Successful Engraftment of T-Cell–Depleted Haploidentical “Three-Loci” Incompatible Transplants in Leukemia Patients by Addition of Recombinant Human Granulocyte Colony-Stimulating Factor–Mobilized Peripheral Blood Progenitor Cells to Bone Marrow Inoculum

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Patients who undergo transplantation with haploidentical “three-loci” mismatched T-cell–depleted bone marrow (BM) are at high risk for graft failure. To overcome the host-versus-graft barrier, we increased the size of the graft inoculum, which has been shown to be a major factor in controlling both immune rejection and stem cell competition in murine models. Seventeen patients (mean age, 23.2 years; range, 6 to 51 years) with end-stage chemoresistant leukemia were transplanted by a combination of BM with recombinant human granulocyte colony-stimulating factor–mobilized peripheral blood progenitor cells from HLA-haploidentical “three-loci” incompatible family members. The average concentration of colony-forming units–granulocyte-macrophage in the final inoculum was sevenfold to 10-fold greater than that found in BM alone. The sole graft-versus-host disease (GVHD) prophylaxis consisted of T-cell depletion of the graft by the soybean agglutination and E-rosetting technique. The conditioning regimen included total body irradiation. The means of overcoming graft failure elucidated in the experimental model can be applied in the clinical setting by combining approaches that increase both the conditioning of the host and the size of the stem cell inoculum.

To this end, we designed a conditioning regimen that added antithymocyte globulin (ATG) and thiopeta, a powerful myeloablative agent, to cyclophosphamide and total body irradiation (TBI) in a single fraction at a fast dose rate to enhance both immunosuppression and myeloablation. It has recently been shown that the administration of recombinant human granulocyte colony-stimulating factor (rhG-CSF) or recombinant human granulocyte-macrophage colony-stimulating factor (rhGM-CSF) can mobilize a sufficient number of peripheral blood progenitor cells (PBPCs) to permit the collection of a transplant inoculum.\textsuperscript{13-15} Infusion of these cytokine-mobilized cells has resulted in rapid marrow recovery and sustained hematopoiesis in autologous\textsuperscript{16-18} and syngeneic transplants.\textsuperscript{19} Two cases of allogeneic PBPC transplant have also been reported.\textsuperscript{20,21} Moreover, studies on PBPC transplants in animals have indicated that PBPCs can provide long-term multilineage hematopoiesis.\textsuperscript{22-24} Therefore, we attempted to increase the overall number of colony-forming units–granulocyte-macrophage (CFU-GM) infused into the recipients by an order of magnitude by add-

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Table 1. Patient’s Characteristics and Donor-Host Relationship

<table>
<thead>
<tr>
<th>Patients</th>
<th>Donors</th>
<th>HLA Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>UPN</td>
<td>Age/Sex</td>
<td>Disease</td>
</tr>
<tr>
<td>306</td>
<td>22/M</td>
<td>AML</td>
</tr>
<tr>
<td>313</td>
<td>37/M</td>
<td>AML</td>
</tr>
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<tr>
<td>317</td>
<td>31/F</td>
<td>ALL</td>
</tr>
<tr>
<td>319</td>
<td>15/M</td>
<td>CML</td>
</tr>
<tr>
<td>320</td>
<td>22/M</td>
<td>CML</td>
</tr>
<tr>
<td>321</td>
<td>28/M</td>
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<tr>
<td>329</td>
<td>6/M</td>
<td>ALL</td>
</tr>
<tr>
<td>331</td>
<td>27/M</td>
<td>CML</td>
</tr>
<tr>
<td>333</td>
<td>23/M</td>
<td>ALL</td>
</tr>
<tr>
<td>334</td>
<td>51/F</td>
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<tr>
<td>401</td>
<td>14/M</td>
<td>AML</td>
</tr>
<tr>
<td>402</td>
<td>26/M</td>
<td>AML</td>
</tr>
<tr>
<td>404</td>
<td>34/M</td>
<td>ALL</td>
</tr>
<tr>
<td>407</td>
<td>13/M</td>
<td>ALL</td>
</tr>
<tr>
<td>408</td>
<td>13/M</td>
<td>ALL</td>
</tr>
<tr>
<td>409</td>
<td>13/M</td>
<td>ALL</td>
</tr>
</tbody>
</table>

Abbreviations: BT, blastic transformation; REL, relapse; BL, blank.

ing to the T-cell–depleted BM PBPCs obtained from the donor after the administration of rhG-CSF. These cells were subjected to the same T-cell–depletion procedure. No post-grafting immunosuppressive treatment was administered.

