Unrelated Bone Marrow Donor Transplants for Children With Leukemia or Myelodysplasia

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Allogeneic bone marrow transplantation is the treatment of choice for many childhood leukemias. The donor of choice—an HLA matched sibling—is only available about 30% of the time. Unrelated donors are an alternative choice. In this report, we describe the results of unrelated donor bone marrow transplants (BMT) in 50 children with leukemia (25 acute lymphoblastic leukemia [ALL], 3 acute myeloid leukemia [AML], 3 juvenile chronic myelogenous leukemia [JCLM], 10 chronic myeloid leukemia [CML]) or myelodysplastic syndrome (MDS; 9). The median age of the 31 male and 19 female patients was 9 years (range 2 to 18). Only 13 patients were serologically matched at HLA-A, B, DR, and DQ with their donors; 6 of these were reactive in mixed lymphocyte culture. The other 37 patients were mismatched for one (36 patients) or more (1 patient) HLA antigens. Pretransplant conditioning included cytosine arabinoside, cyclophosphamide, fractionated total body irradiation (TBI) (with lung, liver, and more recently, kidney shielding), and methylprednisolone. High-risk patients also received busulfan. Graft-versus-host disease (GVHD) prophylaxis consisted of T-cell depletion with IgM monoclonal antibody T1089 plus complement and posttransplant cyclosorpin-A. Forty-nine patients (98%) engrafted. Median times to greater than 500 polymorphonuclear leukocytes (PMNs) /µL and greater than 25,000 platelets/µL were 18 and 20 days, respectively. Acute GVHD grade II occurred in 16 patients (33%); 13 (81%) of these died. Chronic GVHD developed in 30 of 40 patients at risk, but was extensive in only 5. Event-free survival (EFS) for all patients was 44% ± 7% (median follow-up was 49 months), and overall survival was 50 ± 7%. Patients with low-risk disease (ALL or AML in first or second remission and CML in chronic phase) had a better EFS than children with high-risk disease (60% vs 34%, P = .07). There was no significant difference in EFS between patients who were serologically matched with their donors (46%) and those who were partially mismatched (43%) (P = .97). These data compare favorably with published reports for children transplanted with HLA-matched sibling donors and should encourage earlier consideration of unrelated donor BMT in children with leukemia or myelodysplasia.

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Allogeneic Bone Marrow transplantation (BMT) is the treatment of choice for certain childhood leukemias, either at diagnosis or after recurrence of disease. The most desirable marrow donor is an HLA-identical sibling. However, with the decline in family size, only about 30% of patients have an HLA-identical sibling. In the past decade, our group and others have attempted to use either partially-matched family members or, more recently, unrelated donors so that patients who lack a matched sibling could be offered an allogeneic transplant. Potential problems with the use of donors who are not HLA-matched include increased risk of nonengraftment, graft-versus-host disease (GVHD), and infection. In the past, locating a matched unrelated donor was hampered by the lack of large donor registries. With the creation of the National Marrow Donor Program and other international registries, the probability of identifying an unrelated donor has increased. Here, we report results for the first 50 children who received an unrelated donor BMT for leukemia or myelodysplasia at the Medical College of Wisconsin. The data show that unrelated donor BMT is an effective treatment for these diseases and should be considered when BMT is indicated and an HLA-matched sibling is not available.

Materials and Methods

Patient population. Fifty consecutive patients (age: <19 years) with leukemia or myelodysplasia received bone marrow transplants from unrelated donors at the Children's Hospital/Medical College of Wisconsin between March 1986 and November 1991. Histocompatibility testing included serotyping for HLA-A, -B, -DR, and -DQ antigens and the mixed lymphocyte culture (MLC) assay. The most recent 28 patients were also typed for DRB1 and DQB1, using the more sensitive polymerase chain reaction (PCR)-based oligonucleotide techniques. If a genotypically HLA-identical sibling donor or a single antigen-mismatched related donor was not available, a search for an unrelated donor was initiated. Clinical features of the patients are shown in Table 1. Simultaneous relapses (ie, BM and central nervous system [CNS]) were considered as a single relapse. One patient with acute lymphoblastic leukemia (ALL) who was refractory to initial induction therapy entered remission with secondary treatment and was considered as a first complete remission (CR). ALL for the analysis. Three patients with Philadelphia chromosome/positive (Ph+) ALL had evidence of cytogenetic relapse at the time of transplant, but were in hematologic remission (1 CR1, 2 CR2). “Low risk” refers to patients with ALL or acute myeloid leukemia (AML) in a first or second remission or chronic myeloid leukemia (CML) in chronic phase (CP). All other patients were considered “high risk.”

