

## TREATMENT OF HIGH-RISK ACUTE LEUKEMIA WITH T-CELL-DEPLETED STEM CELLS FROM RELATED DONORS WITH ONE FULLY MISMATCHED HLA HAPLOTYPE

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### ABSTRACT

**Background** In this study we tried to achieve successful transplantation in patients with acute leukemia with the use of hematopoietic stem cells from donors who shared only one HLA haplotype with the recipient (a "full-haplotype mismatch"). To prevent graft failure, large doses of T-cell-depleted hematopoietic stem cells were transplanted after a conditioning regimen of enhanced myeloablation and immunosuppression was administered to the recipient.

**Methods** Forty-three patients with high-risk acute leukemia who were scheduled for transplantation received total-body irradiation, thiotepea, fludarabine, and antithymocyte globulin. The graft consisted of peripheral-blood progenitor cells that had been mobilized in the donor with recombinant granulocyte colony-stimulating factor and also, in 28 cases, bone marrow. Bone marrow from the donor was depleted of T lymphocytes by processing with soybean agglutinin and E-rosetting. T-cell depletion of peripheral-blood mononuclear cells was achieved by E-rosetting followed by positive selection of CD34+ cells. No post-transplantation prophylaxis against graft-versus-host disease (GVHD) was administered.

**Results** In all the patients, full donor-type engraftment was achieved. In none of the patients who could be evaluated did acute or chronic GVHD develop. Regimen-related toxicity was minimal. Eleven of the 23 patients with acute lymphoblastic leukemia had a relapse, as did 2 of the 20 patients with acute myeloid leukemia. Transplantation-related mortality was 40 percent. After a median follow-up of 18 months (range, 8 to 30), 12 of the 43 patients were alive and free of disease. All surviving patients had a good quality of life.

**Conclusions** The main limitations of transplantation of bone marrow from donors who are matched with the recipient for only one HLA haplotype — GVHD and graft failure — can be overcome. Since most patients have a relative with one haplotype mismatch, advances in this method will increase the availability of hematopoietic-cell transplantation as curative therapy for acute leukemia. (N Engl J Med 1998;339:1186-93.)

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**T**RANSPLANTATION of bone marrow from an HLA-matched related<sup>1</sup> or unrelated<sup>2-5</sup> donor is a potentially curative treatment for patients with acute leukemia. Despite the existence of a worldwide registry that includes more than 3 million HLA-typed volunteers, about 40 percent of patients do not have an HLA-compatible donor. The HLA genes are tightly linked and are inherited in a genetic unit called a haplotype. Haplotypes can be identified by testing for alleles at three loci: HLA-A, HLA-B, and HLA-DR. A child inherits one haplotype from each parent. For this reason, two siblings have a 25 percent chance of inheriting the same two parental haplotypes and thus of being HLA identical.

For patients for whom an HLA-identical donor is not found, there is an excellent chance of identifying a family member who shares one HLA haplotype with the patient but whose second HLA haplotype is different. This situation is called an HLA full-haplotype mismatch, or an HLA-haploidentical mismatch at three loci.

During the 1980s, transplantation of bone marrow from family donors who were not fully histocompatible with the recipients was unsuccessful because of graft failure and severe graft-versus-host disease (GVHD), at times affecting as many as 90 percent of recipients.<sup>6,7</sup> Thorough depletion of T cells from the donor's bone marrow succeeded in preventing GVHD in children with severe combined immunodeficiency disease,<sup>8,9</sup> but the results of this procedure were disappointing in patients with leukemia, because the benefit of preventing GVHD was offset by graft failure.<sup>10-13</sup> Resistance to engraftment appears to be mediated by host-derived cytotoxic T-lymphocyte precursors that survive supralethal condition-

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ing.<sup>10,11,14</sup> Experiments in mice have shown that histocompatibility barriers can be overcome by infusing high doses of T-cell-depleted marrow cells,<sup>15,16</sup> even in sublethally irradiated animals, in which numerous T lymphocytes have survived the radiation.<sup>17</sup> Engraftment is also improved by the addition of selective anti-T-cell agents<sup>18</sup> or myeloablative drugs such as thiotepe<sup>19</sup> to total-body irradiation.

