Chimerism and clinical outcomes of 110 recipients of unrelated donor bone marrow transplants who underwent conditioning with low-dose, single-exposure total body irradiation and cyclophosphamide


We hypothesized that low-dose (550-cGy), single-exposure, high dose rate (30 cGy/min) total body irradiation (TBI) with cyclophosphamide as conditioning for HLA-compatible unrelated donor (URD) bone marrow transplantation (BMT) would result in donor chimerism (DC) with a low risk for serious organ toxicity and treatment-related mortality (TRM). Twenty-six patients with good risk diagnoses (acute leukemia in first complete remission [CR] and chronic-phase chronic myelogenous leukemia [CML]) and 84 with poor risk diagnoses underwent this regimen and URD BMT. Unsorted marrow nucleated cells were assessed for chimerism using VNTR probes. All DC occurred in 78 (86%) of 91 evaluable patients at 1 or more follow-up points. Graft failure occurred in 7 (7.7%) patients. Fatal organ toxicity occurred in only 2% of patients. TRM rates through 2 years of follow-up were 19% and 42% in those with good and poor risk diagnoses, respectively. Overall and disease-free survival rates in the good risk group were 47% and 40%, respectively, and in the poor risk group they were 25% and 21%, respectively, at a median follow-up for living patients of 850 days (range, 354-1588 days). This regimen resulted in 100% DC in most patients undergoing URD BMT with a relatively low risk for fatal organ toxicity and TRM. (Blood. 2005;105:3035-3041)

Introduction

Treatment-related mortality (TRM) is a frequent complication of unrelated donor (URD) bone marrow transplantation (BMT). In more than 1400 patients with chronic myelogenous leukemia (CML) undergoing URD BMT facilitated by the National Marrow Donor Program (NMDP), the overall risk for TRM was 58%.1 Most adverse events occurred within 1 year of transplantation. Respiratory failure and other organ toxicity, graft-versus-host disease (GVHD), and infections were the most common causes of TRM. TRM rates are even higher in patients with poor risk diagnoses.2 Most regimens for URD BMT include total body irradiation (TBI).1 In related donor (RD) BMT, TBI is usually given in total doses of 1000 cGy or greater, in fractionated schemas, and at low dose rates (7-10 cGy/min). In comparison, URD BMT conditioning regimens usually consist of a higher TBI total dose or additional immunosuppressive agents to reduce the risk for graft failure. These more intense regimens result in an increased risk for serious organ toxicity and contribute to the higher risk for TRM observed with URD BMT.

Strategies to reduce the risk for regimen-related organ toxicity could reduce the risk for TRM after donor transplantation. In dog models, 450 cGy TBI alone given at a single exposure at high dose rates (70 cGy/min) resulted in durable dog leukocyte antigen (DLA)-identical littermate donor cell engraftment with minimal organ toxicity.3 In humans undergoing HLA-matched RD BMT, preliminary experience from the University of Toronto group supported the feasibility of a TBI-based conditioning regimen that incorporated a similar strategy of low-dose TBI (500 cGy) given as a single exposure at a high dose rate (median, 58 cGy/min; range, 42-91 cGy/min).4 Based on these data, we altered the conventional TBI and cyclophosphamide–conditioning regimen for HLA-matched RD peripheral blood stem cell (PBSC) transplantation by using a low dose (550 cGy) of TBI administered as a single dose and at a high dose rate (30 cGy/min).5 Patients conditioned with this regimen had complete and durable donor cell engraftment, absence of graft failure, and low risk (2.5%) for fatal organ toxicity. The TRM rates through a minimum of 2 years of follow-up were 7% and 19% in patients with good and poor risk diagnoses, respectively. These outcomes compare favorably with the 14.8% risk for fatal organ toxicity and 21% risk for TRM at 2 years of follow-up recently reported using conventional myeloablative conditioning regimens for patients with good and poor risk diagnoses who underwent HLA-matched RD PBSC transplantation.6 Thus, we hypothesized that a similar strategy of administering low-dose (550-cGy), single-exposure TBI at a high dose rate (30 cGy/min) with cyclophosphamide would result in consistent and durable engraftment of HLA-compatible URD stem cells with low risk for serious organ toxicity and TRM.

