Persistence of Circulating Blasts After 1 Week of Multiagent Chemotherapy Confers a Poor Prognosis in Childhood Acute Lymphoblastic Leukemia

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Early response to therapy, typically assessed by bone marrow status, is predictive of outcome in childhood acute lymphoblastic leukemia (ALL). Less is known about the significance of early clearance of blast cells in peripheral blood. We reviewed medical records of all patients with ALL enrolled on St Jude Total Therapy Study XI (February 1984 to September 1988) to determine the presence of blast cells in peripheral blood at diagnosis and after 1 week of intensive induction therapy. Of the 358 patients, 59 lacked evidence of circulating blast cells at diagnosis, and data were unavailable for 2 patients. The prognostic significance of persistent circulating blast cells in the remaining 297 patients was assessed in a multivariate analysis that included known adverse prognostic factors. Persistent circulating leukemic blasts were present at day 8 in 41 patients (14%). Compared with the "blast-negative" group, these patients had a significantly higher frequency of several adverse clinical features (leukocyte count >50 × 10^9/L, mediastinal mass, central nervous system leukemia, T-cell phenotype, lack of CD10 expression, and L2 morphology) and a significantly poorer 5-year event-free survival (34% ± 8% [SE] *v* 77% ± 3%, $P <$ .01). By multivariate analysis, blast cell persistence at week 1 was the most significant adverse feature in the overall cohort (relative risk, 2.9; 95% confidence interval, 1.8 to 4.8) and in an analysis limited to B-lineage cases (relative risk, 3.6; 95% confidence interval, 1.9 to 7.1). Patients identified by this simple, noninvasive measure may benefit from early modification of therapy.

THE IDENTIFICATION OF clinical and biologic features that predict treatment outcome has permitted the design of risk-directed clinical trials in childhood acute lymphoblastic leukemia (ALL).[1,2] These trials aim to reduce acute and long-term side effects in patients with a favorable prognosis by avoiding unnecessarily toxic treatment, while improving outcome for patients with high-risk leukemia by intensifying therapy. Effective risk-based therapy produces complete clinical remissions in at least 95% of patients. However, 25% of these children will subsequently relapse.[3] Additional early predictors of treatment failure are needed to identify this subgroup of patients.

Previous studies have ascribed negative prognostic significance to the presence of leukemic blast cells in bone marrow aspirates after 1 or 2 weeks of remission induction chemotherapy,[4-7] and to circulating leukemic blast cell counts >1,000/μL after a week of single-agent steroid therapy.[8,9] However, little is known about the significance of early peripheral blast cell clearance in the context of contemporary multiagent remission induction therapy. Therefore, we assessed the prognostic impact of persistent circulating blast cells after 1 week of chemotherapy among patients treated with an effective risk-based regimen.[10]

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MATERIALS AND METHODS

Patient selection. We studied patients with previously untreated ALL who were enrolled on St Jude Total Therapy Study XI between February 1984 and September 1988. The diagnosis of ALL was based on standard morphologic studies and cytochemical staining of leukemia cells. Blast cell immunophenotype, karyotype, and DNA index were determined by previously described methods.[1,2] This protocol was approved by the hospital's institutional review board and signed informed consent was obtained from parents or guardians.

Medical records of all 358 patients enrolled on this study were reviewed to determine the results of leukemic blast cell counts in peripheral blood smears performed at diagnosis and on day 8 of remission induction therapy (or day 7, in 24 patients who did not have a differential white blood cell count on day 8). The presence of circulating blasts was confirmed by an experienced hematopathologist.

Patients were excluded from the primary statistical analyses if their records did not indicate the presence of circulating leukemic blast cells at diagnosis (n = 59) or if blood cell counts at week 1 were unavailable (n = 2). The remaining 297 patients were the focus of this study.

Treatment. The treatment regimen has been described in detail previously.[10] Briefly, induction therapy consisted of prednisone (40 mg/m^2/day in three divided doses for 28 days), vincristine (1.5 mg/m^2 weekly for 4 weeks), asparaginase (10,000 U/m^2 three times weekly for 3 weeks), daunorubicin (25 mg/m^2 weekly for 2 or 3 weeks), and teniposide (200 mg/m^2) plus cytarabine (300 mg/m^2) on days 22, 25, and 29. Patients enrolled during the first 15 months of the study received teniposide plus cytarabine instead of daunorubicin in the first week of remission induction. This change in therapy did not affect remission induction rates.[10] Triple intrathecal therapy (methotrexate, hydrocortisone, and cytarabine) was administered on days 1, 22, and 43 for all patients and weekly for the first 4 weeks in patients with central nervous system (CNS) leukemia at diagnosis. Thus, at the end of week 1, patients had received 21 doses of prednisone, three doses of asparaginase, one dose each of vincristine and daunorubicin (195 of the evaluable patients), or of teniposide plus cytarabine (102 patients), and one course of triple intrathecal therapy.

