Low-Dose Versus High-Dose Methotrexate During Remission Induction in Childhood Acute Lymphoblastic Leukemia (Protocol 81-01 Update)


We evaluated event-free survival (EFS) and leukemia-free interval (LFI) of children treated for acute lymphoblastic leukemia (ALL). Patients were randomized to receive either a low dose or high dose of methotrexate (MTX) as a single agent at the time of diagnosis. Five days later, multidrug therapy began. We assessed the early antileukemic efficacy of the two doses of MTX, as well as toxicity and long-term efficacy. An increase in cell kill, as indicated by a larger decrease in the percentage of viable cells in the bone marrow between days 0 and 5, was observed for the high-dose MTX group when compared with the low-dose MTX group ($P = .04$). At 7.1 years of median follow-up, the 38 children randomized to receive high-dose MTX had a better EFS and LFI compared with the 39 patients randomized to receive low-dose MTX. The 7-year percentages ($\pm SE$) for EFS were $82\% \pm 6\%$ for high-dose MTX and $69\% \pm 7\%$ for low-dose MTX ($P = .13$). The 7-year percentages for LFI were $91\% \pm 5\%$ and $69\% \pm 7\%$, respectively ($P = .01$). We recommend that high-dose MTX be considered as an effective addition to induction therapy in childhood ALL.

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dence of a mediastinal mass, and no clinical signs or cytologic evidence of CNS leukemia. All others were in the high-risk group. By these criteria, 110 children (38%) were in the standard-risk and 179 children (62%) were in the high-risk group. Treatment for high-risk patients included higher cumulative doxorubicin (345 mg/m²) and asparaginase doses, as well as higher doses of prednisone (120 mg/m²/d for 5 days every 3 weeks) and cranial irradiation doses compared with the standard-risk group. Therapy was electively discontinued for all patients after 2 years of continuous complete remission (CCR). Informed consent was obtained before the initiation of therapy.

Laboratory measurements during remission induction. Pretreatment laboratory measurements included a complete blood count, differential cell counts of BM aspirates after Wright-Giemsa staining, and evaluation of the cellularity of BM biopsies. BM cell viability was measured by uptake of the fluorescent dye rhodamine-123 (Rh-123), known to be selectively accumulated by the mitochondria of living cells. For Rh-123 studies, mononuclear cells of BM aspirates were separated over Ficoll gradients, stained with the dye, and analyzed by flow cytometry as previously described. All laboratory measurements were repeated at hour 120 after the beginning of MTX administration. BM aspirates on each of the 2 days had approximately the same percentage of lymphoblasts (usually > 90%).

Statistics. For EFS, events were defined as failure to achieve remission, death during induction of remission, death during remission, or relapse at any site (whichever occurred first). For LFI, events were defined as failure to achieve remission within 60 days (exclusive of induction deaths) or recurrence of leukemia after remission had been achieved. Induction deaths and remission deaths were censored for evaluation of LFI. EFS and LFI distributions were estimated by the Kaplan-Meier method and standard errors (SE) were estimated using Greenwood's formula. The two-sided log rank procedure stratified by risk group was used to assess the statistical significance of differences between EFS and LFI distributions. The treatment group difference in the decrease in Rh-123 values between days 0 and 12 after the initiation of therapy was evaluated by the one-sided Wilcoxon rank sum test (nonparametric) and by the one-sided t-test (parametric).

RESULTS

As of April 1991, with a median follow-up of 6.7 years (range, 3.0 to 9.5 years), the EFS (±SE) at 7 years was
72% ± 3% overall, 83% ± 4% for the standard-risk group, and 65% ± 4% for the high-risk group (Fig 2). Among the 110 standard-risk patients, there were 18 events (Table 1), five (28%) of which occurred later than 4 years after induction of complete remission. Of the 179 high-risk patients, there were 63 events, eight (13%) of which occurred later than 4 years. These results, both overall (P < .0001; Fig 3) and separately by risk group (standard risk, P = .0001; high risk, P = .0009), represent significant improvement in outcome compared with our previous treatment results for children diagnosed between 1973 and 1980.

We assessed the influence of the modifications of early therapy on long-term outcome. Standard- and high-risk groups were combined for this analysis because patient distribution in each of the modified regimens resembled that of the whole study population, and the groups were balanced for individual risk factors such as age and WBC (Table 2). Analysis of EFS comparing the 38 patients randomized to high-dose MTX with the 39 randomized to low-dose MTX suggested an improved outcome for the high-dose MTX group at a median follow-up of 7.1 years (7-year EFS % ± SE = 82% ± 6% v 69% ± 7%; P = .13, Fig 4). The EFS was 86% ± 6% for patients who received 4 g/m² and 67% ± 16% for patients who received 33 g/m². There were more remission deaths (none of which could be directly attributable to high-dose MTX) among patients randomized to the high-dose MTX treatment (Table 1). Analysis of LFI (censoring induction deaths and remission deaths, and thus providing a specific measure of antileukemic efficacy) was statistically significantly different between the two randomized dose levels. The 7-year LFI percentages were 69% ± 7%, 91% ± 4%, and 76% ± 15% for the 40 mg/m², 4 g/m², and 33 g/m² MTX groups, respectively. As shown in Fig 5, patients randomized to receive high-dose MTX had a significantly improved LFI compared with those randomized (P = .01) and to those assigned (P = .05) to low-dose MTX.