Applying these results in animals to patients, we observed a high rate of engraftment in recipients of T-cell-depleted mismatched transplants who were also given high numbers of hematopoietic stem cells from bone marrow and peripheral blood.<sup>20</sup> To enhance the conditioning regimen of immunosuppression and myeloablation, we added antithymocyte globulin and thiotepe to the combination of total-body irradiation and cyclophosphamide. Although no immunosuppressive treatment was given after transplantation, acute GVHD of grade II, III, or IV occurred in only 18 percent of the patients.

In the present study, to eliminate GVHD, the number of T lymphocytes in the transplants from related donors who had one mismatched haplotype was reduced to a mean of approximately  $3 \times 10^4$  cells per kilogram of the recipient's body weight, equivalent to 1/10 the number administered in our previous study. In a murine model, total-body irradiation plus fludarabine caused a degree of immunosuppression similar to that caused by total-body irradiation plus cyclophosphamide.<sup>21</sup> Therefore, in the effort to lower extramedullary toxicity, we substituted fludarabine for cyclophosphamide in the conditioning regimen.

## METHODS

### Patients

Between October 1995 and August 1997, 43 patients with high-risk acute myeloid leukemia (AML) or acute lymphoblastic leukemia (ALL) received hematopoietic stem-cell transplants from family donors with only one matched HLA haplotype at the Bone Marrow Transplant Unit, University of Perugia, Perugia, Italy (Table 1). The study patients were selected because they had no suitable unrelated donor or because their need for transplantation was urgent. Written informed consent was obtained from the patients or their guardians and from the donors.

### HLA Typing

Family members were assessed for HLA compatibility by serologic typing. In 22 cases the donor was a parent, in 18 cases a sibling, and in 3 cases a child of the recipient. All pairs of donors and recipients were identical for one HLA haplotype (haploidentical) and incompatible at three loci (HLA-A, B, and DR) of the unshared haplotype. The donors and the recipients were heterozygous for HLA-A, B, and DR — that is, all the donor-recipient pairs were mismatched in both directions: host versus graft and graft versus host.<sup>6</sup>

### Conditioning Regimen

Pretransplantation treatment included total-body irradiation and administration of thiotepe, antithymocyte globulin, and fludarabine (Fig. 1). Total-body irradiation was given on day -10 (10 days before transplantation) in a single fraction at an instantaneous dose rate of 16 cGy per minute. The dose rate was calculated at

**TABLE 1. PATIENT CHARACTERISTICS ACCORDING TO STATUS OF LEUKEMIA AT TRANSPLANTATION.**

STATUS OF DISEASE	NO. OF PATIENTS (N=43)	MEDIAN AGE	MEDIAN TIME SINCE DIAGNOSIS (RANGE)
		(RANGE)	(RANGE)
		yr	mo
Acute lymphoblastic leukemia	23	17.5 (4-40)	17.5 (4-66)
First complete remission	4*		
Second or third complete remission	11		
Advanced relapse	8		
Acute myeloid leukemia	20	30 (7-53)	10 (4-55)
First complete remission	3†		
Second or third complete remission	11		
Advanced relapse	6		

\*For four patients with acute lymphoblastic leukemia in first remission, the prognosis was poor: in one first-line induction therapy had failed, two had the t(9;22) translocation, and one had the T phenotype with bulky disease.