High-dose methotrexate (2 g/m^2 per week for 2 weeks) with leucovorin rescue was administered as consolidation therapy. Patients were then stratified by risk classification and randomized to receive different schedules of continuation therapy, as previously described.[10] Cranial irradiation and five courses of triple intrathecal therapy were added after 1 year of complete remission for higher-

Table 1. Presenting Features According to the Presence or Absence of Persistent Blasts After 1 Week of Remission Induction

Feature*	Persistent Blasts (n = 41)	No. of Blasts (n = 256)	*P* Value
Black race	13 (32%)	27 (11%)	<.001
Leukocyte count (≥50 × 10^9/L)	28 (68%)	66 (26%)	<.001
Median (range)	125.8 (4.4-917.0)	16.1 (1.2-549)	
Hemoglobin level (≥8 g/dL)	27 (66%)	103 (40%)	<.01
Mediastinal mass	14 (34%)	23 (9%)	<.001
CNS leukemia	8 (20%)	20 (8%)	.038
T-cell phenotype	23 (56%)	34 (13%)	<.001
CD10 negativity†	24 (59%)	33 (13%)	<.001
L2 morphology	13 (32%)	29 (11%)	<.01

* Only significant associations are listed. Other variables analyzed were liver and spleen size, presence of chromosomal translocation, leukemic cell ploidy and DNA index, age, sex, and treatment arm.

† Data not available for 2 patients.

risk patients (18 Gy) and for those with CNS leukemia (24 Gy). The total duration of continuation therapy was 120 weeks.

Statistical analysis. The distributions of clinical and biologic features for patients with or without persistent circulating blast cells at week 1 were compared by the chi-square or two-tailed Fisher exact test for categorical variables and the Kruskal-Wallis test for continuous variables. Life-table estimates of event-free survival (EFS) were derived by the method of Kaplan and Meier[11] and compared using the stratified log-rank test.[12] Early death and failure to enter remission were considered events at time zero. The influence of potential prognostic factors on EFS was estimated with the Cox proportional hazards model.[13] The factors tested included age, gender, race, leukocyte count, hemoglobin level, liver and spleen size, CNS leukemia, mediastinal mass, French-American-British (FAB) classification, leukemic cell ploidy and DNA index, presence of chromosomal translocation, immunophenotype, leukemic cell CD10 expression, treatment arm, and peripheral blast cell count at the end of week 1. Each factor was first tested as a single regressor variable in the Cox model (univariate analysis). A stepwise multivariate regression approach was then used to identify the most important predictor variables. A P value ≤.10, after adjustment for the effects of other variables, was required for retention in the model. The relative risks of failure and the associated 95% confidence interval were calculated with the coefficient and standard error from the Cox analysis; P values are from the likelihood ratio test.

RESULTS

After 1 week of multiagent remission induction treatment, 41 of the 297 study patients (14%) had persistent circulating leukemic blast cells. The frequency of this finding did not differ between patients who received daunorubicin and those who received teniposide/ara-c during the first week of therapy (29 of 195 *v* 12 of 102 patients; P = .60, by Fisher's exact test). Compared with the 256 patients whose peripheral blood smears showed blast cell clearance, the 41 "blast-positive" patients were more likely to have the following previously identified adverse presenting features: black race, increased leukocyte count and hemoglobin level, mediastinal mass, CNS leukemia, T-cell phenotype, lack of CD10 expression, and FAB L2 morphology (Table 1). The two groups

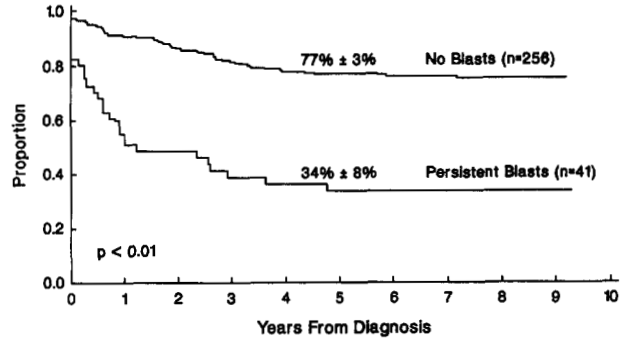

Fig 1. EFS according to the presence or absence of leukemic blast cells in peripheral blood at day 8 of remission induction therapy.

did not differ in age, DNA index or ploidy, or presence of chromosomal translocation.