The primary cause of failure among the 38 patients who received high-dose MTX was toxicity, which occurred after remission induction and could not be directly attributable to the MTX. There were four remission deaths (salmonella sepsis, Pneumocystis carinii pneumonia, pneumococcal sepsis, myocarditis) and three relapses (one BM, one CNS, and one CNS and lymph node) (Table 1). Among the 39 patients randomized to receive low-dose MTX, there were four induction failures (one undifferentiated leukemia, one Philadelphia chromosome-positive disease, and two other patients with refractory disease), eight relapses (three BM, one CNS, and two CNS and lymph node).
were treated with a dose of 4 g/m². Two of the 29 children suffered severe mucositis, two had generalized seizures, one experienced hypertension, one had significant elevation of liver function tests, and another developed abnormal kidney function tests. Low-dose MTX was associated with minimal toxicity, with one child showing transient renal impairment in the presence of a disseminated intravascular coagulopathy.

To address the question of whether the initial single dose of high-dose MTX resulted in increased cell kill compared with a single dose of low-dose MTX, Rh-123 uptake studies for marrow cell viability were performed. Marrows from 40 of the 77 randomized patients (23 high-dose MTX and 17 low-dose MTX patients) were evaluable with complete studies before and 120 ± 12 hours after initiation of MTX therapy. As shown in Table 3, more cell kill (as indicated by a larger decrease in Rh-123) was observed for patients who received high-dose MTX (P = .04). Analyses attempting to correlate the amount of decrease in Rh-123 with outcome had insufficient statistical power because only seven of the 40 evaluable patients had suffered an event.

**DISCUSSION**

Long-term follow-up of this treatment program (Protocol 81-01) showed an EFS that exceeded 70% for the entire population (72% ± 3% 7-year EFS). Our previous report published at 4 years median follow-up showed a 77% ± 3% 4-year EFS. Events occurring between years 4 and 7 thus account for an additional 5% decrease in the EFS curve.

Our assessment of early cytoreductive therapy, especially the use of low-dose or high-dose MTX during induction, suggested a dose effect as measured by EFS that did not reach statistical significance. However, when nonleukemic events, such as induction deaths and remission deaths, were censored, and LFI was used as the measure of antileukemic efficacy, we found that high-dose MTX was superior to low-dose MTX (P = .01) in a randomized comparison. Because none of the nonleukemic events were directly attributable to the high-dose MTX, we think that the enhanced LFI for the group treated with high-dose MTX suggests the potential importance of this drug in the early treatment of childhood ALL. The fact that no leukemic events occurred among the standard-risk group randomized to receive high-dose MTX prompted us to design our...
current, ongoing studies to re-evaluate the importance of high-dose MTX.

The intensification of therapy resulted in some treatment-related toxicity that had not been anticipated from other studies of high-dose MTX. In fact, in Protocol 81-01 the treatment arm with the highest MTX dose (33 g/m²), as well as that with the COAP treatment modification, were closed because of excessive morbidity. The use of high-dose MTX for initial cytoreductive therapy, at a time when the leukemic tumor burden and subsequent cell lysis is most problematic, may be one reason for the excessive toxicity encountered with our use of MTX at a dose of 33 g/m², especially when compared with the successful use of that dose in other trials.

These long-term results represent a marked improvement in outcome for all patient groups compared with our previous studies. The major therapeutic modification that distinguished Protocol 81-01 from its predecessors was that most patients received an induction dose of MTX, a drug that had been established as one of the most active agents in the treatment of childhood ALL. Another major difference between this study population and its predecessor was the elimination of postinduction doxorubicin therapy for standard-risk patients. This was done because of concern about late cardiac complications. We believe that the intensive use of asparaginase in all patients accounts for much of the improved outcome compared with previous trials.

German investigators had previously shown that the in vivo response to corticosteroid therapy before multiple-drug induction therapy was an important risk factor for early failure. In our study, a single exposure to high-dose MTX was associated with both a better outcome in terms of LFI and a better leukemic cell kill, as measured by changes in Rh-123 uptake when compared with standard-dose MTX. A correlation between Rh-123 measures of cell kill and clinical outcome could not, however, be established, due to the small number of patients evaluated for this analysis who had adverse events. As shown in Table 3, there was greater leukemic cell cytotoxicity (as measured by a decrease in Rh-123 fluorescence) in the BM of patients who received high-dose MTX. There was no statistically significant difference in EFS or LFI between patients who received either of the two high doses of MTX. A possible mechanism by which a single dose of a drug might produce substantially improved long-term LFI might be related to more rapid reduction in leukemic burden, preventing mutation to resistant phenotype or entrance of leukemic cells into extramedullary sites.

The comparative evaluation of MTX doses reported here was based on a small number of randomized patients, and the results of the randomized low-dose MTX group were slightly worse than anticipated compared with the assigned low-dose MTX group. Furthermore, early follow-up did not provide enough data to detect treatment outcome differences in 1987, when we initiated our subsequent program, Protocol 87-01. We therefore designed that study to formally address the question of whether increased antileukemic efficacy could be achieved with high-dose MTX during induction compared with standard-dose MTX. While awaiting the results of Protocol 87-01, which has accrued over 300 patients, we recommend that high-dose MTX be considered a potentially effective addition to induction therapy for children with ALL.

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