Patients, materials, and methods

Patient eligibility

Patients with the following diagnoses were eligible: acute myeloid leukemia (AML) or acute lymphoid leukemia (ALL) in first complete remission.
(CR1) with high risk for relapsed disease or with relapsed disease, CML in chronic or accelerated/blast phase, myelodysplastic syndrome (MDS), poor risk non-Hodgkin lymphoma (NHL) as previously defined,3 recurrent chronic lymphocytic leukemia (CLL), multiple myeloma (MM), myelofibrosis (MF), severe aplastic anemia (SAA), polycthyemia vera (PVera), and essential thrombocytocemia (ET). Good risk diagnoses were defined as acute leukemia in CR1 or CML in chronic phase (CP), whereas poor risk diagnoses included all others. Patients with good risk diagnoses and those at high risk for relapse were previously defined but also included patients with CML in late CP (diagnosis more than 1 year earlier).5 Exclusion criteria included age older than 65 years, performance status greater than 2 (Eastern Cooperative Oncology Group [ECOG]), left ventricular ejection fraction (LVEF) less than 40%, diffusion lung capacity of carbon monoxide corrected for hemoglobin less than 40% of predicted, serum creatinine greater than 2.0 mg/dL, liver enzymes greater than 5 times normal, active infection, HIV positivity, or positive pregnancy test results. The Institutional Review Board of Washington University School of Medicine approved the study, and each patient gave written informed consent. The study accrued 116 patients from November 1997 through July 2001, of whom 110 were evaluable and were included in this analysis. Reasons for exclusion included use of PBSCs (vs bone marrow [BM]) in 2 patients and incomplete/available medical records in 4.

Donor selection

Unrelated donor selection and BM collection were facilitated by the National Marrow Donor Program (NMDP). Primary criteria for donor selection included HLA compatibility, defined as a 6 of 6 antigen match (at A, B, and DRB1 loci) or a 5 of 6 antigen match (single-antigen mismatch at A, B, or DRB1 locus), with preference for the former. Secondary criteria included preference for donors who were ABO compatible, cytomegaloivirus (CMV) seronegative, and male. Confirmatory HLA typing was performed at this institution. HLA class 1 antigens were serologically typed using standard microlymphocyte-toxicity assay methods,7 whereas high-resolution DRB1 typing was performed by polymerase chain reaction (PCR) amplification using single-stranded oligonucleotide probes (SSO probes).8 Class 1 antigens were typed using low-resolution DNA methods beginning in December 2000.8

Bone marrow cells were not T-cell depleted but were red blood cell and plasma reduced, as appropriate, if the donor and patient were ABO incompatible. Marrow cells were infused while fresh into the recipient within 24 hours of collection. The date of marrow infusion was defined as transplantation day 0.

Conditioning regimen

The regimen included cyclophosphamide 60 mg/kg per day, given on days −3 and −2, followed by 550 cGy TBI administered in a single dose on day −1. The median dose rate was 32.4 cGy/min (range, 26.8-36.6 cGy/min). TBI was delivered in parallel opposed lateral fields with 6-MeV photons using a Clinac 600 CD (Varian Medical Systems, Alpharetta, GA), as previously published.5

GVHD prophylaxis, evaluation, and treatment

GVHD prophylaxis included cyclosporine given to day 100 as a continuous intravenous infusion of 3 mg/kg per day beginning on day −2, with dose adjustments made to achieve the drug level of 200 to 400 ng/mL. At day 100, cyclosporine was switched to oral dosing and was tapered at a rate of 5% per week in patients without active GVHD. Methotrexate was administered intravenously on day 1 (10 mg/m2) and on days 3 and 6 (7.5 mg/m2). Methylprednisolone (1 mg/kg per day) was given from days 7 to 28 and then was tapered over 6 weeks. Forty-eight patients participated in a phase 2 study investigating the addition of hydroxychloroquine to the GVHD prophylaxis regimen.9

Acute GVHD (aGVHD) was graded according to Glucksberg et al10 and the International Bone Marrow Transplant Registry (IBMTR) Severity Index.11 Chronic GVHD was graded as per Shulman et al.12 Diagnoses of GVHD were based on clinical manifestations and histologic examination of biopsy specimens of affected tissues. Therapy for GVHD was previously defined.5

Supportive care

Transfusion support, hydration and mesna, antimicrobials, and CMV surveillance monitoring and treatment were performed as previously described,3 with the following exceptions: granulocyte-colony-stimulating factor (G-CSF) (10 µg/kg per day subcutaneously) was administered to patients beginning on day 7, and prophylactic intravenous ciprofloxacin, ciprofloxacin, and rifampin were given.