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UNRELATED BMT IN CHILDREN

Table 1. Characteristics of Bone Marrow Transplant Patients

<table>
<thead>
<tr>
<th>Patients</th>
<th>50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>50</td>
</tr>
<tr>
<td>Range (yrs)</td>
<td>2-18</td>
</tr>
<tr>
<td>Median</td>
<td>9</td>
</tr>
<tr>
<td>Male</td>
<td>31</td>
</tr>
<tr>
<td>Female</td>
<td>19</td>
</tr>
</tbody>
</table>

Diagnoses

| ALL | 25 |
| First CR | 4 |
| Second CR | 8 |
| Remission | 7 |
| Relapse | 6 |
| AML | 3 |
| Second CR | 1 |
| Third CR | 1 |
| Second relapse | 1 |
| CML | 10 |
| Chronic | 5 |
| Accelerated | 3 |
| Blast | 2 |
| JCML | 3 |
| Myelodysplasia | 9 |

Searches for unrelated donors and matching of donors and recipients. Searches for unrelated donors were processed through the Marrow Donor Program of the Blood Center of Southeastern Wisconsin, the National Marrow Donor Program, the Anthony Nolan Registry, and European Registries. Donor candidates were identified for patients according to HLA-A and -B specificities. The donors underwent extended serotyping for HLA-A, -B, -DR, and -DQ specificities by microcytotoxicity assays with updated typing trays containing local, American Workshop, and International Workshop reagents. After extended serotyping, potential unrelated donors who were phenotypically matched (n = 13) for HLA-A, -B, -DR, and -DQ or mismatched for one (n = 36) or more (n = 1) HLA-A, -B, or -D determinants were evaluated with other donors by means of MLC. For these assays, two or more cryopreserved peripheral blood mononuclear cell samples obtained at different times were tested against similarly cryopreserved lymphocytes from donors and controls. Results were expressed as percent relative response. Each donor-recipient pair was evaluated for matching of six loci: two for HLA-A, two for HLA-B, and two for the HLA-D region. D-region compatibility was initially defined as the combination of HLA-DR and -DQ seromatching. For the most recent 28 donor-recipient pairs, molecular genetic analysis for HLA-DR and -DQ polymorphisms was performed by sequence-specific oligonucleotide-probe hybridization (SSOHP) after amplification of DNA by PCR. Any disparity that could be detected experimentally (including those recognized by workshop reagents for newly defined antigens or splits, or molecular genetic studies) was considered a mismatch. The length of time from initiation of a formal search until donor selection was 3 to 7 months.

Pretransplantation chemoradiotherapy. Informed consent was obtained from the patients or their guardians. All treatments were administered under protocols approved by institutional review committees.

Standardized pretransplantation conditioning and GVHD prophylaxis were used (Fig 1). All patients received high-dose cytosine arabinoside, cyclophosphamide, and intravenous methyl-prednisolone. Patients with neoplastic disease who were at high risk of relapse also received oral busulfan (2 to 4 mg/kg/d, on days -9 and -8). All dosages were calculated based on ideal body weight for height. Total body irradiation (1,400 cGy) was delivered in nine fractions over 3 days at a dose rate of 8 to 25 cGy/min. Radiation doses to the lungs, liver, and, more recently, the kidneys were attenuated using partial transmission blocks. The area beneath the lung blocks was boosted with low-energy electrons to achieve a uniform dose to the ribs. Patients with either CML or myeloproliferative disorders and enlarged spleens underwent preconditioning splenectomy.

GVHD prophylaxis and treatment. T-cell depletion of donor marrow with the monoclonal antibody (MoAb) T10B9 plus complement was performed as described elsewhere. The antibody T10B9 has previously been shown to preferentially bind to T-cells expressing the αβ receptor and result in ~1.6 log10 T-cell depletion. Intravenous cyclosporine was begun the day before marrow infusion. Cyclosporine levels (assessed with a polyclonal therapeutic drug system method, TDX [Abbott Laboratories, Chicago, IL]) were kept between 200 and 500 ng/mL. In patients without GVHD, the dose of cyclosporine was tapered over 3 to 4 months beginning on day 40 to 50.

