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 chemotherapy-resistant leukemia were transplanted of 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 unit–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 in a single fraction at a fast dose rate, antithymocyte globulin, cyclophosphamide and thiotepa to provide both immunosuppression and myeloablation. One patient rejected the graft and the other 16 had early and sustained full donor-type engraftment. One patient who received a much greater quantity of T lymphocytes than any other patient died from grade IV acute GVHD. There were no other cases of GVHD ≥grade II. Nine patients died from transplant-related toxicity, 2 relapsed, and 6 patients are alive and event-free at a median follow-up of 230 days (range, 100 to 485 days). Our results show that a highly immunosuppressive and myeloablative conditioning followed by transplantation of a large number of stem cells depleted of T lymphocytes by soybean agglutination and E-rosetting technique has made transplantation of three HLA-antigen disparate grafts possible, with only rare cases of GVHD.

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EXTENSIVE EXPERIENCE in transplants for patients with severe combined immune deficiency (SCID) has shown that both acute and chronic graft-versus-host disease (GVHD) is largely preventable, even in mismatched bone marrow transplant (BMT), when a 3-log T-cell depletion of the donor BM is achieved (ie, with the soybean agglutination and E-rosetting technique). However, the use of T-cell–depleted haploidentical grafts in leukemia patients has had poor success because of the high risk of graft failure. Graft failure is presumably a result of the immunologic rejection of donor hematopoietic cells by the host residual immune system, but it could also be mediated by other mechanisms, including competition between donor and residual host stem cells for the limited available niches in the BM stroma as well as the availability of facilitating cells in the donor inoculum. In the mouse model, the immunologic rejection of T-cell–depleted histoincompatible BMT can be overcome by increasing radiation or by adding selective measures with minimal toxicity, such as splenic irradiation or in vivo treatment with anti-T monoclonal antibodies (MoAbs), to the conditioning regimen. Stem cell competition can be manipulated in favour of donor type cells by increasing the size of the T-cell–depleted BM inoculum or by adding myeloablative drugs (busulfan and thiopeta) in the radiation therapy.

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. Infusion of these cytokine-mobilized cells has resulted in rapid marrow recovery and sustained hematopoiesis in autologous and syngeneic transplants. Two cases of allogeneic PBPC transplant have also been reported. Moreover, studies on PBPC transplants in animals have indicated that PBPCs can provide long-term multilineage hematopoiesis.

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-
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 X 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/day from day -6 to day -2 and resumed at 5 mg/kg from day 4 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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**Table 1. Patient's Characteristics and Donor-Host Relationship**

<table>
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<tr>
<th>UPN</th>
<th>Age/Sex</th>
<th>Disease</th>
<th>Status</th>
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<th>Age/Sex</th>
<th>Relationship</th>
<th>HLA Analysis</th>
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**Abbreviations:** BT, blastic transformation; REL, relapse; BL, blank.
All blood products were filtered and irradiated before infusion.

**Engraftment and immunologic studies.** Time to engraftment was assessed by determining the day after transplant on which patients achieved $0.5 \times 10^9$ 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 and resistant targets was evaluated by a 51Cr release assay as described. T-cell proliferation was assessed by stimulating cells with anti-CD3 MoAb and pulsing the cultures with $[^{3}H]$-thymidine for 12 hours at the end of a 72-hour culture period. For detection of interleukin-2 (IL-2) activity in culture supernatants, the CTLL murine cell line assay was used.

All BM preparations were depleted of T lymphocytes by the combined soybean agglutination and E-rosetting technique, as previously described. This procedure results in a 3- to 3.5-log10 reduction in the number of T lymphocytes. Depletion of T lymphocytes from the PBMCs in the first 7 cases was achieved by two-step E-rosetting procedure, whereas the combined soybean agglutination and E-rosetting technique was used in the last 10 cases. Aliquots were taken for differential cell counts, MoAb staining, and GFU-GM assay at each stage of processing. T-cell-depleted BM and PB mononuclear cell (PBMC) collection. Donor BM cells were obtained under general anesthesia by multiple aspirations from the iliac crests bilaterally and cryopreserved, as described below. rhG-CSF (12 µg/kg/d) was administered to donors by continuous subcutaneous infusion from 24 hours after BM harvesting and continued for 5 to 7 days. Two to four leukaphereses were performed between days 4 and 7 in the first 7 donors, who received rhG-CSF for 6 to 7 days; whereas the remaining 10 donors, who were treated with rhG-CSF for 5 to 6 days, underwent two to three leukaphereses (days 4, 5, and 6).