MATERIALS AND METHODS

Patients. Seventeen patients (15 men and 2 women; median age, 23.2 years; range, 6 to 51 years) with advanced chemoresistant leukemia (5 acute myeloid leukemia [AML], 9 acute lymphoblastic leukemia [ALL], and 3 chronic myeloid leukemia in blastic phase) received transplants between March 1993 and March 1994. All patients received grafts from HLA-haploidentical “three-loci” incompatible family members. Six donors were siblings and 11 were parents (Table 1). Antidonor lymphocyte antibodies, as assayed in a complement-dependent microcytotoxicity crossmatch test, were not detected in any of the 12 evaluated cases.

According to the guidelines established by the Umbria Region Public Health Service ethical committee, written informed consent was obtained from patients or their parents as well as from the donors.

Conditioning regimen. All patients received 8 Gy TBI in a single fraction at a fast dose rate (16 cGy/min midplane) from an 18-MV photon beam linear accelerator on day −5. Lungs were shielded by individual lead moulds; the corrected mean total lung dose was 7 Gy. Thiotepa (10 mg/kg) was administered intravenously (IV) in two divided doses (4 hours for each infusion) on day −4. From days −4 to −1, 5 mg/kg rabbit ATG (Fresenius AG, Oberursel, Germany) was infused over 8 hours followed by 60 mg/kg cyclophosphamide on days −3 and −2. No immunosuppressive therapy was administered as GVHD prophylaxis after transplant.

Supportive care. Patients were cared for in laminar air-flow rooms until the neutrophil count recovered to 1 × 10^9/L. All patients received prophylactic trimethoprim-sulfamethoxazole for Pneumocystis carinii, ciprofloxacin for selective gut decontamination, fluconazole for fungal prophylaxis, Ig (0.5 g/kg/wk from day −5 to day +90), and total parenteral nutrition. Fever during the period of neutropenia was treated with broad-spectrum antibiotics; amphotericin B was added if fever persisted. Cytomegalovirus (CMV) prophylaxis consisted of ganciclovir (10 mg/kg/d from day −6 to day −2 and resumed at 5 mg/kg/d from day 7 to day +21, followed by maintenance treatment with 5 mg/kg thrice weekly until day +90). Foscarnet (90 mg/kg/d) was administered from day −1 to day +10. All but 1 patient (UPN 306) received G-CSF (5 μg/kg/d) for a mean of 4.7 days (range, 2 to 9 days) in the immediate posttransplant phase.

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All blood products were filtered and irradiated before infusion.

**Engraftment and immunoologic studies.** Time to engraftment was assessed by determining the day after transplant on which patients achieved $0.5 \times 10^7$ neutrophils/L and $25 \times 10^9$ platelets/L independent of transfusion support. Chimerism was assessed by karyotyping of PB lymphocytes and the analysis of restriction fragment length polymorphism (RFLP) in both PB and BM. The degree of acute GVHD was assessed using standard clinical criteria.

Posttransplant lymphoid cell subsets were identified by two-color immunofluorescence and flow cytometry. Cytotoxicity against a panel of natural killer (NK)-cell-sensitive cells were evaluated by a 51Cr release assay as described.

The combined leukapheresis products contained a mean of 11.62 ± 4.74 × 10^6/kg CD34+ cells (range, 5.47 to 18.99 × 10^6/kg CD34+ cells) and 73.182 ± 40.8 × 10^6/kg CFU-GM (range, 13 to 132.53 × 10^6/kg CFU-GM).

**T-cell depletion of BM and PBPCs.** Table 2 reports the mean number (per kilogram of body weight) of mononuclear cells, CFU-GM, CD34+ cells, and CD3+ cells in BM, PB, and the combined products administered to the patients after T-cell depletion.

The median dose of T cells infused was greater in the first 7 patients (group I) who received E-rosette-depleted PBMCs than in those (group II) whose leukapheresis product was depleted of T lymphocytes by the combined soybean agglutination and E-rosetting technique. In both groups, the average concentration of CFU-GM in the combined product was 7- to 10-fold greater than that found in BM alone.

**Engraftment.** One patient (UPN331) rejected the graft on posttransplant day 18 after initial myeloid engraftment. RFLP analysis of granulocytes confirmed that they were donor-derived on day 14 (data not shown). This early rejection was associated with the abrupt emergence of host T cells that exhibited donor-specific cytotoxic reactivity.

