High-dose Cytosine Arabinoside and Fractionated Total-Body Irradiation: An Improved Preparative Regimen for Bone Marrow Transplantation of Children With Acute Lymphoblastic Leukemia in Remission

By Peter F. Coccia, Sarah E. Strandjord, Phyllis I. Warkentin, Nai-Kong V. Cheung, Erlinda M. Gordon, Louis J. Novak, Donald C. Shina, and Roger H. Herzig

Twenty children with acute lymphoblastic leukemia in second (18 patients) or third (two patients) complete remission after bone marrow relapse received allogeneic bone marrow transplants from histocompatible sibling donors. The preparative regimen for marrow transplantation consisted of 12 doses of 3,000 mg/m² cytosine arabinoside twice daily for six days followed by 1,200 cGy total-body irradiation (six doses of 200 cGy twice daily for three days). The preparative regimen was well tolerated, and all patients showed marrow engraftment promptly. Twelve patients are alive in complete remission 12 to 79+ months posttransplant; eight patients are over 48 months posttransplant. Six patients died 1 to 9 months posttransplant of nonleukemic causes: (two each of graft-vs-host disease, interstitial pneumonitis, and infection). Two patients developed recurrent leukemia at 15 and 30 months posttransplant. Both have died at 19 and 36 months posttransplant. Life table analysis reveals an actuarial survival and event-free survival rate of 58% and a marrow relapse rate of 17%. These results suggest that high-dose cytosine arabinoside and fractionated total-body irradiation is a relatively nontoxic and highly effective preparative regimen for allogeneic bone marrow transplantation for acute lymphoblastic leukemia that deserves further evaluation.

REMARKABLE PROGRESS has been made in the treatment of children with acute lymphoblastic leukemia (ALL) in the past 20 years. In clinical trials initiated since 1978, it is reported that over 95% of children can be induced into complete remission and 65% to 80% of patients are in continuous complete remission upon completion of 2 to 3 years of therapy. It is expected that over 60% of these patients will ultimately be cured of their leukemia.1,4

Unfortunately, in the 20% to 30% of children who develop recurrence of leukemia and especially in those who relapse in the bone marrow while still receiving therapy, subsequent leukemic control and survival are disappointing, with most children dying of refractory leukemia.7,4 This poor outcome after bone marrow relapse has led to an extensive evaluation of high-dose bone marrow ablative therapy followed by allogeneic bone marrow transplantation (BMT)9-26 in second or subsequent remissions as an alternative to chemotherapy. In studies comparing allogeneic BMT directly with maintenance chemotherapy,9,12,16,17 transplant results are significantly better, especially in those studies with a prolonged follow-up.10,12 When cyclophosphamide and total-body irradiation are used to prepare patients for allogeneic BMT, the recurrence rate of leukemia is 40% to 50%, and the disease-free survival rate is 30% to 40%.

In an attempt to decrease the marrow relapse rate and to improve long-term survival after allogeneic BMT for recurrent ALL in remission, we evaluated replacing high-dose cyclophosphamide with high-dose cytosine arabinoside at the established maximum tolerated dose and duration in the preparative regimen with total-body irradiation. Since 1981 we have performed BMT on 20 children with recurrent ALL and matched sibling donors, 18 in second remission and two in third remission, after preparation with high-dose cytosine arabinoside followed by fractionated total-body irradiation. Early results of the evaluation of this preparative regimen have been previously reported2-23 and included the first 12 patients enrolled in the current study.

METHODS

Between March 1981 and October 1986, 20 consecutively evaluated children (with no exclusions) less than 16 years of age who met the following criteria were treated with BMT: (a) diagnosis of ALL in second (18 patients) or third (two patients) complete remission (less than 5% blasts on bone marrow aspirate and biopsy specimen and no evidence of active extramedullary disease at the time of BMT) after a bone marrow relapse while receiving therapy or within 12 months of completion of maintenance chemotherapy and (b) availability of an HLA-A,-B,-C, and -DR genotypically identical and mixed lymphocyte culture nonreactive sibling as a bone marrow donor.

Four of the patients received all of their pre-BMT therapy at Rainbow Babies and Childrens Hospital, whereas 16 were referred from other institutions. All patients had ALL by standard morphological, cytochemical, and immunologic criteria. Most were initially treated and reinduced after relapse on Childrens Cancer Study Group protocols; the remainder were treated similarly on Pediatric Oncology Group or local institutional protocols. Eight of 20 children had received systemic cytosine arabinoside before BMT. Pertinent patient characteristics are shown in Table 1. One patient (unique patient no. [UPN] 9032) had an identical twin donor.