Evaluation, end points, and statistics

Pretransplantation disease status was defined as CR1, CR2 or beyond, or relapse. Posttransplantation evaluations were performed on days 30, 100, 180, year 1, and annually thereafter. Each evaluation included an assessment of the study end points: marrow chimerism, disease status (CR or relapse), survival status (alive or dead), treatment-related toxicity and TRM, and GVHD.

Chimerism was assessed using a variable number of tandem repeat (VNTR) probes, as described.5,11,14 DNA was isolated from unseparated white blood cells from pretransplantation donor and recipient peripheral whole blood samples and posttransplantation recipient unfractionated BM. Sensitivity of detection by this method was 1% to 5%. One informative marker was selected for the study of posttransplantation engraftment patterns in all follow-up specimens. Separation of white blood cells into subsets for chimerism analysis was not performed. Engraftment was defined as 100% if unseparated marrow cells were only of donor origin, as determined by VNTR probes, or mixed chimerism (MC) if marrow cells were of donor and host origin. Primary and secondary graft failures were previously defined.5 Patients with relapsed disease were not evaluable for engraftment or graft failure during or after relapse.

Treatment-related organ toxicity was graded using the National Cancer Institute (NCI) criteria for BMT studies, in which grade 0 indicated no organ toxicity, grade 5 indicated fatal toxicity, and grades 1 to 4 indicated increasing levels of toxicity.15 Idiopathic interstitial pneumonitis was previously defined.5 With the exception of disease relapse, TRM was defined as death from any cause after transplantation. Patients who died before neutrophil engraftment without evident GVHD were not evaluable for aGVHD. Patients who died or had relapses before day 100 were not evaluable for chronic GVHD.

Relapsed disease was defined as morphologic or radiographic evidence of disease occurring at any single posttransplantation evaluation. Relapse of CML was previously defined.5 Disease-free survival (DFS) and overall survival (OS) were assessed using the Kaplan-Meier (KM) technique16 and were determined from the day of transplantation (day 0). Follow-up was performed through August 10, 2002. Data analysis was performed using the StatView program (version 4.5; Abacus Concepts, Berkeley, CA).

Results

Patient and BM characteristics

One hundred ten evaluable patients were accrued to this study. Patient characteristics are detailed in Table 1. The median age was 43.5 years (range, 19-62 years). Twenty-six of the 110 patients had good risk diagnoses, and 84 patients had poor risk diagnoses. Most (18 of 26) patients with good risk diagnoses had characteristics considered high risk for relapse. Median (range) numbers of marrow total nucleated cells (TNCs)/kg, CD34+ cells/kg, and CD3+ cells/kg transplanted were 2.81 (0.24-16.9) × 106, 3.17 (0.66-15.9) × 106, and 4.44 (0.77-39.0) × 107, respectively.

HLA matching

HLA-A,-B, and -DRB1 (6 of 6) matching occurred in 89 patients, for whom class 1 antigen typing was performed by serologic (69
Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. patients or units (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total patients</td>
<td>110</td>
</tr>
<tr>
<td>Median age, y</td>
<td>43.5 (19-62)</td>
</tr>
</tbody>
</table>

**Diagnosis**
- Acute leukemia: 55
- CR: 17
- CR2: 15
- CML: 20
- CP*: 9
- AP: 4
- BP: 7
- MDS: 16
- RA: 10
- Other: 6
- NHL: 12
- CR: 2
- Persistent disease: 10
- Other†: 7

**Diagnostic risk group‡**
- Acute leukemia: 55
- CR: 17
- CR2: 15
- CML: 20
- CP*: 9
- AP: 4
- BP: 7
- MDS: 16
- RA: 10
- Other: 6
- NHL: 12
- CR: 2
- Persistent disease: 10
- Other†: 7

**Follow-up for living patients, d**
- Day 30: 71
- Day 100: 47
- Day 180: 44
- Year 1: 32
- Year 2: 13
- Year 3: 4

**No. patients (range)**
- 850 (354-1588)

*pIncludes 6 and 3 patients who underwent transplantation less than or more than 2 years from diagnosis, respectively.

†Good indicates acute leukemia in CR1 or CML in CP. Poor indicates acute leukemia in relapse or CR2 or beyond, CML in accelerated phase (AP) or blast phase (BP), MDS, NHL, CML, AAL, or MF.

