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%. 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. Most regimens for URD BMT include total body irradiation (TBI). 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. 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). 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). 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. 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), polycythemia vera (PVRA), and essential thrombocythemia (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/ unavailable 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, cytomegalovirus (CMV) seronegative, and male. Confirmatory HLA typing was performed by polymerase chain reaction (PCR) amplification using single-stranded oligonucleotide probes (SSO),5 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 (IBMT R) Severity Institute.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, 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 itraconazole, ciprofloxacin, and rifampin were given.

Evaluation, end points, and statistics

Pretransplantation disease status was defined as CR2, CR3, 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.5,13,14 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 CD34+ cells/kg transplanted were 2.81 (0.24-16.9) × 10⁹, 3.17 (0.66-15.9) × 10⁹, 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
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.

Chimerism and graft failure

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% (79 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 6 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 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; $P = .60$).

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 +30 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.

Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>No. patients or units (range)</th>
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<th>Median age, y</th>
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</tr>
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</tr>
<tr>
<td>CR0/3</td>
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</tr>
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<td></td>
<td></td>
</tr>
<tr>
<td>Persistent disease</td>
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<td></td>
</tr>
<tr>
<td>Other†</td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

**Diagnostic risk group‡**

| Good§                   | 26             |              |              |
| Poor                    | 84             |              |              |

| Follow-up for living patients, d | 850 (354-1588) |

Table 2. Chimerism of unseparated marrow nucleated cells

<table>
<thead>
<tr>
<th>No. patients per percentages of donor cells at evaluation</th>
<th>No. of patients per percentages of donor cells at evaluation</th>
<th>No. of patients per percentages of donor cells at evaluation</th>
</tr>
</thead>
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<tr>
<td>Posttransplantation evaluation</td>
<td>100%*</td>
<td>90%-99%</td>
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<tr>
<td>Day 30</td>
<td>71</td>
<td>8</td>
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<tr>
<td>Day 100</td>
<td>47</td>
<td>9</td>
</tr>
<tr>
<td>Day 180</td>
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<tr>
<td>Year 1</td>
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<td>Year 2</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>Year 3</td>
<td>4</td>
<td>0</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.

Fetal organ toxicity

Fatal organ toxicity (NCI grade 5) occurred in only 2 of 110 patients, whereas life-threatening (grade 4) organ toxicity occurred...
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, 7, 31, 14, 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/\muL was 14 (10-24 days) and a platelet count greater than 50 000/\muL 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>HLA-A, -B, -DRB1 (6 of 6) match</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>Yes</td>
<td>7</td>
<td>75 (57 of 76)</td>
<td>.18</td>
<td>25 (19 of 76)</td>
</tr>
<tr>
<td>No</td>
<td>0</td>
<td>93 (14 of 15)</td>
<td>.18</td>
<td>7 (1 of 15)</td>
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<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>
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<tr>
<td>No</td>
<td>3</td>
<td>82 (28 of 34)</td>
<td>.60</td>
<td>18 (6 of 34)</td>
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<tr>
<td>HLA-C match*</td>
<td>Yes</td>
<td>4</td>
<td>77 (51 of 66)</td>
<td>.99</td>
</tr>
<tr>
<td>No</td>
<td>3</td>
<td>79 (19 of 24)</td>
<td>.99</td>
<td>21 (5 of 24)</td>
</tr>
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</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>
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<tr>
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<tr>
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<td>32</td>
<td>46</td>
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<tr>
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<td>0</td>
<td>1</td>
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</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.
tion or donor lymphocyte infusion (DLI) can increase the number
of patients with CML in blast phase. Patients with CML in accelerated phase, and 0% and 0% for 22% for patients with CML in chronic phase, 50% and 25% for patients in relapse, respectively. OS and DFS rates were 44% and 100% DC by day 28.17 Subsequent withdrawal of immunosuppres-
sion (eg, cyclophosphamide, we recently reported that 100% DC was achieved in 96% of patients at day 30 and that DC was durable through 3 years of follow-up.5 These data formed the rationale for investigating the feasibility of this conditioning regimen for patients undergoing URD BMT. Our results demonstrate the efficacy of this regimen to result in 100% DC in unseparated marrow nucleated cells in most patients undergoing HLA-compatible URD BMT. We observed 100% DC in 78% of evaluable patients at day 30, and 86% of patients achieved 100% DC at 1 or more follow-ups after transplantation. Donor cell engraftment was durable, as evidenced by 100% DC through 3 years after transplantation. Six of 6 and 10 of 10-HLA matched patients had the same rate of 100% DC at day 30 (75%), and this outcome did not appear to be significantly affected by the single-antigen mismatches allowed for enrollment in the study. We were unable to make comparisons between our chimerism data and those observed after conventional conditioning for URD BMT; the latter were reports of patients who underwent T cell–depleted transplantation or reports that included living and disease-free patients.