The preparative regimen for BMT was identical in all cases. Cytosine arabinoside was given as a one-hour intravenous infusion every 12 hours beginning on the morning of the ninth day before marrow infusion and was completed on the evening of the fourth day.

From the Departments of Pediatrics (Division of Oncology-Hematology), Pathology, and Radiology (Division of Radiation Therapy), Case Western Reserve University, Cleveland. Submitted May 28, 1987; accepted November 13, 1987. Supported in part by Grant CA-20320 from the National Cancer Institute. Presented in part at the American Society of Clinical Oncology Meeting, San Diego, May 1983, and at the American Society of Hematology Meeting, Miami Beach, December 1984.

Address reprint requests to Peter F. Coccia, MD, Department of Pediatrics, University of Nebraska Medical Center, 42nd and Dewey Ave, Omaha, NE 68105.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

© 1988 by Grune & Stratton, Inc.

before marrow infusion. Total-body irradiation was given over the subsequent three days as twice daily fractions at a dose of 200 cGy/treatment for a total dose of 1,200 cGy. The dose rate was 15 to 20 cGy/min measured midplane at the umbilicus using 6 to 15 meV photons generated by a linear accelerator and lateral opposed fields to a cumulative midplane dose of 1,200 cGy. Corrections were made to limit the off-axis dosage to 1,200 cGy, but no lung correction or shielding was used. On the day after the completion of irradiation (day 0) donors had bone marrow harvested from both posterior iliac crests under general anesthesia. A minimum of 10 mL/kg recipient body weight was collected, processed, and infused as previously established criteria.† Treatment of acute GVHD consisted of systemic corticosteroids in moderate or high doses as described by Sullivan et al. Treatment of chronic GVHD was as recommended by Sullivan et al. Statistical methods used included Kaplan-Meier product-limit estimates for life table analyses, Student’s t test, and the chi-square test for the analysis of variance. All results are analyzed to October 1, 1987.

All patients had indwelling central venous catheters in place and were nursed in standard single-occupant rooms; reverse isolation with gown, mask, and hand washing was used from initiation of the preparative regimen until the absolute granulocyte count was above 500/μL for three consecutive days. Fourteen patients received glucocorticoid eye drops every six to eight hours during cytosine arabinoside administration. All patients received parenteral hyperalimentation to supply caloric requirements. Platelet transfusions were given to maintain the platelet count above 20,000/μL, and packed RBC were given to maintain the hematocrit value above 30%. All blood components were irradiated (3,000 cGy) before transfusion. Appropriate broad-spectrum antibiotics were given to patients with presumed infection. Patients received trimethoprim-sulfamethoxazole from day –9 before BMT to at least day +100 after BMT.

Toxicity of the preparative regimen and CNS toxicity were evaluated as previously described. Engraftment was documented in nine patients by cytogenetic analysis and in three patients by using other blood genetic markers. The time to engraftment was monitored by evaluation of peripheral blood counts and bone marrow examinations in all patients.

Graft-v-host disease (GVHD) prophylaxis was not given to nine children.† Intravenous methotrexate alone at 10 mg/m² intravenously (on days 1, 3, 6, 11, 18, and 25 after BMT) was used in two children, prednisolone at 2 mg/kg/d in three divided doses for the 14 days after BMT followed by a seven-day taper in two children and methotrexate and prednisolone at the same doses in seven children. Diagnosis and grading of acute and chronic GVHD were by previously established criteria. Treatment of acute GVHD consisted of systemic corticosteroids in moderate or high doses as described by Woods et al. Treatment of chronic GVHD was as recommended by Sullivan et al.

### RESULTS

The current status of the transplant patients is detailed in Table 1. Twelve of 20 are alive in continuous complete remission from 12+ to 79+ months after BMT, with eight patients beyond 48 months post-BMT without an adverse event. Six patients have died of complications: UPN 9037 on day 21 of idiopathic diffuse interstitial pneumonitis without evidence of GVHD; UPN 9039 on day 66 of multifocal osteomyelitis and gram-positive sepsis after resolution of...
grade II acute GVHD; UPN 9042 on day 72 of severe skin and gastrointestinal GVHD with preterminal gram-negative sepsis; UPN 9081 on day 267 of multiple complications of acute and chronic GVHD, opportunistic infections, and bleeding; UPN 9089 on day 195 of idiopathic diffuse interstitial pneumonitis while receiving low-dose corticosteroids for chronic skin GVHD; and UPN 9091 on day 111 of aspergillosis while receiving corticosteroids for treatment of acute GVHD.

