided for 5 years of conventional maintenance therapy, during which time a patients' white blood cell count (WBC) was rarely reduced to $<1000/\mu l$ or to a platelet count of $<75\,000/\mu l$ (Fig. 1a) [7]. Between 1977 and 1980, protocol 970701 was used to treat patients (Fig. 1b) [6]. This protocol provided for the administration of three courses of intensive consolidation therapy, each of which reduced the patients' white blood cell count to $<500/\mu l$ and the platelet count to $<20\,000/\mu l$. The intensive consolidation phase was followed by 3 years of maintenance therapy. From 1981 to 1983, protocol 998028 was used to treat patients (Fig. 1c) [6, 8, 10]. This protocol provided for four intensive courses of consolidation chemotherapy, followed by no further therapy. Each course of consolidation therapy lowered the patients' WBC counts to $<500/\mu l$ and their platelet counts to $<20\,000/\mu l$. This protocol was preceded by a pilot study of consolidation therapy conducted in arm A only in Fig. 1c. The patients treated in this pilot study and those treated according to protocol 998028 are reported together. The earlier pilot study of arm A is the reason for the somewhat longer follow-up time in Fig. 4 for these patients than for arm B patients.

Despite our efforts, not all patients treated according to these protocols were studied for araCTP retention. Reasons for non-study included too few marrow cells obtained for study, private physicians' refusal to permit study of their patients, technician unavailability, and other similar reasons. For all three treatment protocols the percentage of patients studied for araCTP retention was virtually identical: 61.5%, 61.7%, and 57.4%. Further evidence for the nonselection of patients is found in the fact that a comparison of the remission duration curves for all patients treated according to each protocol and for those patients whose cells were studied for araCTP retention revealed no