‡Includes patients with good risk diagnoses having high-risk characteristics as defined in “Patients, materials, and methods” (14), high peripheral blast count at diagnosis [2], late CP [2].

RA indicates refractory anemia.

Table 2. Chimerism of unseparated marrow nucleated cells

<table>
<thead>
<tr>
<th>No. patients per percentages of donor cells at evaluation</th>
<th>No. nonevaluable patients †</th>
</tr>
</thead>
<tbody>
<tr>
<td>Posttransplantation evaluation</td>
<td>100%</td>
</tr>
<tr>
<td>Day 30</td>
<td>71</td>
</tr>
<tr>
<td>Day 100</td>
<td>47</td>
</tr>
<tr>
<td>Day 180</td>
<td>44</td>
</tr>
<tr>
<td>Year 1</td>
<td>32</td>
</tr>
<tr>
<td>Year 2</td>
<td>13</td>
</tr>
<tr>
<td>Year 3</td>
<td>4</td>
</tr>
</tbody>
</table>

*Method used for limits of detection by VNTR was 1% or more recipient cells.

†Nonevaluable because of relapse, death, evaluation not performed, or short follow-up.

Most patients had 100% donor cell engraftment in unseparated nucleated BM cells at each time point assessed after transplantation. The percentages of evaluable patients with 100% donor chimerism (DC) at days 30, 100, 180, year 1, year 2, and year 3 after transplantation were 78%, 76%, 88%, 94%, 93%, and 100%, respectively (Table 2). Overall, 86% (78 of 91) of evaluable patients achieved 100% DC at 1 or more of the follow-up time points. Of the 91 evaluable patients, primary and secondary graft failure occurred in 86% and 1%, respectively. Of the 6 patients with primary graft failures, 3 had CML, 2 had MDS, and 1 had NHL.

The only case of secondary graft failure occurred in a patient with CML. None of the 56 patients with acute leukemia experienced graft failure; however, 9 of 17 evaluable patients with CML experienced graft failure (4) or MC (5) at 1 or more time points after transplantation. Of the 7 patients who experienced graft failure, 4 were HLA-A, -B, -C, -DRB1, and -DQB1 (10 of 10) matched, whereas 3 were not (all 3 were mismatches at HLA-C).

Relapse outcomes of the 23 patients (25% of those evaluable) who experienced MC at 1 or more follow-up time points after transplantation were reviewed (Figure 1). Eleven of these patients subsequently converted back to 100% DC, with 3 (27%) of those patients later experiencing disease relapse. Of the other 11 patients who had persistent MC at last follow-up, 6 (55%) had relapses. Additionally, 1 patient experienced secondary graft failure. Overall, the rate of relapse in patients who on day 30 had 100% DC or had MC was 30% (21 of 71) and 57% (8 of 14), respectively (P = .06). Rates of risk for relapse in patients who had MC at day 30 compared with those who achieved 100% DC at day 30 but had MC at a later follow-up were 57% and 12.5% (P = .03), respectively. Notably, the proportions of patients with good and poor risk diagnoses in the 100% DC and MC cohorts were not significantly different (data not shown).

The potential effect of HLA matching on 100% DC at day 30 was analyzed (Table 3). At day 30, 100% DC was achieved in 75% of HLA-A, -B, and -DRB1 (6 of 6) matched and 93% of mismatched (P = .18) patients. The likelihood of achieving 100% DC at day 30 in HLA-A, -B, -C, -DRB1, and -DQB1-matched (10 of 10) patients was similar to that observed in those who were not 10 of 10 matched (75% vs 82% respectively; P = .60)

**Treatment-related organ toxicity**

Fatal organ toxicity (NCI grade 5) occurred in only 2 of 110 patients, whereas life-threatening (grade 4) organ toxicity occurred in 32 patients or low-resolution DNA (20 patients) methods. Of these 89 patients, 62 were also HLA-C and -DQB1 (10 of 10) matched. Single-antigen A/B or DRB1 mismatching occurred in 16 and 4 patients, respectively, and 1 patient was mismatched at a single antigen at the A and the DRB1 loci. Of those mismatched at A/B, class 1 antigen typing was performed using serologic (14) or low-resolution DNA (3) methods. Overall, HLA-C mismatching occurred in 32 patients, including 25 and 7 patients typed by serologic and DNA methods, respectively.