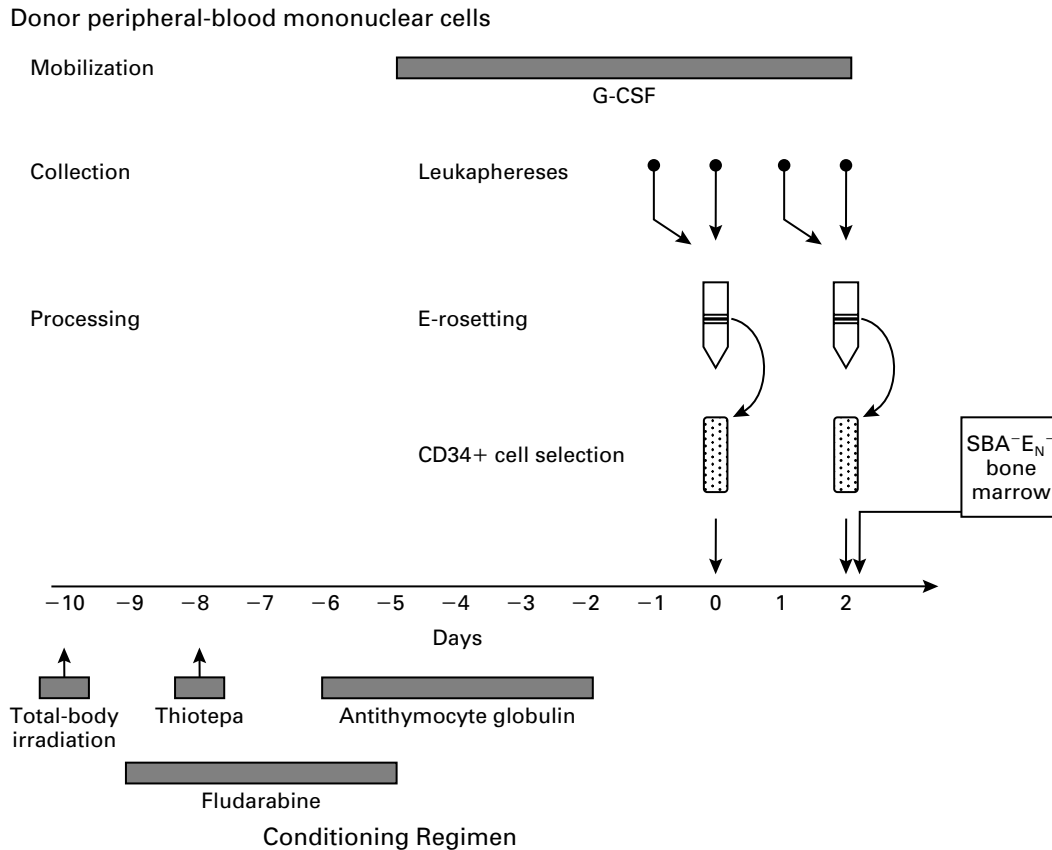
†For three patients with acute myeloid leukemia in first remission, the prognosis was poor: two who had myelodysplasia and one in whom first-line induction therapy had failed.

five body points (cranium, mediastinum, umbilicus, left lung, and pubic symphysis) on a coronal plane at half-body thickness. The total dose of 8 Gy was delivered in 90 minutes at a mean dose rate of 9 cGy per minute. The lungs were shielded so that the first 10 patients received 6 Gy and the others 4 Gy to reduce toxicity to the lungs (see below). The four children less than seven years old received 12 Gy (9 Gy to the lungs), fractionated over a three-day period. Thiotepe (13 mg per kilogram of body weight in the first 11 patients and 10 mg per kilogram in the others) was administered in two doses on day -8. On days -6 to -2, antithymocyte globulin (Fresenius, Oberursel, Germany) was infused at a dosage of 5 mg per kilogram daily over an eight-hour period. Fludarabine was given at a dose of 240 mg per square meter of body-surface area over a six-day period in the first 11 patients and at 200 mg per square meter over a five-day period in the others. The last 30 patients also received cyclosporine (1 mg per kilogram) daily as a continuous intravenous infusion from days -10 to -3.

### Stem-Cell Processing and Transplantation

Donor bone marrow was depleted of T lymphocytes by soybean agglutination and two rounds of E-rosetting<sup>22</sup> and was then cryopreserved. Recombinant human granulocyte colony-stimulating factor (G-CSF, filgrastim) was administered to donors subcutaneously at a dosage of 16  $\mu$ g per kilogram daily for 7 days, beginning a median of 12 days after bone marrow harvesting (Fig. 1). Starting on day 4 of G-CSF administration, peripheral-blood mononuclear cells were collected by leukapheresis for four days with a discontinuous blood-cell separator (Haemonetics, Braintree, Mass.). The product of the first leukapheresis was stored at 4°C overnight and pooled the next day with the product of the second. T-cell depletion was achieved by E-rosetting with sheep erythrocytes, followed by selection of CD34+ cells with use of an immunoadsorption biotin-avidin column<sup>23,24</sup> (Ceptrate SC, Cell Pro, Bothell, Wash.). The entire procedure took nine hours. The pooled cells were infused immediately (day 0). The products of the third and fourth leukaphereses were pooled, processed, and infused, as described above, 48 hours later (day 2).