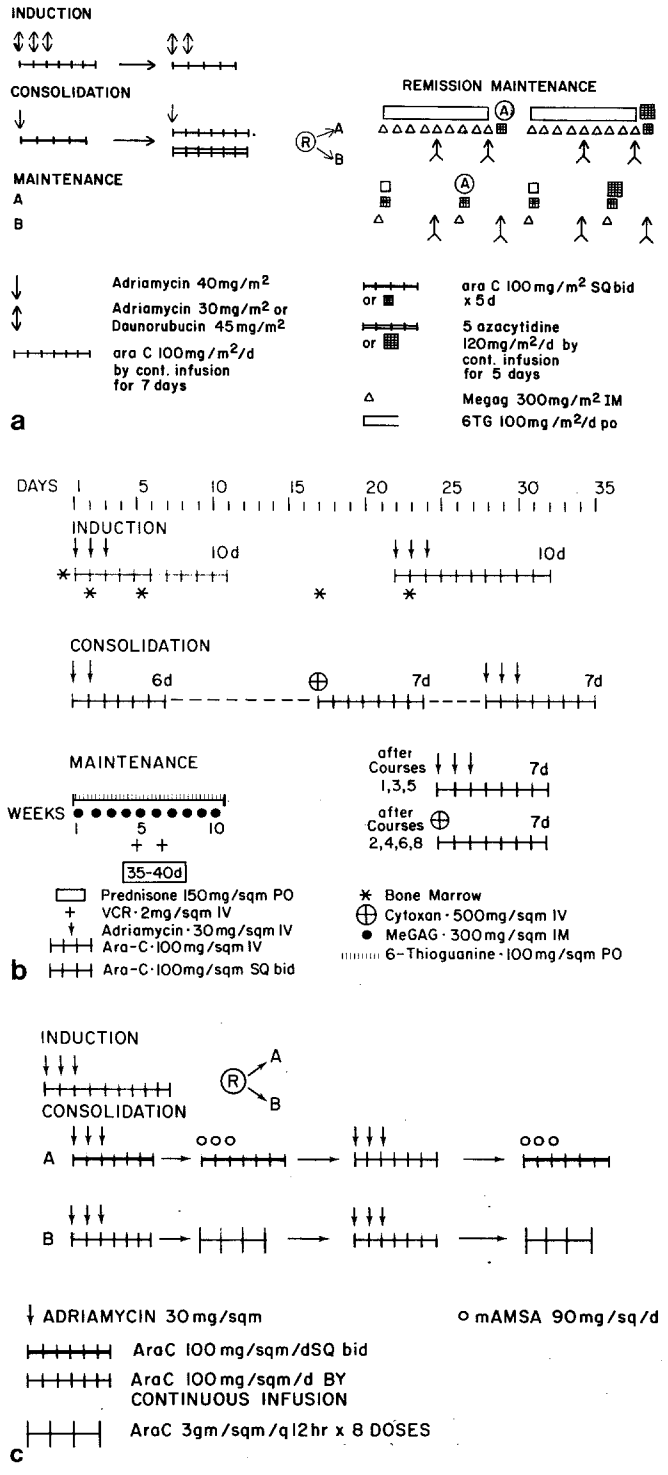

Fig. 1.a–c. Schemas for protocols used to treat patients. **(a)** Protocol 950501; **(b)** protocol 970701; **(c)** protocol 998028

statistically significant differences. It should be pointed out, however, that while statistically significant differences were not present, the median duration of remission for all patients treated according to protocol 998028 is 22 months.

Assessment of retention of cytosine arabinoside triphosphate. Bone marrow (5 ml) was aspirated from the posterior iliac crest into a syringe containing 2 cm^3 10% sodium citrate. In early studies, erythroid cells were lysed by exposure hypotonic NH_4Cl [17], but recently density cut centrifugation has been used to remove erythrocytes. In brief, the marrow cells were layered over a Ficoll-Hypaque solution (spec. gr. 1077) and centrifuged for 10 min at 1000 RPM. The interface cells were recovered, washed, and studied. All specimens studied consisted of >50% leukemic cells.

Three to ten million leukemic bone marrow cells were suspended in 2–5 ml RPMI 1640 made 10% (v/v) with dialyzed heat inactivated fetal calf serum, 2% 4-(2-hydroxyethyl)-1-piperazinethanesulfonic acid-2-(*N*-morphidine) ethanesulfonic containing 1 μg/ml araC and 20 μCi 5-^{3}H-cytosine arabinoside (^{3}Hara-C, 18 ci/mmol). The cells were incubated at 37 °C in a shaking water bath for 30 min and then centrifuged at 500 *g* for 2 min, washed twice with RPMI 1640, and resuspended in 5 ml complete medium free of araC (above medium less araC is complete medium). One aliquot was immediately processed for araCTP extraction, while the other was incubated at 37 °C for 4 h in the araC-free medium. Cells were then centrifuged and washed, and the cell pellets were extracted for analysis of AraCTP by high-performance liquid chromatography (HPLC).

To extract araC and its metabolites, the cells were pelleted by centrifugation and extracted with 50–100 μl 6 *N* perchloric acid and the supernatant neutralized to pH 7.0 with 2 *N* potassium hydroxide. The acid-soluble fraction was analyzed for araC anabolites using previously reported methods [15]. "Zero" time was defined as the time immediately after the initial 30-min incubation of cells with araC. The percentage retention was calculated using the following equation:

$$\frac{\text{araCTP present at 4 h}}{\text{araCTP present at zero time}} \times 100$$

Analysis of data. In our previous studies and in the present study there was no relationship between leukemic cell retention of araCTP and the outcome of remission induction therapy [9, 17]. Hence, these negative data will not be presented. Only the relationship between araCTP retention and remission duration will be discussed. The two patients who expired while in remission were censored at their time of death. While the protocols were designed for newly diagnosed patients, one relapsed patient was inadvertently studied and treated and is included in the data analysis. The criteria of complete remission were those defined by Cancer and Acute Leukemia Group B [14].

