Low-Dose Total Body Irradiation and Fludarabine Conditioning for HLA Class I-Mismatched Donor Stem Cell Transplantation and Immunologic Recovery in Patients with Hematologic Malignancies: A Multicenter Trial

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HLA-mismatched grafts are a viable alternative source for patients without HLA-matched donors receiving ablative hematopoietic cell transplantation (HCT), although their use in reduced intensity conditioning (RIC) or nonmyeloablative (NMA) conditioning HCT has been not well established. Here, we extended HCT to recipients of HLA class I-mismatched grafts to investigate whether NMA conditioning can establish stable donor engraftment. Fifty-nine patients were conditioned with fludarabine (Flu) 90 mg/m² and 2 Gy total body irradiation (TBI), followed by immunosuppression with cyclosporine (CsA) 5.0 mg/kg twice a day and mycophenolate mofetil (MMF) 15 mg/kg 3 times a day for transplantation of granulocyte colony-stimulating factor (G-CSF)-mobilized peripheral blood stem cells (PBSCs) from related (n = 5) or unrelated donors (n = 54) with 1 antigen ± 1 allele HLA class I mismatch or 2 HLA class I allele mismatches. Sustained donor engraftment was observed in 95% of the evaluable patients. The incidence of grade II-IV acute and extensive chronic graft-versus-host disease (aGVHD, cGVHD) was 69% and 41%, respectively. The cumulative probability of nonrelapse mortality (NRM) was 47% at 2 years. Two-year overall and progression-free survival (OS, PFS) was 29% and 28%, respectively. NMA conditioning with Flu and low-dose TBI, followed by HCT using HLA class I-mismatched donors leads to successful engraftment and long-term survival; however, the high incidence of aGVHD and NRM needs to be addressed by alternate GVHD prophylaxis regimens.


KEYWORDS: Nonmyeloablative allogeneic hematopoietic stem cell transplantation, HLA-class I mismatched donor, Low-dose total body irradiation, Fludarabine

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) from HLA-matched donors is a well-established curative strategy for patients with hematopoietic malignancies; however, only 20% to 30% of patients needing HSCT have a genotypically matched sibling donor. Although unrelated donor registries have grown enormously, currently potential donors cannot be identified for 20% of Caucasian recipients and more than 60% of African-American recipients. Moreover, patients with rare haplotypes are unlikely to find HLA-matched unrelated donors (MUDs) in a timely fashion. There is a need for a suitable transplantation procedure that can extend the application of HSCT with reduced-intensity conditioning (RIC) or nonmyeloablative (NMA conditioning to patients who have no readily available HLA-matched donor.
Alternative sources, including HLA-mismatched unrelated donors, have been explored to extend the donor pool [1,2].

The use of HLA class I-mismatched unrelated donors is associated with an increased risk of graft rejection in the myeloablative (MA) setting [3-5]. The intensity of conditioning for HSCT is also associated with successful engraftment. Thus, the increased risk of graft rejection in the HLA-mismatched NMA setting compared with the MA setting may result from less-intensive conditioning. The degree of mismatch that would lead to unacceptable levels of rejection in recipients of NMA HSCT is not known.

Based on preclinical studies [6], we have successfully applied a NMA conditioning regimen involving fludarabine (Flu) 90 mg/m², 2 Gy of total body irradiation (TBI), and postgrafting immunosuppression with mycophenolate mofetil (MMF) and cyclosporine (CsA) as conditioning for grafts from either HLA-matched related or MUD in more than 1200 patients ineligible for high-dose HSCT because of advanced age or comorbidities [7-12]. In this multicenter phase I/II trial, we extended NMA HSCT to include recipients of related or unrelated granulocyte colony-stimulating factor (G-CSF) mobilized peripheral blood stem cell (PBSC) grafts from donors who were mismatched for 1 HLA-class I antigen with or without 1 allele-level HLA-class I mismatches, or donors who were mismatched for 2 HLA-class I alleles. In addition, we evaluated immune reconstitution after HSCT in a subset of the unrelated recipients.