The clinical diagnosis of GVHD was graded as 0 through IV for acute GVHD, and as none, limited, or extensive for chronic GVHD. Skin GVHD was treated with topical corticosteroids and/or steroids. For patients requiring both agents, the regimen proposed by Sullivan et al for high-risk extensive chronic GVHD was used.

Supportive care. Patients were hospitalized in single rooms with high-efficiency particulate air filtration. Trimethoprim-sulfamethoxazole (2.5 mg of trimethoprim per dose) was given twice daily as prophylaxis for Pneumocystis carinii during pre-BMT conditioning and resumed 3 days per week post-BMT when the PMN count was greater than 1,000/μL. Mouth care consisted of chlorhexidine mouth wash and Nystatin (UDL Laboratories, Rockford, IL) or Mycelex (Miles Pharmaceutical, West Haven, CT) troches. Broad-spectrum antibacterial and antifungal antibiotics were administered empirically for episodes of fever. Patients seronegative for cytomegalovirus (CMV) received blood components from CMV-seronegative donors. All patients received acyclovir as prophylaxis against herpesvirus infections, (5 mg/kg every 8 hours intravenously if seronegative for CMV, and 10 mg/kg if seropositive). Intraocular Ig (400 mg/kg/wk) was administered prophylactically for at least the first 120 days. Patients with chronic GVHD received monthly infusions there-
Trilineage engraftment was documented by examining the bone marrow. No fatal events such as acute GVHD and engraftment. Survival were censored at the time of death in the product limit analysis of in BM, peripheral blood, or extramedullary sites greater than 25,000/μL and increasing without platelet transfusions. Trilineage engraftment was documented by examining the bone marrow. A relapse was indicated by morphologic evidence of leukemia in BM, peripheral blood, or extramedullary sites or by cytogenetic recurrence of a neoplastic clone. Cytopenias that occurred after confirmed leukemic relapse were not considered evidence of graft failure, but were attributed to disease recurrence. Similarly, cytopenias that resulted from therapy with 9 (1,3-didydroxy-2-propoxymethyl) guanine were not considered graft failure.

Statistical analysis. Endpoints were calculated with the date of latest follow-up being January 1, 1994. The median duration of follow-up for the patient population was 49 months (range, 27 to 85). Cumulative actuarial probabilities of engraftment, event-free survival (EFS) (death or recurrent leukemia), overall survival, and acute GVHD were calculated by product limit analysis.41 Patients were censored at the time of death in the product limit analysis of nonfatal events such as acute GVHD and engraftment. Survival curves were compared by the log-rank test.44 Fischer’s exact test was used to compare incidence rates of acute GVHD.44 Student's t-test and chi-square analysis were used to compare differences in prognostic features between groups of patients.32,46

RESULTS

Engraftment. Forty-nine of the 50 patients (98%) engrafted. There were no late graft failures. The median time to neutrophil engraftment was 18 days (range, 12 to 35) (Fig 2). Platelet engraftment was attained in a median of 20 days (range, 10 to 32). The median number of nucleated cells infused after T-cell depletion was 1.6 X 10^6 cells/kg of patient weight (range, 0.6 to 4.0 X 10^6 cells/kg). There was no correlation between the number of cells infused and rapidity of engraftment. The single patient who did not engraft had myelodysplasia and did not receive busulfan. She received a cell dose of 1.7 X 10^6 cells/kg.

Unrelated donors. Unrelated donors were obtained from the Marrow Donor Program of the Blood Center of Southeastern Wisconsin (n = 15), the National Marrow Donor Program (n = 29), the Anthony Nolan Registry (n = 2), and European Registries (n = 4). Thirteen patients had donors who were serologically matched for HLA-A, -B, -DR, and -DQ. Six of these had a relative response index in the MLC of greater than 10% in either the donor-recipient direction or the recipient-donor direction. Thirty-seven patients were mismatched with their donors. The majority of the mismatches were between class I antigens (n = 29); seven were class II mismatched, and one was mismatched with the donor at both class I and II (Table 2). Initially, the only additional test to assess histocompatibility was the MLC assay. The median relative response index in either direction was 7%, but the range was wide (0% to 98%). Furthermore, reactivity in either one direction or both was not interpretable for 20% of the patients because of poor cell viability and/or high background secondary to the patient’s underlying disease.