For 15 patients, the transplants consisted only of peripheral-blood progenitor cells, because if bone marrow had been added the mean number of T lymphocytes would have exceeded the



**Figure 1.** Pretransplantation Conditioning Regimen, Donor Hematopoietic Stem-Cell Processing, and Times of Infusions. G-CSF denotes recombinant human granulocyte colony-stimulating factor, and SBA<sup>-</sup>E<sub>N</sub><sup>-</sup> denotes lectin soybean agglutinin– and E-rosette–separated.

projected dose of  $3 \times 10^4$  cells per kilogram. In 28 patients the T-cell–depleted bone marrow was given after infusion of the peripheral-blood progenitor cells.

The inoculum was evaluated by measuring the quantity of granulocyte–macrophage colony-forming units (CFU-GM), CD3+ cells, and CD34+ cells in the bone marrow harvest, in the leukapheresis products after purification, and at each phase of cell manipulation, as described elsewhere.<sup>25,26</sup>

No immunosuppressive treatment was given after transplantation for prophylaxis against GVHD. All but three recipients received G-CSF (5  $\mu$ g per kilogram per day) for a median of eight days starting on the fourth day after transplantation. Antifungal prophylaxis consisted of amphotericin B (0.5 mg per kilogram) or liposomal amphotericin B (AmBisome, NeXstar, Boulder, Colo.; 1 mg per kilogram) or both, from day 0 to day 30. Prophylaxis against cytomegalovirus infection included ganciclovir (5 mg per kilogram per day) from day 5 to day 20. Maintenance treatment, given two or three times weekly, continued until day 210.

#### Assessment of Engraftment and Immunologic Studies

Engraftment was assessed according to published criteria.<sup>27</sup> Chimerism was detected by karyotyping peripheral-blood lymphocytes and by polymerase-chain-reaction amplification of a panel of variable-number tandem-repeat regions with different DNA polymorphism patterns.<sup>28–30</sup> Subtypes of lymphoid cells present after transplantation were identified by two-color immunofluorescence and flow cytometry. The frequencies of T cells responding

to polyclonal activators were evaluated with a sensitive limiting-dilution assay in the presence of phytohemagglutinin and feeder cells.<sup>31</sup>

The diagnosis and the degree of acute and chronic GVHD were assessed according to consensus criteria.<sup>32</sup>

#### Statistical Analysis

Patients were considered able to be evaluated for acute GVHD at the time of engraftment and for chronic GVHD beginning 100 days after engraftment. Rates of relapse and event-free survival were evaluated by Kaplan–Meier analysis. The log-rank test was used for comparisons.

The reported outcomes are as of April 30, 1998.

## RESULTS

#### Recipients and Donors

Table 1 summarizes the characteristics of the patients. Their median age was 22 years (range, 4 to 53); 11 patients were less than 16 years old, and 5 were older than 40. The median age of the donors was 41 years (range, 18 to 59). Only one donor–recipient pair was negative for cytomegalovirus. All seven patients undergoing transplantation during their first complete remission (three patients with AML and

four with ALL) had poor prognostic features. Most of the 22 patients in their second or third remission had a high risk of leukemic relapse or transplantation-related death; 7 had had a relapse after autologous transplantation. Fourteen patients (six with AML and eight with ALL) were in chemoresistant relapse at the time of transplantation. Anti-donor-lymphocyte antibodies were detected in one patient who underwent plasmapheresis and successful engraftment.

Harvesting of bone marrow followed by mobilization of peripheral-blood progenitor cells was well tolerated by the donors. Moderate bone pain occurred in 43 percent and fever in 13 percent. It was not necessary to decrease the dose of G-CSF because of side effects. Two donors required central venous access.

**Bone Marrow Fractionation**

The final product of bone marrow fractionation contained  $3.3 \times 10^4$  to  $39.1 \times 10^4$  CFU-GM per kilogram of the recipient's body weight (mean  $\pm$ SD,  $18.2 \times 10^4 \pm 10.9 \times 10^4$ ) and  $0.2 \times 10^6$  to  $5.4 \times 10^6$  CD34+ cells per kilogram (mean,  $1.2 \times 10^6 \pm 1.1 \times 10^6$ ). T-cell subgroup analysis demonstrated a  $3.5 \log_{10}$  depletion of CD3+ cells (the difference between the starting number of cells and the final number in the inoculum, expressed logarithmically).