The other 16 patients had early and sustained engraftment. They achieved PB neutrophil counts greater than $0.5 \times 10^9$/L and greater than $1.0 \times 10^9$/L at a mean of 10.2 days (range, 9 to 17 days) and 11.5 days (range, 10 to 22 days), respectively. Platelet counts of $25 \times 10^9$/L and $50 \times 10^9$/L were reached at a mean of 17.2 days (range, 10 to 29 days) and 30 days (range, 14 to 60 days), respectively. The time course of engraftment is illustrated in Fig 1.

The curves represent the time required for T-cell-depleted "three-loci" incompatible transplants to reach 0.5 and 1.0 × 10^9/neutrophils/L, compared with our own historical control group of 23 patients who received autologous chemotherapy/cytokine-mobilized PBPCs (Tabiilo et al, unpublished observations, 1994) and 93 patients receiving transplants of T-cell-depleted HLA genotypically identical BM cells. RFLP analysis documented full donor-type chimerism in both the PB and BM of the 16 engrafted patients (data not shown).

**Immune reconstitution.** Phenotypic and functional analyses of posttransplant lymphocyte subsets were performed and compared with those obtained in HLA-matched T-cell-depleted BMT recipients. Whereas essentially identical data were obtained for B-cell and T-cell subsets in the two BMT settings (data not shown), a twofold increase in the early (1 to 2 months postgrafting) NK cell wave was noted in mismatched as compared with matched transplants. The
Table 2. Characteristics of Transplanted BM and PB Cells After T-Cell Depletion

<table>
<thead>
<tr>
<th>Group I</th>
<th></th>
<th></th>
<th></th>
<th>Group II</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BM</td>
<td>PBMC</td>
<td>Total</td>
<td>BM</td>
<td>PBMC</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>MNC (x10^6/kg)*</td>
<td>0.31</td>
<td>5.96</td>
<td>6.27</td>
<td>0.27</td>
<td>2.98</td>
<td>3.25</td>
<td></td>
</tr>
<tr>
<td>CFU-GM (x10^5/kg)</td>
<td>12.77</td>
<td>71.55</td>
<td>84.32</td>
<td>4.56</td>
<td>35.80</td>
<td>40.36</td>
<td></td>
</tr>
<tr>
<td>CD34+ (x10^6/kg)</td>
<td>1.9</td>
<td>12</td>
<td>13.9</td>
<td>ND</td>
<td>16</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>CD3+ (x10^6/kg)</td>
<td>0.32</td>
<td>5.91</td>
<td>6.23</td>
<td>0.19</td>
<td>1.24</td>
<td>1.43</td>
<td></td>
</tr>
</tbody>
</table>

For group I (7 donors), BM was T-cell-depleted by SBA and one-step E-rosette. PBMCs were T-cell-depleted by only two-step E-rosette. Donors underwent two to four leukaphereses. For group II (10 donors), BM and PBMCs were T-cell-depleted by SBA and two-step E-rosette. Donors underwent two to three leukaphereses.

Abbreviation: ND, not determined.

* Recipient body weight.

number of CD56+/CD16−/CD3− NK cells reached a peak value of 707 ± 212/µL after mismatched BMT and 306 ± 19/µL after matched BMT.

GVHD. One patient (UPN317) developed grade IV acute GVHD that was fatal. It is worth nothing that she received a greater quantity of T lymphocytes (11.13 × 10^7/kg) than did any of the other patients. There were no other cases of acute GVHD ≥grade II.

Toxicity and clinical outcome. In almost all patients, thiopeta caused a sunburn-like erythema that gradually faded and peeled off; mild, reversible oral mucositis developed in all patients. Mild diarrhea was generally seen within 2 to 4 days of completion of the conditioning regimen and resolved spontaneously. Transient hemorrhagic cystitis complicated the course of 4 patients and resolved with hydration and continuous bladder irrigation. Moderate venoocclusive disease of the liver occurred in 2 patients; their bilirubin levels ranged from 2 to 7 mg/dL and returned to normal in 10 days with sodium restriction and diuretics. The median time to onset of VOD was 6 days posttransplant.

Six patients developed interstitial pneumonitis between days +14 and +160 and died from respiratory failure (Table 3). No infectious cause could be identified in 2 patients (UPN 319 and 404), whereas CMV was the causative agent in 4 patients (UPN 315, 320, 321, and 334). Hematologic remission and full donor-type chimerism was documented in all 6 cases at the time of death. CMV-related gastroenteritis occurred in 5 patients but resolved with ganciclovir treatment.