Discussion

For patients undergoing HLA-matched RD PBSC transplantation with low-dose (550 cGy) single-exposure TBI given at a high dose rate (30 cGy/min) with cyclophosphamide, we recently reported that 100% DC was achieved in 96% of patients at day 30 and that DC was durable through 3 years of follow-up.5 These data formed the rationale for investigating the feasibility of this conditioning regimen for patients undergoing URD BMT. Our results demonstrate the efficacy of this regimen to result in 100% DC in unseparated marrow nucleated cells in most patients undergoing HLA-compatible URD BMT. We observed 100% DC in 78% of evaluable patients at day 30, and 86% of patients achieved 100% DC at 1 or more follow-ups after transplantation. Donor cell engraftment was durable, as evidenced by 100% DC through 3 years after transplantation. Six of 6 and 10 of 10-HLA matched patients had the same rate of 100% DC at day 30 (75%), and this outcome did not appear to be significantly affected by the single-antigen mismatches allowed for enrollment in the study. We were unable to make comparisons between our chimerism data and those observed after conventional conditioning for URD BMT; the latter were reports of patients who underwent T cell–depleted transplantation or reports that included small numbers of patients. However, in a study of 52 patients undergoing URD BMT who underwent conditioning with a nonmyeloablative regimen (200 cGy TBI and fludarabine, only 13 (25%) patients experienced 100% DC by day 28.17 Subsequent withdrawal of immunosuppression or donor lymphocyte infusion (DLI) can increase the number of patients with 100% DC.18-22 However, achieving 100% DC early (versus late) would likely benefit a greater number of patients, based on observations by Childs et al22 that achieving 100% DC often precedes disease regression. In contrast to that observed in nonmyeloablative regimens, 100% DC was achieved early in most patients undergoing URD BMT after this low-dose (550 cGy), TBI-based conditioning regimen.

With this low-dose (550 cGy), single-exposure, TBI-based regimen, a significant minority (25%) of evaluable patients experienced MC after transplantation. The risk for MC early after transplantation has not been well characterized following conven-
tional conditioning for URD BMT. With a nonmyeloablative regimen of 200 cGy TBI and fludarabine, most patients had MC within the first 2 months of transplantation, and achieving 100% DC (in T cells) required another 6 months or more.17 The clinical significance of MC after allogeneic transplantation is unclear because correlations between MC and relapse have been discordant and controversial.23-29 We observed a trend toward greater risk for relapse in patients who achieved MC compared with 100% DC at day 30 (57% vs 30%, respectively; P = .06). If confirmed, chimerism data on day 30 could be used to test whether subsequent intervention (eg, donor lymphoid infusion [DLI]) may reduce the risk for relapse. These data further support the importance of achieving 100% DC early (versus late) after transplantation. In addition, we observed a higher 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 (57% vs 12.5%, respectively; P = .03). These data suggest that the relationship of MC to relapse may be a time-dependent variable and could, in part, explain the controversy within the literature surrounding this topic.