The persistence of circulating leukemic blast cells was associated with a significantly poorer 5-year EFS (34% ± 8% *v* 77% ± 3%; P < .01), as shown in Fig 1. Separate analyses by immunophenotype (Fig 2) yielded similar results among cases with B-lineage (5-year EFS = 29% ± 10% *v* 79% ± 3%; P < .01) and T-lineage ALL (39% ± 11% *v* 64% ± 9%; P = .049).

Table 2 compares types of failure for the week 1 blast-positive and blast-negative groups. As expected, the majority of treatment failures in both groups were related to resistant leukemia. The remission induction rate was significantly lower in the blast-positive group (34 of 41 *v* 250 of 256; P < .01). An analysis that censored induction failures in both groups also showed an inferior EFS (P = .01) for patients

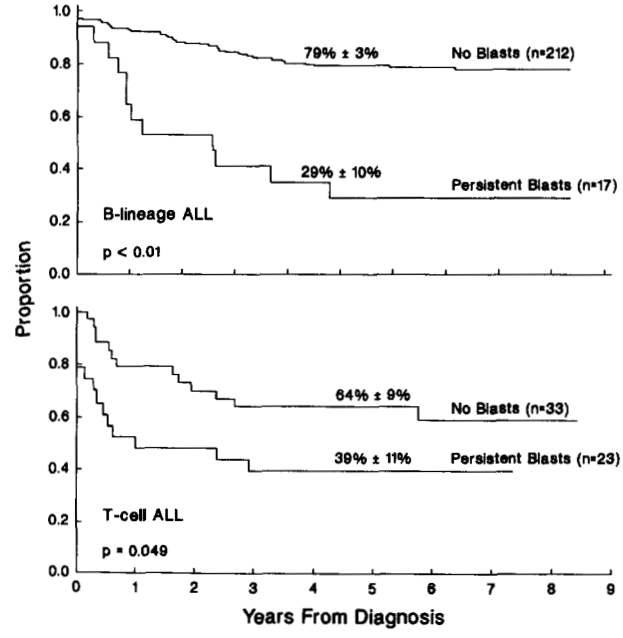

Fig 2. EFS in B-lineage and T-cell ALL by the presence or absence of circulating blast cells at day 8 of remission induction therapy.

Table 2. Adverse Events by Circulating Blast Cell Status at Day 8

Types of Event	Persistent Blasts (n = 41)	No Blasts (n = 256)
Induction failure	7	6
Hematologic relapse	12	27
CNS relapse	3	14
Testicular relapse	0	1
Combined relapse	1	3
Death in remission	0	5
Secondary AML	4	6

with persistent circulating blasts at day 8. Secondary acute myeloid leukemia was more common in the blast-positive group, and deaths in remission were seen only in blast-negative cases. When cases in these two categories of failure were censored in a separate analysis, the persistence of circulating blast cells remained significantly associated with a poorer EFS ($P < .01$; data not shown).

By multivariate analysis, after adjustment for competing covariates, blast cell persistence at week 1 emerged as the most significant adverse prognostic factor in this cohort of patients as a whole and in B-lineage patients analyzed separately. Presenting leukocyte count entered both models, albeit with marginal significance, in the overall cohort. Age at diagnosis (<1 year or ≥10 years) retained significance only for the B-lineage cases (Table 3). The small number of patients precluded meaningful multivariate analysis for cases of T-cell ALL.

DISCUSSION

In this large series of children treated with effective, risk-based therapy for ALL, the persistence of circulating leukemic blast cells after 1 week of multiagent remission induction chemotherapy was an important early predictor of treatment failure. Only 34% of patients with this feature were alive and free of disease 5 years after diagnosis, compared with 77% of patients whose peripheral blast cells had cleared by day 8. Multivariate analysis confirmed the independent prognostic significance of circulating blast cells at day 8. Of the other potential prognostic factors studied, including leukemic cell DNA index, CNS leukemia, and absence of CD10 expression, only initial leukocyte count and, in B-lineage cases, patient age, achieved significance in this model.