**Mobilization and Processing of Peripheral-Blood Progenitor Cells**

Table 2 lists the number of mononuclear cells, CFU-GM, CD34+ cells, and CD3+ cells collected in four leukaphereses and recovered at each stage of the purification procedure. The final product con-

tained approximately 74 percent of the starting population of CD34+ cells, with a purity of 70 percent. A T-cell depletion of  $4.3 \log_{10}$  was achieved.

In 15 patients, the final inoculum (containing only peripheral-blood progenitor cells) contained  $7.4 \times 10^6$  to  $46.9 \times 10^6$  mononuclear cells per kilogram (mean,  $19.9 \times 10^6 \pm 12.0 \times 10^6$ ),  $3.8 \times 10^6$  to  $33.7 \times 10^6$  CD34+ cells per kilogram (mean,  $14.0 \times 10^6 \pm 8.7 \times 10^6$ ), and  $0.8 \times 10^4$  to  $7.5 \times 10^4$  CD3+ cells per kilogram (mean,  $2.7 \times 10^4 \pm 2.0 \times 10^4$ ). In 28, the final inoculum (containing bone marrow and peripheral-blood progenitor cells) contained  $16.5 \times 10^6$  to  $112.1 \times 10^6$  mononuclear cells per kilogram (mean,  $39.8 \times 10^6 \pm 21.7 \times 10^6$ ),  $3.1 \times 10^6$  to  $25.0 \times 10^6$  CD34+ cells per kilogram (mean,  $10.6 \times 10^6 \pm 5.4 \times 10^6$ ), and 0 to  $21.5 \times 10^4$  CD3+ cells per kilogram (mean,  $3.5 \times 10^4 \pm 4.2 \times 10^4$ ).

**Engraftment**

In 41 of the 43 patients, the primary transplantation resulted in durable engraftment, with achievement of neutrophil counts higher than 1000 per cubic millimeter at a median of 11 days (range, 8 to 19). Platelet counts of 50,000 per cubic millimeter were reached at a median of 29 days (range, 13 to 124). Two patients (both with ALL) had primary graft failure between 16 and 22 days after transplantation. One patient (a seven-year-old child) had received bone marrow and peripheral-blood cells containing  $25.0 \times 10^6$  CD34+ cells per kilogram and  $21.5 \times 10^4$  CD3+ cells per kilogram. In both cases, rejection was reversed by the transplantation of T-cell-depleted peripheral-blood progenitor cells from a different family member after further immunosuppression with

**TABLE 2. CELL CONTENTS OF UNFRACTIONATED LEUKAPHERESIS PRODUCTS AND AT EACH STAGE OF PURIFICATION.\***

CELLS	LEUKAPHERESIS PRODUCTS	AFTER E-ROSETTING	AFTER POSITIVE SELECTION OF CD34+ CELLS
Mononuclear cells ( $\times 10^{-6}$ )			
Mean	161,380 $\pm$ 42,440	73,390 $\pm$ 22,750	856.6 $\pm$ 317.2
Range	82,000–247,000	29,600–118,600	289.3–1650
Granulocyte-macrophage colony-forming units ( $\times 10^{-6}$ )†			
Mean	135.5 $\pm$ 100.6	—	51.7 $\pm$ 56.0
Range	14.1–543	—	0.08–237.4
CD34+ cells ( $\times 10^{-6}$ )			
Mean	873.8 $\pm$ 380.2	786.0 $\pm$ 338.0	609.1 $\pm$ 173.4
Range	213.5–1956	184.9–1605.1	135.4–1352.5
CD3+ cells ( $\times 10^{-6}$ )			
Mean	23,550 $\pm$ 9970	209.9 $\pm$ 155.6	0.8 $\pm$ 1
Range	6100–50,600	29.6–688.8	0–4.6

\*Plus-minus values are means  $\pm$ SD. Purification was carried out by T-cell depletion with sheep erythrocytes and CD34+ immunoselection.

†Granulocyte-macrophage colony-forming units were not counted after E-rosetting.

cyclophosphamide (80 mg per kilogram over a two-day period) and antithymocyte globulin (25 mg per kilogram over a five-day period). Thus, sustained engraftment was achieved in all patients. No late rejection of a graft has been observed. Analysis of DNA polymorphism documented full donor-type chimerism in both the peripheral blood and the bone marrow of all the patients (data not shown).