Statistical methods. The patients were divided into *low-(<20%)* and *high*-retention (≥20%) groups [9, 17], and the Kaplan-Meier estimate of the survivor function for each group was obtained [4]. The estimates of the survivor functions were compared using the log-rank (Mantel-Cox) test. An additional approach to analysis of the data presented here is made possible by the use of proportional hazard modeling [4]. In this approach, the assumption is made that the hazard functions in the six patient groups (< and ≥20% araCTP retention for patients treated on 3 different protocols) differ only by a constant, which is not a function of time. Since proportionality of the hazard is an assumption, before proceeding with this analysis a test for nonproportionality of the hazards was performed and found to have a chi-square statistic of 3.07 with 5 degrees of freedom ($P=0.69$). Therefore, proportionality could not be rejected and this model was used.

Table 1. Patient characteristics, araCTP retention and remission duration

All protocols	No. of pts	Age[b]	Sex (M/F)	% Retention[b]	Remission duration[b]
(A) All patients					
All patients	80	52 (14–71)	41/39	18.4 (0–73.2)	21.4 (1.5–104.6+)
< 20 %[a]	44	45.5 (14–71)	24/20	13.9 (0–19.4)	12.2 (2.6–74)
≧ 20 %	36	56.5 (16–66)	17/19	42.0 (20–73.2)	44.8 (1.5–104.6+)
(B) Protocol 950501					
All patients	16	41.5 (22–66)	6/10	49 (0–73.2)	12.2 (1.5–104.6+)[c]
< 20 %	6	28 (22–52)	2/ 4	16.5 (0–19.2)	7.2 (2.7–21.2)
≧ 20 %	10	50 (28–66)	4/ 6	56.5 (36.4–73.2)	34.3 (1.5–104.6+)
(C) Protocol 970701					
All patients	29	56 (21–71)	15/14	17.0 (4.6–50.7)	16.7 (2.6–74+)
< 20 %	20	55 (21–71)	10/10	13.1 (4.6–19.0)	11.3 (2.6–74+)
≧ 20 %	9	57 (23–66)	5/ 4	41.5 (20–50.7)	none (6.2–68.7+)
(D) Protocol 998028					
All patients	35	53 (14–66)	20/15	19.4 (1.8–61.1)	37 (4.7–45.1+)
< 20 %	18	46 (14–66)	12/ 6	14.7 (1.8–19.4)	23.0 (6.8–45.1+)
≧ 20 %	17	57 (16–65)	8/ 9	32.5 (20.3–61.1)	none (4.7–40+)

[a] Patients divided into those whose leukemic cells retained < or ≥ 20% of the initially formed araCTP
[b] Median (range)
[c] The remission duration curves for patients treated on the two maintenance arms are indistinguishable

Results

AraCTP retention was measured for pretherapy leukemic marrow obtained from 80 patients. Table 1 provides these data, together with the age and sex distributions, for all patients studied and the corresponding data for the patients treated according to each of the three treatment regimens. The patients are almost equally divided between high- and low-araCTP-retention categories (36 and 44 patients, respectively). The median age of the patients reported here is 52 years, with the median ages for patients in the high- and low-araCTP retention categories being 56.5 and 45.5 years, respectively. These differences in age distribution are not statistically significant.

The remission duration curves for patients treated with each of the treatment regimens can be found in Fig. 2. The median durations of remission are 12, 17, and 37 months, respectively, for patients treated according to protocols 950501, 970701, and 998028. Before proceeding it should be noted that when patients who did not have araCTP retention studies performed and additional patient entries are considered the median duration of remission for all patients treated according to protocol 998028 is 22 months. When treated according to protocol 950501 or 970701, patients whose leukemic cells exhibit high retention of araCTP have longer remissions than individuals in the low-retention category. Table 1 also provides these data. With respect to protocol 950501, patients in the high-retention category have a median duration of remission of 34.3 months, with 20% ± 13% of patients still in remission at >5 years. The median duration of remission for patients in the low-araCTP-retention category is 7.2 months, with no pa-