**Figure 1. Outcomes of patients with MC.** One other patient (not included in Figure 1 because of residual ALL on day +30) had 90% donor chimerism by DNA analysis (VNTR) and 100% donor chimerism by cytogenetic analysis. After discontinuation of immunosuppression, this patient had 95% donor chimerism and no evidence of ALL. Thus, the patient was considered evaluable for engraftment on day 30. A-CR, patients alive in complete remission; A-R, alive in relapse; D-R, death due to relapse; D-TRM, death due to transplant-related mortality; f/u, follow-up. *Patient who remained MC on day +100 and then experienced secondary graft failure after day +100. This patient had CML and underwent a sex-mismatched transplant. The routine cytogenetics showed 46 XX and the fluorescent in situ hybridization (FISH) and reverse transcriptase-polymerase chain reaction (RT-PCR) for bcr/abl were negative following the secondary graft failure.
in 5 patients (Table 4). One patient with cardiomyopathy before transplantation died on day 5 because of cardiac toxicity. One patient died of renal toxicity caused by thrombotic thrombocytopenic purpura. All 3 cases of grade 4 liver-related toxicities were caused by veno-occlusive disease. Grade 4 mucositis and grade 4 bladder toxicity (hemorrhagic cystitis) occurred in 1 patient each.

The frequency of the maximum regimen-related organ toxicity (RROT) experienced by each of the patients was grades 0 to 1 in 27 patients, grades 2 to 3 in 77 patients, and grades 4 to 5 in 6 patients. Grade 3 central nervous system (CNS) toxicity occurred in 11 patients and was attributed to cyclosporine. CNS toxicity resolved in 9 of 11 patients with the discontinuation of cyclosporine. Two patients died because of other causes before CNS toxicity resolved.

Infection
Forty-one patients contracted infectious pneumonia; 21 of them had active GVHD. The causes of the pneumonias were bacterial (14), viral (11), fungal (4), and culture negative (12). Of these 41 patients, 6 were alive at last follow-up, 9 died of disease relapse, and 26 died of TRM.

GVHD
Nine patients were not evaluable for aGVHD because of primary graft failure (6) or death before engraftment (3). Of the 101 evaluable patients, 38 experienced no aGVHD. The numbers of patients who experienced grades 1, 2, and 3-4 aGVHD were 30, 15, and 18, respectively. Using the IBMTR Severity Index, the numbers of patients who developed index 0, A, B, C, and D aGVHD were 38, 14, 7, 11, and 11, respectively. Sixty-four patients were evaluable for chronic GVHD. Limited and extensive chronic GVHD occurred in 7 (11%) and 38 (59%) patients, respectively.

Hematologic recovery, febrile days

The median (range) of posttransplantation days to recovery of an absolute neutrophil count (ANC) greater than 500/μL were 14 (10-24 days) and a platelet count greater than 50 000/μL was 26 (14-229 days). During the initial hospitalization, the median number of febrile days was 1 (0-20 days), and the number of days of intravenous antibiotics was 12 (0-56 days). Forty-three patients were afebrile throughout the hospital stay for transplantation.

Survival, TRM, and relapse

The median (range) follow-up for living patients was 850 (354-1588) days after transplantation. Thirty-two patients were alive at last follow-up, 29 were in CR, and 3 had relapsed disease. Seventy-eight patients died (38 because of relapse and 40 because of TRM). Of the 26 good risk patients, 11 (42%) are alive in CR; of the 84 poor risk patients, 18 (21%) are alive in CR, and 3 (4%) are alive with relapsed disease. Relapse of disease occurred in 41 patients, including 10 (38%) from the good risk group and 31 (37%) from the poor risk group.

Fatal RROT was an infrequent (2%) cause of TRM. The most common cause of TRM was bacterial infection (22 patients). Other causes of TRM included GVHD (6), viral (3), fungal infection (4), graft failure (2), and other (1 anaphylaxis from platelet transfusion). Cumulative rates of TRM in the patients with good and poor risk diagnoses were 19% and 42%, respectively. Overall, 21 (19%) patients died of TRM before day 100, and 33 (30%) patients died of TRM before year 1.