The donors reported no untoward effects during or after the administration of rhG-CSF or from the blood collection. All BM preparations were depleted of T lymphocytes using the soybean agglutination and E-rosetting technique, as previously described. This procedure results in a 3- to 3.5-log10 reduction in the number of T lymphocytes. Depletion of T lymphocytes from the PBMCs in the first 7 cases was achieved by two-step E-rosetting procedure, whereas the combined soybean agglutination and E-rosetting technique was used in the last 10 cases. Aliquots were taken for differential cell counts, MoAb staining, and GFU-GM assay at each stage of processing. T-cell-depleted BM and PB cells were frozen in a controlled rate liquid nitrogen freezer and stored in the vapor phase of liquid nitrogen. In some cases, the collections from PB were performed on the day before and on the day of the transplant; these cells were not cryopreserved.

GFU-GMs were measured in whole blood and in the leukapheresis product by plating $0.5 \times 10^9$ mononuclear cells in a 3% agar solution containing 10% of 5637 cell line conditioned medium, 20% fetal bovine serum, and Iscove medium. Colonies of greater than 40 cells (days 4, 5, and 6). The donors reported no untoward effects during or after the administration of rhG-CSF or from the blood collection. All BM preparations were depleted of T lymphocytes using the soybean agglutination and E-rosetting technique, as previously described. This procedure results in a 3- to 3.5-log10 reduction in the number of T lymphocytes. Depletion of T lymphocytes from the PBMCs in the first 7 cases was achieved by two-step E-rosetting procedure, whereas the combined soybean agglutination and E-rosetting technique was used in the last 10 cases. Aliquots were taken for differential cell counts, MoAb staining, and GFU-GM assay at each stage of processing. T-cell-depleted BM and PB cells were frozen in a controlled rate liquid nitrogen freezer and stored in the vapor phase of liquid nitrogen. In some cases, the collections from PB were performed on the day before and on the day of the transplant; these cells were not cryopreserved.

CBF and PBMC processing. All BM preparations were depleted of T lymphocytes using the soybean agglutination and E-rosetting technique, as previously described. This procedure results in a 3- to 3.5-log10 reduction in the number of T lymphocytes. Depletion of T lymphocytes from the PBMCs in the first 7 cases was achieved by two-step E-rosetting procedure, whereas the combined soybean agglutination and E-rosetting technique was used in the last 10 cases. Aliquots were taken for differential cell counts, MoAb staining, and GFU-GM assay at each stage of processing. T-cell-depleted BM and PB cells were frozen in a controlled rate liquid nitrogen freezer and stored in the vapor phase of liquid nitrogen. In some cases, the collections from PB were performed on the day before and on the day of the transplant; these cells were not cryopreserved.

The donors reported no untoward effects during or after the administration of rhG-CSF or from the blood collection. All BM preparations were depleted of T lymphocytes using the soybean agglutination and E-rosetting technique, as previously described. This procedure results in a 3- to 3.5-log10 reduction in the number of T lymphocytes. Depletion of T lymphocytes from the PBMCs in the first 7 cases was achieved by two-step E-rosetting procedure, whereas the combined soybean agglutination and E-rosetting technique was used in the last 10 cases. Aliquots were taken for differential cell counts, MoAb staining, and GFU-GM assay at each stage of processing. T-cell-depleted BM and PB cells were frozen in a controlled rate liquid nitrogen freezer and stored in the vapor phase of liquid nitrogen. In some cases, the collections from PB were performed on the day before and on the day of the transplant; these cells were not cryopreserved.