Two patients have developed recurrent leukemia after BMT. UPN 9067, a 3-year-old male who received a transplant in third bone marrow and CNS remission, developed recurrent leukemia in the bone marrow 30 months post-BMT. His leukemia was successfully reinduced into remission; however, he subsequently relapsed and died 36 months post-BMT. UPN 9083, a 5-year-old female who received a transplant in second remission, relapsed in the bone marrow 15 months after BMT and died 19 months after BMT.

Figure 1 demonstrates a Kaplan-Meier life table analysis for the entire group of 20 children who received BMT. The actuarial survival rate is 58%, and the bone marrow relapse rate is 17%. Analysis of the 18 patients who received BMT while in second complete remission revealed an actuarial survival rate of 59% and a marrow relapse rate of 10%.

The acute toxicity of the preparative regimen is shown in Table 2. No unusual, unexpected, or fatal toxicities occurred. The only patient (UPN 9081) developing cerebellar dysfunction had received 2,400 cGy cranial irradiation for prophylaxis against CNS leukemia 1 month after the diagnosis of ALL. This patient developed a relapse in the CNS 24 months from diagnosis and received an additional 1,200 cGy cranial irradiation and aggressive multiagent intrathecal chemotherapy before BMT. He developed mild cerebellar dysfunction on the last day of cytosine arabinoside administration and completely recovered within 2 weeks. All other preparation-related toxicities resolved within 1 week of completing total-body irradiation. Conjunctivitis, eye pain, and photophobia were less frequent and less severe in patients prophylactically treated with glucocorticoid eye drops during cytosine arabinoside administration. Hepatic dysfunction was limited to only minimal elevations of transaminase levels of short duration after cytosine arabinoside administration.

All patients showed engraftment promptly after bone marrow infusion as measured by the recovery of the peripheral blood counts. The marrow dose infused ranged from 2.1 to $8.4 \times 10^8$ nucleated cells/kg recipient weight (median, 3.8). There was a trend toward more rapid engraftment in patients who received a higher marrow nucleated cell dose; however, this difference was not significant. As shown in Table 3, recovery of the total WBC count to 1,000 cells/µL was significantly related to the administration of methotrexate after BMT. In patients who did not receive methotrexate, the total WBC count, but not the absolute neutrophil count, recovered more rapidly.

No significant difference in the incidence or severity of acute or chronic GVHD was seen whether or not GVHD prophylaxis was administered. In the no-prophylaxis group, eight of nine were evaluable for the development of acute GVHD (the identical twin is nonevaluable). Four of eight had no or minimal skin GVHD (grades 0 to 1), three had moderate GVHD (grade 2), and one had severe GVHD (grades 3 to 4). In the GVHD prophylaxis group, all 11 patients are evaluable, and four of 11 had grade 0 to 1, four had grade 2, and three had grades 3 to 4 acute GVHD. Fifteen allogeneic patients were alive 4 months after BMT and were evaluable for chronic GVHD. One of seven in the no-prophylaxis group and three of eight in the prophylaxis group developed chronic GVHD. Both relapses occurred in patients without significant acute or chronic GVHD.

The quality of life of the 12 patients who remain in continuous complete remission is, in general, excellent. All 12 children are attending school, and most are functioning normally with the following exceptions: (a) UPN 9048 has mild chronic scleroderma-like GVHD, which is resolving; (b)

### Table 3. Influence of Post-BMT Methotrexate on Time to Engraftment

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No Methotrexate</th>
<th>Methotrexate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td>Marrow dose</td>
<td>$3.7 \pm 1.1$</td>
<td>$4.4 \pm 1.9$</td>
</tr>
<tr>
<td>(nucleated cells x $10^8$/kg)</td>
<td>(NS)</td>
<td></td>
</tr>
<tr>
<td>Day to</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WBC $&gt;1,000$/µL</td>
<td>$12.8 \pm 3.4$</td>
<td>$21.2 \pm 4.6$</td>
</tr>
<tr>
<td>ANC $&gt;500$/µL</td>
<td>$17.2 \pm 3.9$</td>
<td>$21.5 \pm 4.5$</td>
</tr>
</tbody>
</table>

Abbreviation: ANC, absolute neutrophil count.
DISCUSSION

Allogeneic BMT is an extremely promising approach for the treatment of recurrent ALL in remission, especially when the patient has an HLA genotypically matched sibling donor.9,20,32,33

In three recently reported studies with a long follow-up,10,12,15 a total of 85 children and young adults received transplants in second, third, or fourth complete remission after preparation for BMT with high-dose cyclophosphamide followed by total-body irradiation. Twenty-five (29%) are in continuous complete remission since BMT and are probably cured; however, 41 (48%) have developed recurrent leukemia after BMT. In a recent update of the Seattle results of allogeneic BMT in 57 children with ALL in second complete remission and with HLA-identical sibling donors, 40% are alive in continuous complete remission, and 42% have relapsed.18 Possible reasons for the high rate of recurrent leukemia after BMT in these studies include the lack of an adequate antileukemic ablative regimen and/or the lack of an adequate graft-vs-leukemia effect in those patients who relapse.18,34-37 We have addressed the first possibility in the current study.