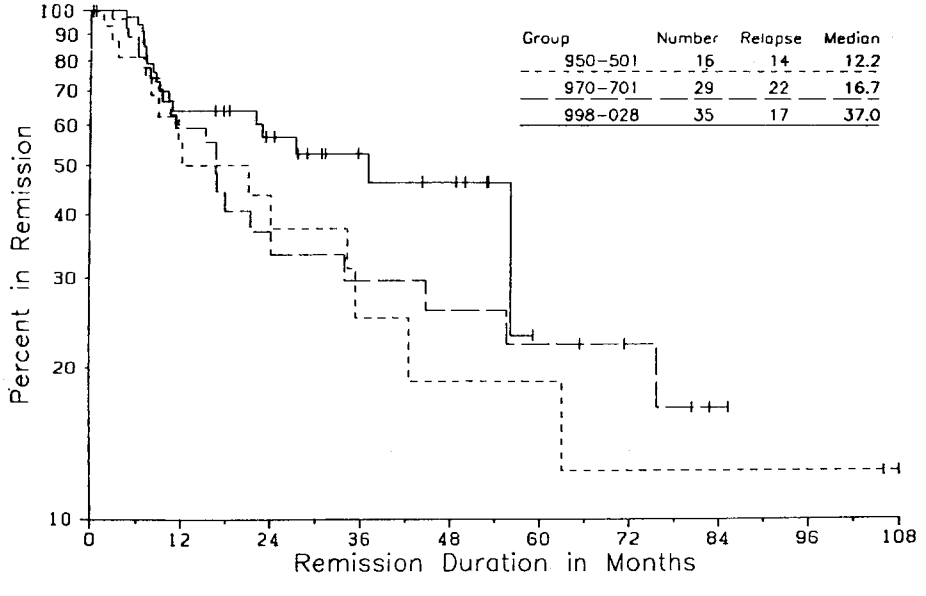

Fig. 2. Overall remission durations for patients treated according to the protocols illustrated in Fig. 1 and whose pretherapy leukemic cell retention of araCTP was studied

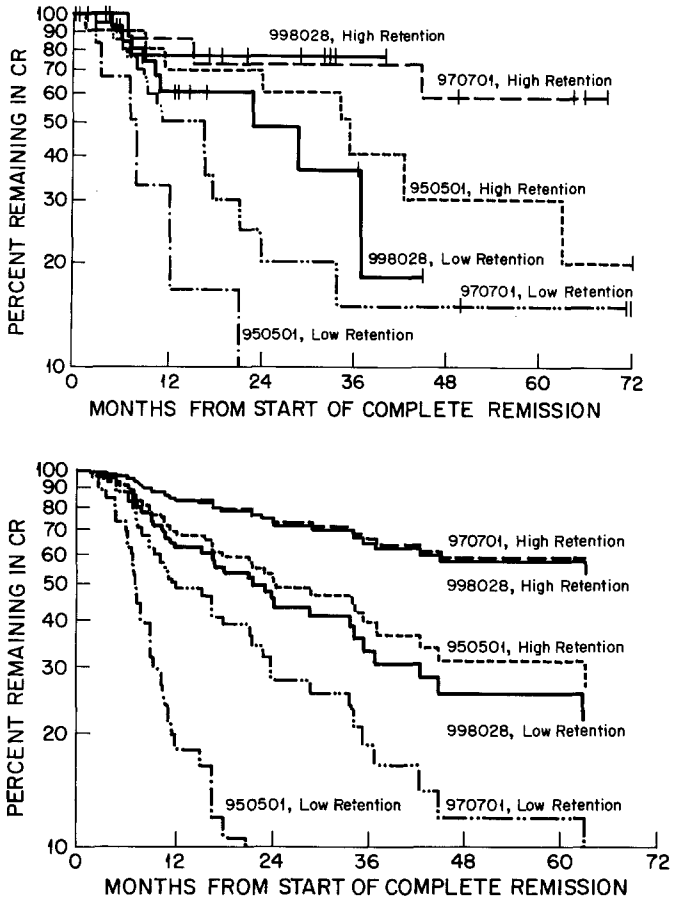

Fig. 3a, b. Simultaneous analysis of the relationship of remission duration to leukemic cell araCTP retention and intensity of therapy. **(a)** Kaplan-Meier estimate; **(b)** proportional hazard estimate