#### GVHD

In none of the patients who could be evaluated did either acute or chronic GVHD develop. To improve immunologic reconstitution, two patients received donor T lymphocytes ( $3 \times 10^4$  per kilogram) three months after transplantation. Despite the low number of T cells infused, severe acute GVHD developed in one patient, and the patient died.

#### Immunologic Reconstitution

Peripheral-blood counts of natural killer cells returned to normal within two to four weeks after transplantation. CD4+ T-cell counts were below 100 and 200 cells per cubic millimeter for as long as 10 and 16 months, respectively. The frequencies of T cells responding to polyclonal activators in a sensitive limiting-dilution assay were approximately 1 in 100 cells in the first month and 1 in 10 cells 10 months after transplantation (with a control frequency of approximately 1 in 2).

#### Death from Causes Other Than Leukemia

Mortality from causes other than leukemic relapse was 40 percent. Sixteen of the 29 patients in remission (7 in a first remission and 22 in a second or third remission) and 1 of the 14 in relapse at the time of transplantation died. Infection was the most frequent cause of death (Table 3). Five deaths were due to systemic bacterial infections (with pseudomonas in three cases and staphylococcus in two), five to fungal infections (aspergillus in three and candida in two), and one to cytomegalovirus-associated pneumonia. High-grade B-cell lymphoproliferative disease occurred in two patients. The other four patients died of toxic causes. One other patient with AML, who underwent transplantation in relapse, died of acute GVHD induced by a donor-lymphocyte infusion ( $3 \times 10^4$  per kilogram) administered three months after transplantation in order to strengthen the antileukemic effect. No difference in transplantation-related mortality was observed between patients with AML and those with ALL.

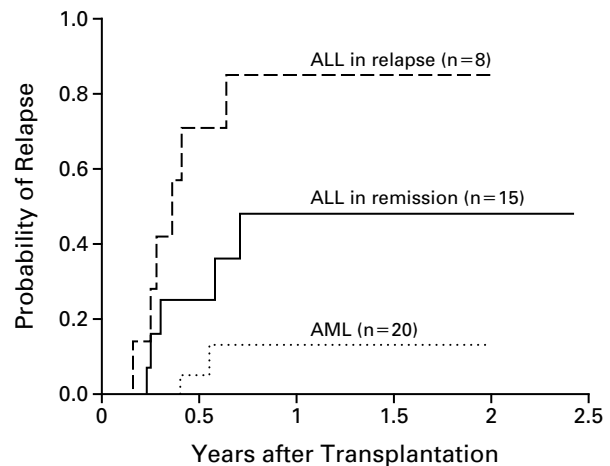
#### Leukemic Relapse

Two of the 20 patients with AML and 11 of the 23 patients with ALL had a relapse. Of these 13 relapses, 7 occurred in patients who were in relapse at the time of transplantation, 4 in patients who were in a second remission, and 2 in patients who were in

a third remission. The probability of relapse after transplantation was  $0.13 \pm 0.08$  for patients with AML and  $0.63$  for patients with ALL ( $P=0.004$ ). The risk of leukemic relapse after transplantation reached borderline significance ( $P=0.056$ ) in patients with ALL who were in remission as compared with those who had had a relapse (Fig. 2).

**TABLE 3. DEATHS FROM CAUSES OTHER THAN LEUKEMIA.**

CAUSE OF DEATH	NO. OF PATIENTS (N=17)
Infection	11
Bacterial	5
Pseudomonas	3
Staphylococcus	2
Fungal	5
Aspergillus	3
Candida	2
Viral	1
Cytomegalovirus	1
B-cell lymphoproliferative disease	2
Other	4
Embolism	1
Renal failure	1
Acute respiratory distress syndrome	2



**Figure 2.** Probability of Relapse.

After two years, the mean ( $\pm$ SD) probability of relapse for the 8 patients with ALL who were in relapse at the time of transplantation was  $0.85 \pm 0.13$ , and that for the 15 patients with ALL who were in remission (4 in first complete remission and 11 in second or third complete remission) at the time of transplantation was  $0.44 \pm 0.17$  ( $P=0.06$ ). The probability of relapse for the 20 patients with AML was  $0.13 \pm 0.08$  during the 1.7-year observation period.