The most recent 28 patients had MHC class II evaluation performed by SSOPH using PCR amplification. Using this sensitive technique, only one patient was mismatched for class II (DR11 subtype disparity). Donors and recipients were sex matched in 24 cases and mismatched in 26 cases.

GVHD. For the 49 patients who engrafted, the actuarial risk of developing grades II-IV GVHD was 33.1%. The risk of severe (grades III-IV) acute GVHD was only 8.2%. HLA matching resulted in a trend toward decreased incidence and severity of GVHD. Only 2 of 13 patients who were serologically HLA matched had GVHD grades II-IV, while 14 of 36 who were partially mismatched had moderate to severe
GVHD (P = .18). However, the benefit of a serologic match did not translate into an increase in EFS. Seventeen of 37 mismatched patients remain disease-free, whereas 6 of 13 matched patients remain disease free (P = .97) (Fig. 3). GVHD was seen more frequently in the high-risk patients (40.9%) as compared with the low-risk group (16.7%, P = .09). This could be explained, in part, by the higher incidence of mismatched patients in the high-risk group (25 of 31) compared with the low-risk group (11 of 18).

EFS was adversely affected by the severity of acute GVHD; only 2 of 16 patients with grades II-IV acute GVHD survive event free (Fig 4). EFS was similar in low- and high-risk patients who did not develop grades II-IV acute GVHD (61% and 58%, respectively). Of the high-risk patients who did develop grades II-IV acute GVHD, only 1 of 14 survives in continued remission, whereas 1 of 2 in the low-risk group remains disease free.

Chronic GVHD developed in 30 of 40 evaluable patients. It was limited in 25 patients and extensive in five. Nine of 11 HLA-matched patients evaluable for chronic GVHD developed limited (n = 6) or extensive (n = 3) disease, whereas 21 of 29 mismatched patients had limited (n = 19) or extensive (n = 2) chronic GVHD.

**Survival.** Twenty-two of the 50 patients remain event free (44 ± 7%) (median follow-up in the surviving patients is 49 months). Disease status at the time of transplant affected outcome. Patients whose disease was considered low risk had a higher rate of EFS (60.6% ± 11.6%) than patients with high-risk disease (34.4% ± 8.4%) (P = .07) (Fig 5).

Twenty-five patients were transplanted for ALL. Three of four patients transplanted in first CR remain event free, whereas 5 of 8 in second CR and 2 of 13 beyond a second CR remain event free. The length of initial remission did not differ between patients who remain event free (median, 2; range, 9 to 49 months) and those who relapsed or died (median, 21; range, 5 to 47 months). For the 21 patients who were not transplanted in first remission, five of the seven event-free survivors have been in remission longer than in any remission before transplant.

Of the nine patients with myelodysplasia who received transplants, two were in blast transformation. Five patients survive (four in remission) 27 to 80 months posttransplant.

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**Table 2. Immunogenetic Matching for 50 Donor/Recipient Pairs**

<table>
<thead>
<tr>
<th>Method</th>
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</tr>
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<tr>
<td>HLA-A, B, DR, DQ matched</td>
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<tr>
<td>Class I mismatch</td>
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<tr>
<td>A locus</td>
<td>17</td>
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<tr>
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<td>Class II mismatch</td>
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<td>DQ locus</td>
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</tr>
<tr>
<td>A and DQ</td>
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<tr>
<td>Cellular (mixed lymphocyte culture)</td>
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<tr>
<td>Relative response index (%RR)</td>
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<tr>
<td>Donor → recipient</td>
<td>50</td>
</tr>
<tr>
<td>0%-98% (median = 7%)</td>
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<tr>
<td>Recipient → donor</td>
<td>50</td>
</tr>
<tr>
<td>0%-56% (median = 7%)</td>
<td></td>
</tr>
<tr>
<td>6 patient assays not evaluable</td>
<td></td>
</tr>
</tbody>
</table>

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![Fig 3](image-url) Probability of EFS for patients who received matched or mismatched marrow from an unrelated donor. Patients currently disease free are represented by tick marks.