tients in remission beyond 2 years ($P=0.006$). The median duration of remission for patients in the high-araCTP-retention category and treated according to protocol 970701 is indeterminate, with 57%±19% of patients still in remission at 5 years. For patients whose leukemic cells exhibit low-araCTP-retention values, their median duration of remission is 11.3 months, with 15%±8% of patients in remission at 5 years ($P=0.043$). In contrast, araCTP retention is not of prognostic significance for patients treated according to protocol 998028. The median duration of remission for patients whose leukemic cells manifested high retention of araCTP is not yet known, while the corresponding value for patients in the low-retention category is 29 months.

The data described above suggest that the administration of intensive consolidation chemotherapy is beneficial for patients in both high and low araCTP retention categories. To evaluate this possibility a simultaneous analysis of remission duration was performed for patients in the high and low-araCTP retention categories during treatment according to all three protocols. Figure 3a provides the simultaneous Kaplan-Meier estimates of the remission durations for these patients. The overall differences between the curves are significant at the $P=0.0023$ level. Since proportional hazard estimates may provide a somewhat clearer description of the differences between the groups, the same data are presented in Fig. 3b using this method of representation. The overall difference between

Table 2. Proportional hazard functions for patients in the high- and low-retention categories relative to the hazard function for the high retention group in protocol 970701

Protocol	AraCTP retention	Relative hazard	Hazard for low retention relative to high retention for each protocol
950501	Low	9.48	4.28
	High	2.22	
970701	Low	3.99	3.99
	High	1.00	
998028	Low	2.57	2.55
	High	1.02	

these curves is at the $P=0.0017$ level. These hazard estimate data are presented in tabular form in Table 2. It can be seen that as the intensity of consolidation therapy is increased the relative hazard function falls, with the greatest decline being manifested in patients in the low araCTP retention category. Inspection of Figure 3b and Table 2 reveals that the major benefit derived from increasing the intensity of therapy is experienced by patients whose leukemic cells are in the low araCTP retention category. The median duration of remission for these patients increased from 7.2 months, to 11.3 months, to 29 months as therapy evolved from protocol 950501 to 970701 to 998028. In contrast, the remission duration curves for patients in the high araCTP retention categories are quite similar regardless of the treatment protocol, being essentially identical for patients treated according to protocols 970701 and 998028.

Given that protocol 998028 actually has two different intensive consolidation arms, a further analysis was carried out to evaluate the efficacy of each arm in patients whose leukemic cells exhibited high or low araCTP retention (Fig. 3). The ability of leukemic cells to retain araCTP has no demonstrable relationship to the remission duration of patients who receive high-dose araC as part of consolidation therapy (arm B). In contrast, patients in the low araCTP retention category treated in arm A appear to have shorter remissions than patients who are in the high araCTP retention category and are treated with the same therapy. Furthermore, arms A and arm B are associated

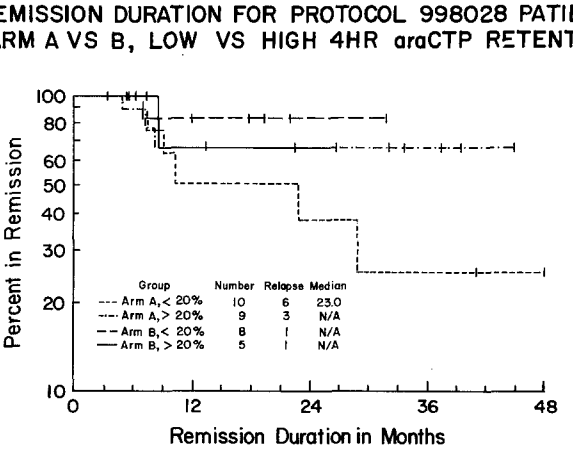

Fig. 4. Comparison of the remission duration of patients treated in arms A and B of protocol 998028, segregated on the basis of leukemic cell retention of araCTP

with indistinguishable remission durations for patients whose leukemic cells retain araCTP well. In contrast, patients in the low araCTP retention group appear to have longer remission durations if treated in arm B. In summary, treatment arms A and B are equally efficacious for patients in the high araCTP retention category, while patients in the low retention category appear to do better when treated with arm B, which includes high-dose araC.