### Survival

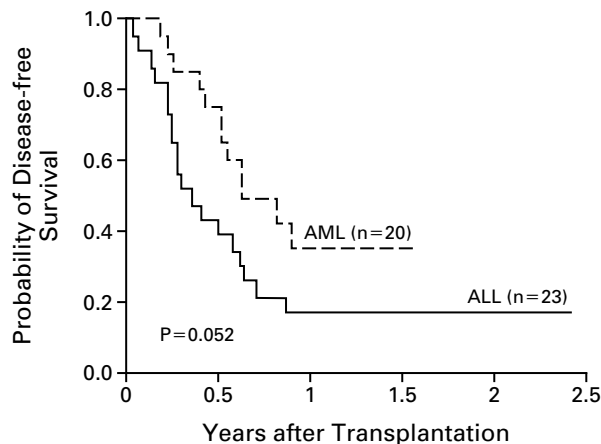
As of April 30, 1998, 12 of the 43 patients (28 percent) were alive and free of disease, with a median follow-up of 18 months (range, 8 to 30). Nine patients had been followed up for more than one year. All surviving patients had a Karnofsky score of 100. The probability of disease-free survival differed between patients with AML and those with ALL ( $P=0.052$ ): it was  $0.36\pm 0.11$  at 1.5 years for the 20 patients with AML and  $0.17\pm 0.07$  at 2.5 years for the 23 patients with ALL (Fig. 3). Of the seven patients at high risk who underwent transplantation during a first remission, three were alive (two with AML and one with ALL). Of the other four, one died of high-grade B-cell lymphoma, two of infections, and one of renal failure.

### DISCUSSION

Our study confirms and extends our previous finding that transplantation of bone marrow from related donors who share only one HLA haplotype with the recipient, preceded by intensive chemoradiotherapy and consisting of the infusion of large numbers of T-cell-depleted hematopoietic stem cells, results in a rate of engraftment<sup>20,33</sup> that overlaps with the rate seen with HLA-matched transplants from unrelated donors.<sup>27</sup> The rapid engraftment seen in the present series was similar to that observed with transplantation of HLA-identical allogeneic peripheral-blood CD34+ cells.<sup>34</sup> No late graft failures occurred, and full donor-type chimerism was achieved in all cases.

Fifteen patients received transplants consisting only of CD34+ cells that had been positively selected from peripheral-blood mononuclear-cell collections. Although the inoculum had been depleted of cell subpopulations that can induce specific tolerance toward donor-type antigens in animals,<sup>35-38</sup> a high rate of engraftment was maintained. This finding provides strong evidence that supplementing peripheral-blood progenitor cells with bone marrow in transplantations from mismatched donors is unnecessary. In addition, it is possible that infusing large numbers of purified CD34+ cells enhances engraftment. A recent study showed that human CD34+ cells were capable of reducing the frequencies of cytotoxic T-lymphocyte precursors directed against the histocompatibility antigens of the infused cells, but not against third-party cells.<sup>39</sup> Likewise, purified early hematopoietic progenitors with the Sca-1+Lin-phenotype successfully overcame the residual immunity of the host in lethally and sublethally irradiated mice.<sup>40,41</sup>

A striking finding was that there was no GVHD in these patients with acute leukemia, most of whom were adults. Low rates of GVHD have been reported in patients with severe combined immunodeficiency who received haploidentical transplants, in



**Figure 3.** Probability of Disease-free Survival in Patients with ALL or AML.

The mean ( $\pm$ SD) probability of disease-free survival was  $0.36\pm 0.11$  for the 20 patients with AML at 1.5 years. For the 23 patients with ALL, it was  $0.17\pm 0.07$  at 2.5 years.

whom a mean threshold dose of  $4\times 10^4$  T cells per kilogram was identified.<sup>42</sup> Ten of our patients who could be evaluated received  $4\times 10^4$  to  $8\times 10^4$  T lymphocytes per kilogram, which was slightly above this threshold, but GVHD did not develop. The antithymocyte globulin, with a plasma half-life of six days, that was administered during conditioning might have helped prevent GVHD by a cytotoxic effect against donor T lymphocytes.