The high frequency of induction failures in the week 1 blast-positive group (17%) suggested that the prognostic significance of this measure might rest on the identification of patients with aggressive, inherently resistant disease (who would be unlikely to benefit from known alternative strategies). When induction failures were censored in the analyses, however, EFS remained significantly poorer in this group. Relapses during continuation therapy, another common cause of failure in patients with circulating blasts at day 8, have also been associated with a dismal outcome. However, if identified prospectively, these patients might benefit from early interventions designed to overcome drug resistance.

Our results are in accord with prior studies showing the importance of early response to therapy in predicting the outcome of treatment for childhood ALL. In two successive trials, the Berlin-Frankfurt-Münster group found that circulating blast cell counts >1,000/μL after 1 week of single-agent prednisone therapy were associated with a significantly poorer EFS, despite assignment of "poor prednisone responders" to more intensive therapy in the second trial.[8,9] However, this approach to identifying high-risk patients is incompatible with our goal of initiating maximally effective therapy as soon as possible after the diagnosis of ALL.

In a study of patients treated between 1975 and 1984, Rautonen et al[14] identified delayed clearance of circulating blast cells (ie, beyond 10 days) as a negative prognostic factor. This study was performed in the context of less intensive treatment, and the outcome was poorer than that achieved in our series (5-year EFS for patients with early blast cell clearance = 60%, compared with 77% for week 1 blast-negative patients in the present study). Hence, although the findings are complementary with the results of our study, they are not directly comparable.

Bone marrow examination, typically at day 14 of remission induction therapy, remains the clinical gold standard for assessment of early treatment response in US studies. The Childrens Cancer Group (CCG) identified an inferior disease-free survival among patient subgroups defined by marrow status M2 (5% to 25% blast cells) or M3 (>25% blast cells) at day 14.[5] In a subsequent study,[4] this group assessed day 7 marrow status in a cohort of high-risk patients (defined by a leukocyte count $>50 \times 10^9$/L or lymphomatous features). Patients with M1 (<5% blast cells) or M2 marrow status had a significantly better 3-year disease-free survival than those in the M3 category. This finding has been further confirmed in a larger cohort of patients with similar high-risk features, treated on two different regimens. Both day 7 and day 14 marrow status were significant prognostic factors.[6] Finally, a CCG study of lower-risk patients showed that M2 or M3 marrow at day 14 was also predictive of a significantly poorer EFS in this patient group.[7]

To our knowledge, the concordance between findings in bone marrow and persistence of blasts in peripheral blood in individual patients has not been defined. We are addressing this question prospectively in our current clinical

Table 3. Independent Adverse Prognostic Features in Patients With ALL

	All Cases (n = 285)*		B-Lineage (n = 212)	
Adverse Feature	Relative Risk (95% CI)	P Value	Relative Risk (95% CI)	P Value
Blast persistence at day 8	2.9 (1.8, 4.8)	<.001	3.6 (1.9, 7.1)	<.001
Initial leukocyte count $>50 \times 10^9$/L	1.8 (1.1, 2.9)	.018	2.9 (1.7, 5.0)	<.001
Age <1 yr or >10 yr	—		2.2 (1.2, 3.8)	.01

*Twelve patients with no immunophenotype data at diagnosis were excluded from the model. The relative risk of treatment failure is in comparison to that in patients without the adverse feature and was calculated after adjusting for the factors in the final model. Risks are shown only for factors that achieved independent significance.

trial for newly diagnosed ALL. If the correlation is sufficiently high, it might be possible to avoid multiple bone marrow examinations during induction therapy—a prospect with considerable appeal for both physicians and patients.

Clearly, there is a subset of high-risk patients who are not identified by either presenting features or blast cell persistence in peripheral blood or bone marrow. A variety of molecular biologic techniques are being evaluated for their usefulness in detecting residual disease that is not evident by clinical criteria.[15-17] However, the ultimate value of these techniques in predicting response remains to be determined. Further, these techniques are labor-intensive and require sophisticated technology and expertise that is not available at all centers. Although detection of circulating blasts does not identify all patients who will subsequently relapse, this approach is simple, noninvasive, and universally applicable.

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