Discussion

The three treatment protocols described here demonstrate the evolution, during the past decade, of the nature of chemotherapy administered to patients with ANLL in remission. Protocol 950501 provided for 5 years of maintenance therapy, protocol 970701 provided for the administration of three courses of intensive consolidation chemotherapy followed by 3 years of maintenance chemotherapy, and protocol 998028 provided for the administration of four courses of consolidation chemotherapy and no maintenance therapy. Despite the reduction in the duration of therapy (from 5 years to 4–6 months), the median duration of remission increased as the intensity of the courses of therapy administered to patients in remission increased. Furthermore, maintenance chemotherapy following three courses of intensive consolidation chemotherapy produced no discernible benefit.

The ability of leukemic cells to retain the active metabolite of araC was the single most important prognostic factor for patients treated according to protocols 950501 and 970701. This is not the case for patients treated according to protocol 998028. Since araC is the predominant chemotherapeutic agent in all three regimens, the logical question arises as to why araCTP retention is of progressively less prognostic significance. In a prior report we postulated that pharmacokinetic considerations were responsible for the prognostic significance of araCTP retention. It appeared likely that leukemia cell araCTP retention was prognostically important, because the dose and schedule of araC administration in protocols 950501 and 970701 provided therapeutic plasma araC levels for only 2 h twice a day. Hence, patients whose leukemic cells retained araCTP for longer periods would have functionally more pharmacologically effective therapy than patients whose leukemic cells retained araCTP poorly [9]. Further, earlier observations suggested that the administration of even a single course of araC by continuous infusion resulted in an improvement in remission duration over that produced by s. c. administration [18]. On the basis of these observations, protocol 998028 was designed to provide all patients with at least one course of araC administered by continuous infusion and to provide one-half of the patients with two courses of high-dose araC therapy. The latter arm was included because during high-dose araC therapy the plasma level never falls below the araC level produced by the standard dose of araC (100 mg/m^2) administered over 24 h by continuous infusion [3].

These changes in araC dose and schedule appear to provide more effective therapy for patients as a whole, and especially for patients whose leukemic cells retain araCTP poorly. The median duration of remission for this subset of patients is 22 months, as against 11 and 7 months for patients treated according to the predecessor protocols. While the number of patients treated in each arm of protocol 998028 is not large, the data suggest that the inclusion of high-dose araC therapy in arm B is especially beneficial for patients in the low araCTP retention category.

Hence, as therapy has evolved, remission durations have increased and by altering the route, dose, and schedule of araC administration we were able to overcome the adverse prognostic significance of low araCTP retention. Recent analyses of the three protocols reported here have demonstrated that as araCTP retention has lost its prognostic significance, cytogenetic characteristics have become the single most important indicator of the likely duration of remission [12]. For protocol 998028 the only additional prognostic factors were the level of the white blood cell count at diagnosis and the percentage of S phase cells in the marrow [13]. Development of an understanding of the reasons for the differing prognosis of leukemias with these different characteristics should lead to adjustments in therapy which, as in the studies described here, will overcome the adverse prognosis associated with these factors.

Acknowledgements. The authors would like to thank. A. Evans, B. Owczarcek, G. Wang, D. Buscaglia, C. Wrzosek, and E. Kelly for their excellent technical assistance, and Drs P. D'Arrigo, T. Doeblin, M. Stein, M. Bloom, and A. Early for allowing us to include their patients in this study.

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Received June 5, 1986/Accepted September 9, 1986