**RESULTS**

**Mobilization and collection of PBPCs.** In the normal donors, under steady-state hematopoiesis (baseline), minimal amounts of circulating CD34+ cells (median, 0.5/L blood; range, 0 to 15/µL) and CD34+ cells (median, 122.7/µL; range, 0 to 268/µL) were detectable. With G-CSF treatment, the donor white blood cell count increased from a median of $6.7 \times 10^9/L$ to $72 \times 10^9/L$ by day 7.

Peak levels of CD34+ cells, as well as of CFU-GM, were reached simultaneously at a median of 5 days. Median values of peak levels were 830.7 CD34+ cells/µL (range, 92.8 to 1,035 CD34+ cells/µL) and 12,347 CFU-GM/µL (range, 549 to 20,126 CFU-GM/µL). The two to four leukapheresis procedures yielded a mean total number of $10.4 \times 10^9$ mononuclear cells/kg of donor body weight (range, 4 to $23 \times 10^9$ mononuclear cells/kg). The combined leukapheresis products contained a mean of $11.62 \pm 4.74 \times 10^9/kg$ CD34+ cells (range, 5.47 to 18.99 × 10^9/kg CD34+ cells) and $73,182 \pm 40.8 \times 10^9/kg$ CFU-GM (range, 13 to 132.53 × 10^9/kg CFU-GM).

**T-cell depletion of BM and PBMCs.** Table 2 reports the mean number (per kilogram of body weight) of mononuclear cells, CD34+ cells, and CD33+ cells present 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 cure is presented as the time required for T-cell–depleted "three-loci" incompatible transplants to reach 0.5 and $10^9$/neutrophils/L, compared with our own historical control group of 23 patients who received autologous chemotherapy/cytokine-mobilized PBPCs (Tahilu 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 BM recipients. Whereas essentially identical data were obtained for B-cell and T-cell subsets in the two BM 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</th>
<th>BM (x10^6/kg)*</th>
<th>PBMC (x10^6/kg)</th>
<th>Total (x10^6/kg)</th>
<th>BM (x10^6/kg)</th>
<th>PBMC (x10^6/kg)</th>
<th>Total (x10^6/kg)</th>
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<tbody>
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<td>6.23</td>
<td>0.19</td>
<td>1.24</td>
<td>1.43</td>
</tr>
</tbody>
</table>

Abbreviation: ND, not determined.

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.

GVHD. One patient (UPN317) developed grade IV acute GVHD that was fatal. It is worth noting 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, thiotepa 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 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.
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.37,38 The risk of graft failure may be 20% or higher.39 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%.3,40

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 BM correlates closely with the presence of residual radiochemoresistant host clonable T cells in the PB.41 Moreover, abrupt emergence of host T cells with specific antidonor cytotoxic activity has been documented in patients who rejected T-cell–depleted mismatched BM.32,43

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.44 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,45 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 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.46 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.47,48,49,50 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,50 the effect of which could be translated to its actual equivalence in cell dose. Furthermore, 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.51 Similar conclusions were also indicated recently in a rat model for transplantation of T-cell–depleted marrow.52

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
T-CELL-DEPLETED INCOMPATIBLE TRANSPLANTS

PBPC transplants 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 mice, 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 transplantats 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 x 10^6, 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 suggested that the threshold dose of clonable T cells that leads to GVHD is about 2 x 10^6/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 or Campath in the conditioning regimens for matched unrelated 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-matched donors. 14

ACKNOWLEDGMENT

We express our appreciation to the nursing staff of the BMT Unit at the Institute of Hematology, University of Perugia; we thank Judy Dale for her assistance in the preparation of this manuscript; and we thank Maria Daniela Asciani and Maria Luisa Pezzuti for excellent secretarial assistance. We are very grateful to Dr. Isabel Cunningham (Bone Marrow Transplant Program, Indiana University, Indianapolis) for critical review of the manuscript.

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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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