The 1 patient (UPN 331) who experienced graft failure and the 1 patient with GVHD died. One patient (UPN 401) died from fungal infection. There have been two relapses, both in patients receiving transplants for ALL, within 2 months from the transplant.

Six patients are alive and well at a median follow-up of 230 days (range, 100 to 485 days) posttransplant, all with a Karnofsky performance status of 100%.

DISCUSSION

More general application of allogeneic BMT for the treatment of patients with hematologic malignancies is restricted by the availability of suitable donors. Less than 30% of patients who might benefit from transplant have genotypically HLA-identical siblings and only 3% to 5% have a one HLA-locus-mismatched relative. The establishment of large registries of HLA-typed individuals during recent years has led to a substantial increase in transplants from unrelated donors. Although 40% to 50% of Caucasian patients in the United States are successful in locating an HLA-A, B, DR-matched unrelated donor, many patients still fail to find an appropriate (either related or unrelated) donor. Moreover, certain ethnic groups have a much lower probability of finding donors, even when equal numbers of blacks and Caucasian are recruited into the registry.

![Time to 500 polys](image1)

![Time to 1000 polys](image2)

Fig 1. Curves represent cumulative proportions of patients reaching 500 and 1,000 neutrophils. (■), Present study; (△), HLA-identical T-cell-depleted BMT; (●) autologous chemotherapy/cytokine-mobilized PBPC transplant.
In contrast, nearly all patients have an HLA-haploidentical relative (parent, child, or sibling) who could serve as a donor. To date, transplantation of unmodified BM from HLA-haploidentical two- or three-loci incompatible donors has been associated with unsuccessful outcome caused by the high incidence (80%) of severe GVHD. The risk of graft failure may be 20% or higher. Extensive T-cell depletion of mismatched donor marrow can be used to effectively prevent GVHD, but the undesirable consequence of such transplants has been an increase in the incidence of graft failure to as high as 50%. Among several different mechanisms implicated in the pathogenesis of graft failure, clinical and experimental data strongly indicate that "conventional T-cell mechanisms," rather than "nonimmune responses," such as those mediated by NK cells or macrophages, are responsible for these rejections. Resistance to T-cell-depleted allogeneic mismatched BMT correlates closely with the presence of residual radio-resistant host clonable T cells in the PB. Moreover, abrupt emergence of host T cells with specific antidonor cytotoxic activity has been documented in patients who rejected T-cell-depleted mismatched BMT.

T-cell depletion of donor marrow may enhance the likelihood that these host cells can survive, because donor T cells present in unmanipulated BM help to eliminate or inactivate any residual host T lymphocytes that survive the preparative regimen. In the hope of eliminating residual radio-resistant host T lymphocytes and thereby promoting sustained engraftment, attempts have been made to employ more intensive pretransplant immunosuppression. Various approaches, such as TBI in a single fraction at a fast dose rate, addition of total lymphoid irradiation (TLI) to TBI, treatment of the recipient with anti-T MoAbs or ATG, addition of cytosine arabinoside or thiopeta (Aversa et al, unpublished observations, 1992) to pretransplant conditioning, have generally been unsuccessful in preventing rejection of T-cell-depleted grafts with high degrees of HL A disparity.

Early studies in rodents using unseparated marrow, although complicated by lethal GVHD, have shown that transplants of mismatched marrow could engraft if a larger BM inoculum was used compared with doses required for syngeneic transplants. Likewise, it has been shown subsequently in a mouse model that, when a certain degree of immunosuppression is achieved by the conditioning regimen, the number of donor BM cells (depleted of T lymphocytes) is a critical determinant for engraftment. Moreover, when different agents were measured in this model for their efficacy to promote engraftment of T-cell-depleted BM allografts, it has become apparent that increasing cell dose by 1 log will probably surpass any other available modality, including the potent myeloablative drugs busulphan or thiopeta or the addition of non-GVHD-producing T cells, the effect of which could be translated to its actual equivalence in cell dose. Further more, even when graduated numbers of host type T cells were added back to heavily conditioned recipients of BM allografts, it has been possible to overcome the allo-responses mounted by these cells against the graft by increasing the size of the T-cell-depleted transplant. Similar conclusions were also indicated recently in a rat model for transplantation of T-cell-depleted marrow.