![Fig 4](image-url) Probability of EFS as it relates to GVHD. Patients currently disease free are represented by tick marks.
For the 10 patients with CML, 7 survive (1 with a cytogenetic relapse) 36 to 74 months posttransplant. At the time of transplant, four of the survivors were in CP, two were in accelerated phase (AP), and one was in blast crisis (BC). Two of three patients with JCML survive (one with recurrence) 38 and 84 months posttransplant. Of the three patients with AML (third CR), one is alive and disease free at 30 months posttransplant.

Three patients are alive with evidence of recurrent disease based on cytogenetic criteria. The first patient (CML transplanted in CP) had 2 of 20 Ph⁺ metaphases in the BM 809 days posttransplant. She remains in a hematologic remission 1,434 days posttransplant, but has severe restrictive lung disease. A second patient (JCML) had recurrence of a malignant clone on day 661 posttransplant, but remains stable 1,140 days posttransplant. The third patient (myelodysplastic syndrome with monosomy 7) had evidence of a second malignant clone (deletion of chromosome 5) on day 321 posttransplant, but remains well at day 1,378. All three patients with recurrent malignant clones are mixed chimeras.

*Adverse events.* The incidence of leukemia relapse was 16% (8 of 50 patients), whereas the actuarial risk of relapse was 23% (Fig 6). The median time to relapse was 7 months with a range of 2 to 27 months. Four of the patients who relapsed had ALL (two transplanted in second relapse, one in second CR, one in third CR), two had CML (one in first CP, one in second AP), one had JCML, and one had a myelodysplastic syndrome.

The causes of death are illustrated in Fig 7. The leading primary cause of death was interstitial pneumonia. This was caused by regimen-related toxicity in five patients and by infection in four patients. Another four patients died from systemic infection. Three patients died of Epstein-Barr virus (EBV)-associated lymphoproliferative disease. A fourth patient developed an EBV lymphoma at day 58; immunosuppression was discontinued and the disease resolved. The patient is alive and well 54 months posttransplant. Although GVHD was the primary cause of death in only one patient, acute GVHD was a contributing factor in eight other deaths.

Fatal veno-occlusive disease (VOD) was the cause of death in two patients. One patient had ALL and was in a third CR, but had previously received cranial-spinal irradiation for CNS disease. The other patient had ALL and was in a fourth CR.

Nonfatal hemolytic uremia syndrome (HUS) occurred in four patients and resolved in two without sequelae. However, two patients continue to have elevated serum creatinine (greater than four times baseline values) and require erythropoietin to maintain their hemoglobin values. Both patients are fully functional, attend school full-time, and are now off erythropoietin. Hemorrhagic cystitis developed in four patients, but was managed with increased fluids and did not require catheterization or bladder instillation of sclerosing agents. One patient has severe chronic pulmonary disease, but is able to attend college full-time. Three patients developed clinical and radiographic evidence of avascular necrosis. One patient developed a CNS glioblastoma, received radiation therapy, and is in CR. The median Karnofsky score of the surviving patients is 100% (range, 70 to 100).

The CMV status of recipients was evaluable in 48 patients. Eight patients were seropositive and seven seroconverted CMV posttransplant, compared with only 2 of 40 who were CMV seronegative (P < 0.002). Six of the eight seropositive patients developed interstitial pneumonia; CMV was the primary cause of death in three patients and was suspected as a contributing cause in two others. There was no significant difference between survival and recipient CMV status.

Adenovirus infections were detected in 14 patients. Nine isolates were from rectal swabs, four were from throat swabs, and one was from lung tissue at autopsy. Adenovirus infection was felt to be a contributing cause of death in three patients.

**DISCUSSION**

The treatment of choice for patients with certain forms of leukemia is BMT using an HLA-matched or single antigen mismatched family member as the donor. Use of greater mismatched family donors and unrelated donors has been marked by an increase in nonengraftment, severe GVHD, and other treatment complications. Our results indicate the feasibility of using matched and mismatched donors.