At a median follow-up of 850 days, the KM estimates of OS and DFS for patients in each diagnostic risk group are shown in Figures 2 and 3. OS and DFS rates were 47% and 40% for good risk patients and 25% and 21% for poor risk patients, respectively. OS

Table 3. Effect of HLA matching on donor cell engraftment

<table>
<thead>
<tr>
<th></th>
<th>Graft failure, no.</th>
<th>100% DC, % (n per total on day 30)</th>
<th>P</th>
<th>MC, % (n per total on day 30)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HLA-A,-B,-DRB1 (6 of 6) match</td>
<td>Yes</td>
<td>7</td>
<td>75 (57 of 76)</td>
<td>.18</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>0</td>
<td>93 (14 of 15)</td>
<td>.18</td>
</tr>
<tr>
<td>HLA-A,-B,-C,-DRB1,-DQB1 (10 of 10) match</td>
<td>Yes</td>
<td>4</td>
<td>75 (43 of 57)</td>
<td>.60</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>3</td>
<td>82 (28 of 34)</td>
<td>.60</td>
</tr>
<tr>
<td>HLA-C match*</td>
<td>Yes</td>
<td>4</td>
<td>77 (51 of 66)</td>
<td>.99</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>3</td>
<td>79 (19 of 24)</td>
<td>.99</td>
</tr>
</tbody>
</table>

Percentages are derived from number of evaluable patients.
— indicates .
*One patient, who was a 5 of 6 match, did not undergo HLA-C typing.

Table 4. Organ toxicity

<table>
<thead>
<tr>
<th>Organ</th>
<th>Toxicity grade</th>
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<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulmonary</td>
<td>108</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Renal</td>
<td>69</td>
<td>23</td>
<td>12</td>
<td>5</td>
<td>0</td>
<td>1</td>
<td>0</td>
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<tr>
<td>CNS</td>
<td>93</td>
<td>2</td>
<td>4</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Bladder</td>
<td>100</td>
<td>2</td>
<td>5</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Oral mucosa</td>
<td>22</td>
<td>32</td>
<td>46</td>
<td>9</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Liver</td>
<td>65</td>
<td>23</td>
<td>13</td>
<td>6</td>
<td>3</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Cardiac</td>
<td>98</td>
<td>1</td>
<td>3</td>
<td>7</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

Grade 0 indicates no toxicity; 1-3, increasing toxicity; 4 life-threatening toxicity; 5, fatal toxicity.
*Toxicity was graded according to NCI common toxicity criteria for BMT.

Figure 2. KM estimate of OS of 110 patients undergoing URD BMT and conditioning with 550-cGy single-dose TBI and cyclophosphamide. Tick marks represent living patients.
and DFS rates were 48% and 51% for patients with acute leukemia in CR1, 38% and 38% for patients in CR2, and 4% and 0% for patients in relapse, respectively. OS and DFS rates were 44% and 20% for patients with CML in accelerated phase, and 0% and 0% for patients in relapse, respectively. OS and DFS rates were 44% and 20% for patients with CML in accelerated phase, and 0% and 0% for patients in relapse, respectively. OS and DFS rates were 44% and 20% for patients with CML in accelerated phase, and 0% and 0% for patients in relapse, respectively. OS and DFS rates were 44% and 20% for patients with CML in accelerated phase, and 0% and 0% for patients in relapse, respectively. OS and DFS rates were 44% and 20% for patients with CML in accelerated phase, and 0% and 0% for patients in relapse, respectively. OS and DFS rates were 44% and 20% for patients with CML in accelerated phase, and 0% and 0% for patients in relapse, respectively. OS and DFS rates were 44% and 20% for patients with CML in accelerated phase, and 0% and 0% for patients in relapse, respectively. OS and DFS rates were 44% and 20% for patients with CML in accelerated phase, and 0% and 0% for patients in relapse, respectively. OS and DFS rates were 44% and 20% for patients with CML in accelerated phase, and 0% and 0% for patients in relapse, respectively. OS and DFS rates were 44% and 20% for patients with CML in accelerated phase, and 0% and 0% for patients in relapse, respectively. OS and DFS rates were 44% and 20% for patients with CML in accelerated phase, and 0% and 0% for patients in relapse, respectively. OS and DFS rates were 44% and 20% for patients with CML in accelerated phase, and 0% and 0% for patients in relapse, respectively. OS and DFS rates were 44% and 20% for patients with CML in accelerated phase, and 0% and 0% for patients in relapse, respectively. OS and DFS rates were 44% and 20% for patients with CML in accelerated phase, and 0% and 0% for patients in relapse, respectively. OS and DFS rates were 44% and 20% for patients with CML in accelerated phase, and 0% and 0% for patients in relapse, respectively. OS and DFS rates were 44% and 20% for patients with CML in accelerated phase, and 0% and 0% for patients in relapse, respectively. OS and DFS rates were 44% and 20% for patients with CML in accelerated phase, and 0% and 0% for patients in relapse, respectively.
MDS were 23.5% and 18%, respectively, whereas in 2 NMDP studies, risk rates for graft failure in patients with CML and MDS conditioned with standard regimens were 15.5% and 13%, respectively. Risk rates for graft failure in patients with CML and MDS after nonmyeloablative regimens and URD BMT have been reported in small studies to be 29% to 44% and 33%, respectively. We are evaluating the impact on graft failure of the addition of pretransplantation alemtuzumab to this regimen and the use of PBSCs (rather than BM).