A potential limitation of our study is that chimerism analysis was performed on unseparated marrow nucleated cells and not on cell subsets. Recent studies explored the significance of assaying DC in specific cell lineages and in unseparated marrow cells and their implications to clinical outcomes. In these studies, achieving DC in T cells of the peripheral blood (PB) correlated with disease regression,22 graft rejection, and aGVHD.30 Achieving DC in unsorted marrow cells was important in predicting graft rejection, but not other clinical outcomes assessed.30 However, these relationships were based on observations in only 15 and 45 patients, respectively. Our results indicate that day-30 chimerism analysis of unseparated marrow cells may be useful in predicting disease relapse, a trend that might have been obscured by small patient numbers in other studies. Thus, although the evidence supports that T cell DC is a predictor of clinical outcomes, the impact of unseparated marrow nucleated cells may also be important. The correlation of DC in unseparated marrow nucleated cells and in PB T cells at any given time after BMT with reduced-intensity regimens has been investigated. Maris et al25 observed that when median mononuclear marrow cell DC was 95%, median PB T cell DC exceeded 80%. However, Childs et al22 reported the percentage of donor T cells in the PB to always be greater than the percentage of donor myeloid PB cells (CD14+ and CD15+) and marrow CD34+ cells. These studies suggest that, at any given time after transplantation with these reduced-intensity regimens, full DC of unseparated marrow nucleated cells is a surrogate marker of predominately DC in T cell subsets.

For patients undergoing HLA-matched RD PBSC transplantation with this regimen, graft failure did not occur. However, in the current study, 7.7% of patients undergoing HLA-compatible URD BMT with this same regimen experienced either primary or secondary graft failure. The risk for graft failure after URD BMT observed in our study compares favorably with that seen after conventional myeloablative and nonmyeloablative conditioning regimens. An NMDP study of more than 5000 patients undergoing myeloablative conditioning for URD BMT since 1987 reported a graft failure rate of 14%; however, the current rate of graft failure after myeloablative conditioning is likely lower as a result of improvements in transplantation technology.23 A recent study of 89 patients with varied diagnoses undergoing nonmyelo-
ablative conditioning for URD BMT reported a graft failure rate of 21%.17 In our study, graft failure did not occur in patients with acute leukemia but did occur almost exclusively in patients with CML and MDS. Rates of absolute risk for graft failure in patients with CML and
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 posttransplantation 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. Over 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 were 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 poor 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 100% vs 13% and 22%, respectively).

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, y</th>
<th>GF, %</th>
<th>Fatal RROT, %</th>
<th>TRM, % 100 d/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>Cornelissen et al&lt;sup&gt;44&lt;/sup&gt; n = 127*</td>
<td>&gt; 1000 cGy/Cyt</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;37&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/Cyt</td>
<td>2.3</td>
<td>7.7</td>
<td>2</td>
<td>18/30</td>
<td>37</td>
<td>69/45/31</td>
<td>60/30/25</td>
</tr>
</tbody>
</table>

GF indicates graft failure; Cyt, cyclophosphamide; Flu, fludarabine; AL, leukemia in CR; NA, not available.

*Included only adults with ALL in CR, CR2, or relapse.
†Regimens were Cy/TBI (67 patients), Cy/TBI + cytarabine (Ara-C) or etoposide (VP16) (34 patients), or other (26 patients).
60% vs 47% and 47% vs 40% and 40%, respectively). Survival was similar in patients with CML and MDS. However, it should be noted that all patients in our study received BM, whereas most patients who underwent conditioning with the 200 cGy TBI-based regimen received PBSCs. In the latter study, the use of marrow (vs PBSCs) was an independent factor associated with significantly lower DFS (hazard ratio, 2.5; P = .006) and a higher risk for graft failure (hazard ratio, 4.7; P = .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.

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