![Fig 6. Probability of relapse as a function of time after transplantation. Patients currently disease free are represented by tick marks.](image-url)

![Fig 7. Primary cause of death after transplantation in 25 patients.](image-url)
unrelated donors in concert with T-cell depletion for the transplantation of children with leukemia and myelodysplasia without significant graft failure or acute GVHD. In our cohort, only one patient failed to engraft. Furthermore, the severity of GVHD did not differ greatly from HLA-matched sibling transplants in children.\textsuperscript{50,51} Only 4 of 49 patients exhibited severe (grades III-IV) acute GVHD, and 33\% experienced acute GVHD of grades II-IV. The long-term disease-free survival rates for the patients reported in this study are comparable with those reported in studies using HLA-matched siblings for the same disease states.\textsuperscript{50}

These encouraging results may be related to a number of factors. The first of these is the GVHD prophylaxis regimen used in the present study. GVHD prophylaxis consisting of T-cell depletion with T10B9 and posttransplant CsA allowed for successful transplantation of marrow from donors who were not fully HLA matched with the recipient. Studies performed at the Medical College of Wisconsin (Milwaukee, WI) and at the University of Kentucky (Lexington, KY) have shown that the antibody is directed to the $\alpha/\beta$ T-cell receptor heterodimer.\textsuperscript{33,34,52} Consequently, the percent $\alpha/\beta$ T cells postpurging is significantly lessened, whereas the percentage of $\gamma/\delta$ T cells is increased.\textsuperscript{52} $\alpha/\beta$-bearing T cells have been implicated in the pathogenesis of acute GVHD.\textsuperscript{53} $\gamma/\delta$ T cells, along with natural killer (NK) cells, may be helpful in ensuring engraftment by eradicating residual host cells.\textsuperscript{54} Analysis of the most recent 125 T-cell depleted marrow grafts continues to show a log 10 T-cell depletion of 1.7 ± 0.35 determined by a limiting dilution analysis (unpublished data, September 1994). In other series, more extensive T-cell depletion has been associated with a higher rate of graft rejection.\textsuperscript{55-57} Thus, the lower rejection rate reported here may reflect both a quantitative and qualitative difference in T-cell depletion techniques.

Another reason for the low incidence of severe acute GVHD, as compared with other unrelated transplant reports, may be the prompt treatment of mild cutaneous GVHD. The overall incidence of chronic GVHD was higher in this study than for pediatric matched sibling transplants,\textsuperscript{59} but similar to other reports using unrelated or mismatched family member donors.\textsuperscript{15,11,17} However, most of the chronic GVHD was very mild cutaneous involvement. Extensive chronic GVHD occurred in only five patients and all of these patients are currently surviving with Karnofsky scores of 70 to 100.

Although its cellular basis is not well delineated, the graft-versus-leukemia (GVL) effect has been extensively documented in murine systems.\textsuperscript{58,59} Evidence for a similar phenomenon in humans has been largely inferred from retrospective clinical studies.\textsuperscript{50-62} Recently, CML patients who relapsed after matched sibling BMT have returned to complete hematologic, cytogenetic, and molecular remission after receiving donor leukocytes, suggesting a direct GVL effect from a cellular mechanism.\textsuperscript{63-65} AML patients transplanted with marrow from identical twin donors have a higher incidence of relapse when compared to patients without GVHD who received matched sibling grafts.\textsuperscript{61} This implies a GVL effect in the absence of clinical GVHD. The low incidence of relapse in the present study (16\%) may reflect a GVL effect despite a relatively low incidence of significant acute GVHD.

Many centers involved in unrelated donor transplants only perform the procedure when they are able to obtain an HLA-A, -B, and -DR matched donor who is MLC nonreactive. Such criteria limit the opportunity to identify an unrelated donor for most patients. Beatty et al\textsuperscript{66} have been successful with minor HLA-mismatches. They reported no difference with regard to survival between patients with complete matches and those with single antigen cross-reactive mismatches in the absence of T-cell depletion. However, the mismatched group of patients did experience a higher incidence of grades II-IV GVHD (95\%). Three quarters of the patients in our study were mismatched and only four had a cross-reaction mismatch. Despite these HLA disparities, the conditioning regimen and GVHD prophylaxis techniques allowed us to expand the acceptable donor pool with an incidence of grade II-IV GVHD of 39\%.