Because availability of human BM cells is limited by the BM aspiration sites (iliac crests) and the necessity of avoiding excessive trauma and hypovolemia in the normal donor, it has been difficult to achieve significant increase in the BM inoculum and the possible effect of progenitor cell dose has not been tested to date in clinical mismatched transplantation. In our study a 7- to 10-fold increase in the dose of the transplant inoculum was achieved by adding T-cell-depleted rhG-CSF-mobilized PBPCs to the T-cell-depleted BM. The very large cell dose we infused after the intensive conditioning regimen was followed by prompt and sustained engraftment in 16 of 17 recipients of haploidentical three-loci' mismatched T-cell-depleted BM. Neutrophil and platelet recovery was very rapid and the engraftment characteristics were very similar to those observed in syngeneic

### Table 3. Clinical Outcome

<table>
<thead>
<tr>
<th>UPN</th>
<th>Disease</th>
<th>Status at Transplant</th>
<th>Blasts (%) in BM</th>
<th>Engraftment</th>
<th>Acute GVHD (grade)</th>
<th>Current Status (June 30, 1994)</th>
</tr>
</thead>
<tbody>
<tr>
<td>306</td>
<td>AML</td>
<td>2nd relapse</td>
<td>80</td>
<td>Yes</td>
<td>0</td>
<td>Alive in CCR on day +485</td>
</tr>
<tr>
<td>313</td>
<td>AML</td>
<td>Induction failure</td>
<td>100</td>
<td>Yes</td>
<td>0</td>
<td>Alive in CCR on day +413</td>
</tr>
<tr>
<td>315</td>
<td>ALL</td>
<td>3rd relapse</td>
<td>100</td>
<td>Yes</td>
<td>0</td>
<td>Died on day 120 from CMV-IP</td>
</tr>
<tr>
<td>317</td>
<td>ALL</td>
<td>2nd relapse</td>
<td>100</td>
<td>Yes</td>
<td>IV</td>
<td>Died on day 60 from GVHD</td>
</tr>
<tr>
<td>319</td>
<td>CML</td>
<td>2nd blast crisis</td>
<td>80</td>
<td>Yes</td>
<td>0</td>
<td>Died on day 90 from Idiopat-IP</td>
</tr>
<tr>
<td>320</td>
<td>CML</td>
<td>3rd blast crisis</td>
<td>15</td>
<td>Yes</td>
<td>0</td>
<td>Died on day 20 from CMV-IP</td>
</tr>
<tr>
<td>321</td>
<td>AML</td>
<td>3rd relapse</td>
<td>100</td>
<td>Yes</td>
<td>0</td>
<td>Died on day 18 from CMV-IP</td>
</tr>
<tr>
<td>329</td>
<td>ALL</td>
<td>3rd relapse</td>
<td>100</td>
<td>Yes</td>
<td>0</td>
<td>Relapsed on day 60, died on day 70</td>
</tr>
<tr>
<td>331</td>
<td>CML</td>
<td>2nd blast crisis</td>
<td>30</td>
<td>No</td>
<td>NE</td>
<td>Died on day 45 from sepsis</td>
</tr>
<tr>
<td>333</td>
<td>ALL</td>
<td>3rd relapse</td>
<td>15</td>
<td>Yes</td>
<td>0</td>
<td>Relapsed on day 50, died on day 60</td>
</tr>
<tr>
<td>334</td>
<td>ALL</td>
<td>2nd relapse</td>
<td>15</td>
<td>Yes</td>
<td>I</td>
<td>Died on day 180 from Idiopat-IP</td>
</tr>
<tr>
<td>401</td>
<td>AML</td>
<td>3rd relapse</td>
<td>100</td>
<td>Yes</td>
<td>0</td>
<td>Died on day 45 from sepsis</td>
</tr>
<tr>
<td>402</td>
<td>AML</td>
<td>Induction failure</td>
<td>100</td>
<td>Yes</td>
<td>I</td>
<td>Alive in CCR on day +157</td>
</tr>
<tr>
<td>404</td>
<td>ALL</td>
<td>2nd relapse</td>
<td>100</td>
<td>Yes</td>
<td>0</td>
<td>Died on day 62 from Idiopat-IP</td>
</tr>
<tr>
<td>407</td>
<td>ALL</td>
<td>2nd relapse</td>
<td>15</td>
<td>Yes</td>
<td>I</td>
<td>Alive in CCR on day +126</td>
</tr>
<tr>
<td>408</td>
<td>ALL</td>
<td>3rd relapse</td>
<td>15</td>
<td>Yes</td>
<td>I</td>
<td>Alive in CCR on day +110</td>
</tr>
<tr>
<td>409</td>
<td>ALL</td>
<td>3rd relapse</td>
<td>10</td>
<td>Yes</td>
<td>I</td>
<td>Alive in CCR on day +100</td>
</tr>
</tbody>
</table>