Another aim of this study was to test a conditioning regimen that would facilitate engraftment of an extensively T-cell-depleted mismatched transplant yet not have excessive nonhematologic toxicity, which has been a considerable problem in previous clinical trials.<sup>43-45</sup> Substituting fludarabine for cyclophosphamide did not reduce the degree of immunosuppression (even at the lower dosage of fludarabine), as indicated by the lack of clonable T cells in peripheral blood at the end of the conditioning regimen and the excellent engraftment. Furthermore, nonhematologic toxicity was minimal: there was no veno-occlusive disease, and the incidence of severe mucositis was low. Because lethal pulmonary decompensation occurred in 2 of the first 10 patients, we reduced the doses of thiopeta and lung radiation. After these modifications, this complication did not occur again. For the last 30 patients, pretransplantation conditioning also included cyclosporine in an attempt to inhibit cytokine release after chemoradiotherapy, which has been observed *in vitro*<sup>46</sup> and *in vivo*.<sup>47</sup> The specific role of cyclosporine in lowering transplantation-related mortality in these patients would be extremely difficult to establish.

A serious problem in the transplantation of T-cell-depleted bone marrow is an increased risk of relapse

after transplantation.<sup>48,49</sup> In our study, relapses occurred in patients with ALL, particularly those in relapse at the time of transplantation. This result was not unexpected, and it mirrored the relapse rate in similar patients with ALL after transplantation with unmanipulated matched marrow.<sup>50</sup> To date, 2 of the 20 patients with AML have had a relapse, even though all were at high risk. Although follow-up is short, in our previous, pilot study the cumulative incidence of relapse after transplantation was  $28 \pm 17$  percent at 4.5 years (minimal follow-up, 3 years) in 12 patients with advanced AML. These results suggest that T-cell-depleted mismatched transplants trigger unique graft-versus-leukemia effector mechanisms. In many of the 43 donor-recipient pairs in the present study, the donor's natural killer cells failed to recognize the recipient's major-histocompatibility-complex allotypes and, consequently, were capable of killing hematopoietic targets from the recipient in vitro. After transplantation, we were surprised to find that the new repertoire of donor natural killer cells contained high frequencies of donor-versus-recipient alloreactive natural killer clones in absence of GVHD.<sup>50</sup> Moreover, a large percentage of reconstituting T cells displayed allotype recognition similar to that of natural killer cells.<sup>51,52</sup> Because AML but not ALL blasts were targets of donor-versus-recipient natural-killer-cell alloreactivity, it is possible that these natural killer cells are partly responsible for the low rate of relapse in patients with AML.

The high incidence of infectious complications was probably due to the delay in the reconstitution of T cells. Because of the extended period of susceptibility to infection, we administered antiviral and antifungal prophylaxis, but bacterial and fungal infections still occurred, resulting in 59 percent of all nonleukemic deaths. Although all but one donor-recipient pair were cytomegalovirus-positive, cytomegalovirus pneumonia was prevented in all patients except one.

The slow rate of T-cell recovery appears to be due to the low T-cell content of the graft. Immunologic reconstitution was much faster in our previous series of patients, who received 10 times as many T lymphocytes. Unfortunately, this approach resulted in an 18 percent incidence of severe GVHD.<sup>20</sup> Previous studies of T-cell-depleted transplants from unrelated donors have been complicated by a high rate of lymphoproliferative disorders related to Epstein-Barr virus.<sup>51,53</sup> In this study, the incidence of these disorders was relatively low (<5 percent), perhaps because of the lack of post-transplantation immunosuppressive therapy or the few B cells in the graft.

In conclusion, our overall results in terms of transplantation-related mortality and disease-free survival compare favorably with the outcome expected in patients who have the same stage of disease and who receive transplants from matched unrelated donors.<sup>27</sup> The high rate of engraftment, the elimination of

GVHD, and the minimal nonhematologic toxicity of the conditioning regimen demonstrate that the main obstacles that limited the use of marrow from relatives with only one matched haplotype have been overcome. Since virtually every patient with hematologic cancer has a haplotype-mismatched relative, further refinements of this strategy will increase the probability of a cure.

Supported in part by the Comitato per la Vita Daniele Chianelli and by grants from the Associazione Italiana Ricerche sul Cancro, the Associazione Italiana Leucemie e Linfomi, and the Istituto Superiore di Sanità Italy-USA Program on Therapy of Tumors.

*We are indebted to Prof. E. Donnal Thomas and Prof. Robert R. Negrin for their critical review of the manuscript; to Tiziana Zei, Roberta Jacucci, and Fabiola Bariletti for their excellent technical assistance; and to Dr. Geraldine Boyd and Catherine Gillies for assistance in the preparation of the manuscript.*

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