The recent proliferation of molecular approaches to HLA typing will continue to alter donor selection criteria. Molecular methods are more sensitive and accurate and have a higher resolution than serotyping.\textsuperscript{24,26} In oligotyping, amplified HLA genes are hybridized with a panel of probes to detect key polymorphic sequences in the amplified products. At the present time, HLA-DR and DQ are routinely evaluated using oligotyping, whereas molecular typing of the class I antigens is proceeding in several research labs. If all HLA typing were to be performed on a molecular level, many cases accepted as serologic matches would likely be identified as HLA mismatches. The clinical importance of these molecular mismatches remains to be determined. Whereas the number of amino acid differences between two antigens may be important, their position within the HLA chains and their ability to induce cytotoxic T cells (allorecognition) may be more important.\textsuperscript{66}

Donors for the last 28 patients in this series were selected on the basis of molecular typing of the HLA-D region. Although these patients have experienced less acute GVHD (\textasciitilde grade II) than the previous 22 patients (22\% vs 45\%), the patient numbers are too small for definitive conclusions when variables such as disease state and degree of donor mismatch for class I HLA antigens are taken into consideration.

The intensity of our conditioning regimen may be a factor in our incidence of graft failure (2\%). Because a T-cell depletion technique was used for prophylaxis of GVHD, total body irradiation (TBI) was considered to be an essential component of the conditioning regimen. Reports in the literature have documented poor engraftment when T-cell depletion is a component of the GVHD prophylaxis for matched sibling transplants.\textsuperscript{55,67,68} However, escalating the total dosage of TBI has improved the engraftment rate.\textsuperscript{69,70} Therefore, it seemed reasonable that if we were to perform mismatched transplants and use T-cell depletion for GVHD prophylaxis, an intensive regimen that included TBI would be necessary. Furthermore, TBI is important not only for its immunosuppressive effects, but also for its value as an ant leukemia agent.\textsuperscript{67,71} In this study, the dose of TBI was 1,400 cGy given
in nine fractions over 3 days with liver, lung, and kidney shielding. Because of the frequency of HUS, especially in the adult population, the dose to the kidneys was decreased from 1,400 to 980 cGy. This has resulted in a marked decrease in the occurrence of this problem.

Busulfan was incorporated into the treatment regimen for patients with myelodysplasia, advanced acute leukemia, and CML in AP or BC. Initially, the dose was 4 mg/kg/d for 2 days, but because of concerns for VOD and the fact that many patients were heavily pretreated, the dose was decreased to 2 mg/kg/d. Pharmacokinetic studies were not performed. Such studies may be important in the future because busulfan kinetics vary with patient age.75,76

The role of splenectomy for patients with CML or JCML and an enlarged spleen, who also receive a T-cell-depleted unrelated donor BMT, is uncertain. The positive effect on disease control is anecdotal, but there is data suggesting that engraftment occurs more quickly in patients who have been splenectomized.75,76 These potential benefits must be weighed against the risks of postsplenectomy infection.77 Splenectomy should be considered before conditioning in patients with significant splenomegaly or evidence of hypersplenism.

Infectious complications were a major source of morbidity in this study. The four patients who were CMV seropositive and received marrow from CMV seropositive donors all died with evidence of CMV infection. Ganciclovir prophylaxis for cases where donor and/or recipient are CMV seropositive has shown promise.78,79 Although detection of CMV at an early stage of reactivation may be possible, treatment in the setting of unrelated donor BMT may be different or more difficult than in the setting of a matched sibling transplant.80

Toxicities related to previous therapy and the conditioning regimen per se have involved the liver, lungs, and kidneys. VOD of the liver was initially a significant problem, but the incidence of VOD has decreased concomitantly with a decrease in the dose of busulfan. Similarly, posttransplant HUS decreased with increased shielding of the kidneys. Interstitial pneumonitis caused by CMV has been discussed previously. Recent reports suggest that human herpesvirus 6 (HHV-6) may be responsible for a number of cases of what is now called idiopathic interstitial pneumonitis, unexplained marrow suppression, or an enchephalopathic condition.81,82

In summary, unrelated donor transplants, even with a major HLA mismatch, can be successful. They offer long-term disease-free survival and leukemia cure in patients who would otherwise die of their disease. Therefore, patients should be considered for such transplants earlier rather than later in their disease course. This field has undergone rapid evolution, and it is likely that with additional knowledge and insight, the remaining gap between sibling and unrelated donor transplantation can be further narrowed.

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