Abbreviations: NE, not evaluable; CCR, continuous complete remission; IP, interstitial pneumonia.
PBPC transplants\textsuperscript{19} or in our historical control group of patients who received autologous chemotherapy/rhG-CSF–mobilized PBPCs (Tabilio et al, unpublished observations, 1994). The short posttransplant course of rhG-CSF treatment may have also contributed to the swift increase in the neutrophil count. However, the patients (UPN 306 and 409) who did not receive G-CSF had a similar progression in the neutrophil count.

The impressive rate of engraftment across the most difficult histoincompatibility barrier shows that, in humans, as in mice, the stem cell dose plays a critical role in the engraftment of T-cell-depleted transplants. This concept is further supported by the finding that the same pretransplant conditioning failed to promote engraftment in any of the 5 patients receiving transplants of conventional doses of T-cell–depleted "three-loci" mismatched BM cells (Aversa et al, unpublished observations, 1992).

One potential major concern raised by the use of a large T-cell–depleted inoculum is an increased risk of GVHD, mainly caused by T-cell contamination of PBPCs. However, greater than grade I GVHD was extremely rare and occurred in only 1 of the evaluable patients who received the largest number of T cells (11.3 \times 10^8, almost twofold more than the average number administered to group I and about 10-fold more than the average of group II). Kerman et al\textsuperscript{51} suggested that the threshold dose of clonable T cells that leads to GVHD is about 2 \times 10^7/kg. Considering that the cloning efficiency is around 20%, our observed threshold falls within the same range. However, it is likely that ATG, administered between days −5 and −2, would contribute to lowering both the frequency and severity of GVHD by exerting a cytotoxic effect against donor inoculum T lymphocytes. The following points support this hypothesis: (1) the plasmatic half-life of rabbit ATG is 6 days; (2) the introduction of ATG\textsuperscript{32} or Campath\textsuperscript{53} in the conditioning regimens for mismatched transplants has been associated with a lower incidence of severe acute GVHD.

Despite the intensive prior therapy and the high leukemic burden at the time of transplant, the transplant-related mortality was very close to those reported by the European (47% at 2 years in CML patients in accelerated phase and 62% in blast crisis) and Italian (62% at 2 years in 46 advanced leukemia patients; Gruppo Italiano Trapianto Midollo Osseo, data unpublished) Registries in end-stage leukemia patients who received marrow grafts from HLA genotypically identical siblings.\textsuperscript{54} On the other hand, using a conditioning regimen very similar to ours and T-cell-depletion with Campath, Bacigalupo et al\textsuperscript{55} obtained a 13% 1-year transplant-related mortality TRM in CML patients (median age, 43 years) who received foscarnet as CMV prophylaxis.

Of more concern is the question of CMV disease. CD8\textsuperscript{+} CMV-specific cytotoxic T lymphocytes (CTLs) are responsible for protective immunity and elimination of active infection. Because CMV-specific CTL responses may require an extended time period after mismatched BMT and are HLA-restricted,\textsuperscript{46} susceptibility for CMV-infections is greater with mismatched than with matched BMT. The rapid hematopoietic reconstitution observed in our series of patients should permit early prophylaxis of CMV infections with ganciclovir and, therefore, contribute to lowering mortality. After the schedule of ganciclovir was modified to begin day +7, no case of CMV pneumonia was documented in the 9 patients (group II).

Given the fact that the patients in this small series received transplants while in refractory end-stage disease and that the mean follow-up is short, no conclusions can be drawn about survival. Nevertheless, the high engraftment rate, virtual elimination of acute GVHD greater than grade II, and acceptable conditioning-related toxicity suggest that this approach should be applied to selected leukemia patients who do not have HLA-matched donors.

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Successful engraftment of T-cell-depleted haploidentical "three-loci" incompatible transplants in leukemia patients by addition of recombinant human granulocyte colony-stimulating factor-mobilized peripheral blood progenitor cells to bone marrow inoculum

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