The risk for TRM after conventional regimens for URD BMT is significant. In more than 1400 patients with CML undergoing URD BMT, the overall risk for TRM was 58%. TRM is even greater in patients with poorer risk diagnoses. Overall incidences of TRM observed after nonmyeloablative regimens for URD BMT ranged from 16% to 55%. Using this low-dose (550 cGy), single-exposure, TBI-based approach for URD BMT, risk rates for TRM in patients with good and poor risk diagnoses were 19% and 42%, respectively. TRM rates through day 100 after nonmyeloablative conditioning regimens have been reported to range from 11% to 37%, comparable to the 19% rate observed in our study. This relatively low risk for TRM likely reflects, in part, the very low (2%) risk for fatal RROT with this regimen. In large reports with conventional and nonmyeloablative regimens for URD BMT, the overall risk rates for fatal RROT were 11% and 4%, respectively. Thus, the risk for TRM after our regimen and other reduced-intensity regimens may be lower than that observed after conventional regimens.

The most common cause of TRM in our study was bacterial infections. In addition, infectious pneumonia was frequent. Comparable rates of these complications have been reported after conventional conditioning for URD BMT. These adverse events largely reflect the intense and lengthy immunosuppression required in all patients to prevent and treat GVHD. Indeed, the use of corticosteroids as GVHD prophylaxis may be associated with an increased risk for bacterial infections. Therefore, reducing the intensity of the conditioning regimen would likely have limited influence on the occurrence of these complications.

A challenge of any reduced-intensity conditioning regimen for URD BMT is to ensure that potential gains in curbing TRM are not counterbalanced by an increased risk for relapse. Using this low-dose, single-exposure, TBI-based regimen, risk rates for relapse in patients with good and poor risk diagnoses was 38% and 37%, respectively. The rate for relapse risk in patients with good risk diagnoses undergoing conventional conditioning for URD BMT is reported to range from 6% to 20%. A case could be made that this novel regimen may be associated with greater relapse risk than conventional conditioning in patients with good risk diagnoses; however, the differences in relapse risk may reflect patient selection bias. In our study, most (69%) patients with good risk diagnoses had characteristics associated with a high risk for relapse. Indeed, after conventional conditioning and allogeneic transplantation, patients with acute leukemia in CR1 with these same high risk features have a rate of relapse risk from 50% to 76%. For patients with poor risk diagnoses, the relapse rate with our regimen appears to be lower than that seen in other studies, where reported rates of relapse were 51% to 74%. Comparisons between our study and others are difficult because of potential confounding factors; however, the relapse risk we observed was generally favorable given the patient characteristics.

In this study, OS and DFS rates at a median follow-up of 850 days for patients with good risk diagnoses were 47% and 40%, respectively, and for poor risk diagnoses they were 25% and 21%, respectively. In a study of more than 300 patients with ALL undergoing conventional conditioning for URD BMT, DFS rates for good and poor risk features were 32% and 20%, respectively. In a study of 17 patients with acute leukemia in first or subsequent remission undergoing nonmyeloablative conditioning for URD BMT, 1-year OS and DFS rates were both 47%; however, the median follow-up was only 13 months. In our study, with a longer median follow-up, the OS and DFS rates of patients with acute leukemia in CR1 were 48% and 51%, respectively, and in CR2 they were 38% and 38%, respectively. In patients with CML undergoing conventional conditioning for URD BMT, the OS rate at 3 years was 37.5%, whereas the DFS rates at 3 years were 43%, 20%, and 10% for patients with CML in chronic, accelerated, and blast phase, respectively. In 14 patients with CML (11 in chronic phase) undergoing nonmyeloablative conditioning for URD BMT, the 1-year OS and DFS rates were 61% and 21%, respectively. In our study, the OS and DFS rates for patients with CML in chronic phase were 44% and 22%, respectively. Graft failure was a major contributor to the lower DFS rate observed in patients with CML in our study and in studies of nonmyeloablative regimens. With our regimen, patients with CML in accelerated or blast phase or with resistant acute leukemia fared poorly. Such patients also fare poorly with conventional or nonmyeloablative conditioning regimens. Comparisons of our data with other reports must be interpreted with caution because of potential confounding variables. The favorable survival outcomes of patients with acute leukemia in remission treated with our regimen warrant further controlled trials for comparison with alternative conditioning regimens.