Although most pretransplant cytoreductive regimens have used only high-dose cyclophosphamide and total-body irradiation, there have been attempts to add additional chemotherapeutic agents to the regimen, which has resulted in a high mortality from drug toxicity that outweighed the potential long-term antileukemic benefit.32,33 We therefore elected to replace cyclophosphamide with cytosine arabinoside because of the excellent antileukemic effect of high-dose cytosine arabinoside alone21-38 or with other chemotherapeutic agents.39-40 Further, systemic high-dose cytosine arabinoside is an effective treatment for meningeal leukemia,41 an important consideration for childhood ALL patients undergoing BMT. In the present study, the regimen of high-dose cytosine arabinoside and total-body irradiation was well tolerated and had a substantial antileukemic effect. The toxicity of the preparative regimen was similar to that seen with high-dose cytosine arabinoside alone.21 The omission of methotrexate for GVHD prophylaxis after BMT resulted in a more rapid recovery of the peripheral WBC count (Table 3), an observation previously reported by both our group27 and by Deeg et al.29 It appeared that the 11 patients who did not receive methotrexate had less mucositis after BMT than the nine who did but that other toxicities were similar. With the regimen only two bone marrow recurrences were seen, and no extramedullary relapse in either the CNS or testes has occurred. Neither patient who relapsed had had prior exposure to cytosine arabinoside, but eight of 18 of the remaining patients did.

Weidorf et al.19 recently reported that patients with ALL in remission who develop acute GVHD are significantly less likely to relapse after BMT. They speculated the reason to be the aforementioned graft-vs-leukemia effect36,37 or the antileukemic efficacy of corticosteroids used to treat the acute GVHD. In our study, patients experienced a relatively high incidence of acute GVHD, possibly due to the nonaggressive approach to GVHD prophylaxis. Eleven of 19 (58%) allogeneic patients developed grades 2 to 4 acute GVHD. Excluding the six children who died within 9 months of BMT of nonleukemic causes, two of eight patients without GVHD have relapsed in contrast to none of six with acute GVHD, which is not significantly different. Sanders et al18 did not find an association between the absence of GVHD and leukemic relapse.

Many authors have addressed the issue of adverse factors that may predict for relapse after BMT for ALL in remission.1,11-14,16,18,19,33 Some of these factors include an elevated WBC count at the initial diagnosis of leukemia, extramedullary leukemia before BMT, a short duration of first remission, and an absence of acute and/or chronic GVHD. In the current study, eight children are alive in remission for more than 48 months after BMT. Three had WBC counts at diagnosis of over 50,000/μL, three had extramedullary disease before BMT, five had initial remissions of less than 24 months, five had no or minimal (grade 1) acute GVHD, and seven had no chronic GVHD. The proportion of high-risk factors in these eight children was not different from the overall group nor different from the percentage of patients with high-risk features in the other reported studies.

In an encouraging report, Dinsmore et al.14 have reported that 14 of 22 patients with ALL in second remission who were treated with hyperfractionated total-body irradiation with partial lung shielding followed by high-dose cyclophosphamide are in continuous complete remission 12 to 34 (median, 24) months after BMT and that only two patients have developed recurrent leukemia. In the present study, 11 of 18 patients with ALL in second remission who were treated with high-dose cytosine arabinoside followed by total-body irradiation are in continuous complete remission 12 to 79 (median, 52) months, and only one patient has developed recurrent leukemia. Both preparative regimens appear extremely promising for significantly reducing the incidence of recurrent leukemia after BMT and deserve further evaluation both in patients with matched sibling donors and in the 60% to 70% of patients without matched donors who are candidates for purged autologous BMT.20,42,43 or mismatched allogeneic BMT.44,45

ACKNOWLEDGMENT

We are indebted to Samuel Gross, MD, and John R. Graham-Pole, MD, for their involvement in the initial development of these studies; to the many referring physicians; and to the house officers, nurses, technologists, and other support staff at Rainbow Babies and Childrens Hospital and The University Hospitals of Cleveland whose many contributions made these studies possible.
REFERENCES


BONE MARROW TRANSPLANTATION FOR ALL


High-dose cytosine arabinoside and fractionated total-body irradiation: an improved preparative regimen for bone marrow transplantation of children with acute lymphoblastic leukemia in remission

PF Coccia, SE Strandjord, PI Warkentin, NK Cheung, EM Gordon, LJ Novak, DC Shina and RH Herzig