In this study, we demonstrated the feasibility of this low-dose (550 cGy), single-exposure, TBI-based regimen to result in 100% DC of unseparated marrow nucleated cells in most patients undergoing URD BMT. The risk for fatal RROT was very low (2%). When placed in the context of other conditioning regimens for URD BMT, we observed TRM rates lower than those reported for conventional (more than 1000 cGy) TBI-based regimens and similar to those for 200 cGy TBI-based regimens (Table 5). The overall risk for disease relapse with our regimen appeared to be lower than that observed with a 200 cGy TBI-based regimen (37% vs 51%, respectively). OS and DFS rates at 1 year in patients with acute leukemia in complete remission were higher with our regimen than was observed with a 200 cGy TBI-based regimen or a conventional (more than 1000 cGy) TBI-based regimen (69% and

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**Table 5. Outcomes of adults undergoing URD BMT after conditioning with various myeloablative and reduced-intensity regimens**

<table>
<thead>
<tr>
<th>Source</th>
<th>Regimen</th>
<th>Median follow-up</th>
<th>GF, %</th>
<th>Fatal RROT, %</th>
<th>TRM, % 100/1/y</th>
<th>Disease recurrence, %</th>
<th>KM OS at 1 y AL/CML/MDS</th>
<th>KM DFS at 1 y AL/CML/MDS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cornellisssen et al&lt;sup&gt;10&lt;/sup&gt; n = 127&lt;sup&gt;†&lt;/sup&gt;</td>
<td>&gt; 1000 cGy/Cy†</td>
<td>2.0</td>
<td>13</td>
<td>30</td>
<td>45/52</td>
<td>18</td>
<td>40/—/—</td>
<td>40/—/—</td>
</tr>
<tr>
<td>Maris et al&lt;sup&gt;17&lt;/sup&gt; n = 89</td>
<td>200 cGy + Flu</td>
<td>1.1</td>
<td>21</td>
<td>8</td>
<td>11/16</td>
<td>51</td>
<td>47/61/29</td>
<td>47/21/25</td>
</tr>
<tr>
<td>Current study n = 110</td>
<td>550 cGy/Cy</td>
<td>2.3</td>
<td>7.7</td>
<td>2</td>
<td>18/30</td>
<td>37</td>
<td>68/45/31</td>
<td>60/30/25</td>
</tr>
</tbody>
</table>

GF indicates graft failure; Cy, cyclophosphamide; Flu, fludarabine; AL, leukemia in CR; —, NA.

<sup>†</sup>Regimens were Cy/TBI (67 patients), Cy/TBI + cytarabine (Ara-C) or etoposide (VP16) (34 patients), or other (26 patients).
of marrow (vs PBSCs) was an independent factor associated with significantly lower DFS (hazard ratio, 2.5; P = 0.006) and a higher risk for graft failure (hazard ratio, 4.7; P = 0.003).

These data strongly suggest that PBSCs should be the preferred source of stem cells with reduced-intensity regimens, and they imply that outcomes with our regimen may be further improved by switching from BM to PBSCs. Using PBSCs instead of BM with our regimen may improve DFS rates among all patients and may reduce the problem of graft failure in the subset of patients with CML and MDS. We are conducting additional clinical trials to evaluate these hypotheses.

References

17. Niederwieser D, Maris M, Shizuru JA. Low-dose total body irradiation (TBI) and fludarabine followed by hematopoietic stem-cell transplantation (HCT) from HLA-matched or mismatched unrelated donors and postgrafting immunosuppression with cyclosporine and methotrexate (MMF) can induce durable complete chimerism and sustained remissions in patients with hematologic diseases. Blood. 2003;101:1620-1629.
Chimerism and clinical outcomes of 110 recipients of unrelated donor bone marrow transplants who underwent conditioning with low-dose, single-exposure total body irradiation and cyclophosphamide