PATIENTS, MATERIALS, AND METHODS

This phase I/II multicenter trial included 7 transplant centers: Fred Hutchinson Cancer Research Center (FHCRC), University of Utah, University of Torino, Medical College of Wisconsin, LDS Hospital, Rocky Mountain Cancer Center, and Veterans Affairs Puget Sound Health Care System, with FHCRC acting as the coordinating center. The study protocol was approved by the institutional review boards of FHCRC and the collaborating performance sites. Written informed consent was obtained from all patients.

Study Endpoints

The primary objective of this trial was to determine whether stable allogeneic engraftment from related and unrelated HLA-mismatched PBSC donors could be safely established using the NMA Flu/2Gy TBI conditioning regimen with or without escalating doses of the anti-CD52 monoclonal antibody (mAb) alemtuzumab in patients with hematologic malignancies. Secondary objectives included evaluation of acute and chronic graft-versus-host disease (aGVHD, cGVHD), infections, disease progression and relapse, and immunologic reconstitution.

Eligibility Criteria

All of the 59 patients enrolled in this study were ineligible for conventional HSCT and had disease expected to be stable for at least 100 days without further chemotherapy. Forty-one patients were aged > 50 years; the 18 patients aged 50 years met the eligibility criteria either because of previous HSCT (autologous, n = 14; allogeneic, n = 1), neurologic toxicity (n = 1), fungal infection and a history of major toxicity to chemotherapy (n = 1), or pancytopenia for 3 months (n = 1).

Histocompatibility testing for donor selection was performed for all patients and donors, using methods described previously [13-15]. HLA-A, -B, -C, -DRB1, and -DQB1 alleles were typed prospectively by oligonucleotide hybridization or DNA sequencing methods [16]. Donor-recipient compatibility was further tested by lymphocyte cross-match (recipient serum vs donor T and B cells) before HSCT [17]. Related or unrelated donors were allowed if matched for HLA-DRB1 and -DQB1 alleles, and mismatched for a single antigen at HLA-A, -B, or -C, with or without an additional single allele mismatch at HLA-A, -B, or -C. Donors were excluded if the recipient was homozygous at the mismatched locus, or if both mismatches were at the same locus (HLA-A, -B, or -C).

Patients were ineligible for the present study if they were pregnant or breast-feeding, or had rapidly progressive intermediate- or high-grade non-Hodgkin lymphoma (NHL), circulating leukemic blasts in the PB detected by standard pathology, central nervous system involvement refractory to intrathecal chemotherapy, infection with the human immunodeficiency virus (HIV), active bacterial or fungal infection unresponsive to therapy, decompensated liver disease, corrected pulmonary diffusion capacity < 35%, cardiac ejection fraction < 35%, poorly controlled hypertension, or a Karnofsky performance score < 50%.

Patient Characteristics

A total of 59 patients were enrolled between February 2002, and October 2008 (Table 1). Classification for risk of relapse, as described previously [18], found 16 patients with high-risk disease, 27 with standard-risk disease, and 16 with low-risk disease. Disease status at the time of HSCT included complete remission (CR) in 29 patients, partial remission (PR) in 14, stable disease (SD) in 4, and refractory or relapsed disease in 12. All patients with acute myelogenous leukemia (AML), secondary AML from myelodysplastic syndrome (MDS/AML), or acute lymphoblastic leukemia (ALL) were in CR at the time of HSCT. Four patients received a planned tandem autologous transplant.
Table 1. Patient and Disease Characteristics

<table>
<thead>
<tr>
<th>Category</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>59</td>
</tr>
<tr>
<td>Median age, years, n (range)</td>
<td>56 (13-72)</td>
</tr>
<tr>
<td>Males/females, n</td>
<td>44/15</td>
</tr>
<tr>
<td>Unrelated/related donor, n</td>
<td>54/5</td>
</tr>
<tr>
<td>Previous courses of chemotherapy, n (range)</td>
<td>6 (2-19)</td>
</tr>
<tr>
<td>Previous transplantations (autologous/allo), n</td>
<td>26/1</td>
</tr>
<tr>
<td>Time to HCT, months, median (range)</td>
<td>36 (5-205)</td>
</tr>
<tr>
<td>Dose of CD34+ G-CSF-mobilized PBSCs, median (range)</td>
<td>7.0 x 10^6 cells/kg (2.1-37.7 x 10^6)</td>
</tr>
<tr>
<td>Dose of CD3+ G-CSF-mobilized PBSCs, median (range)</td>
<td>2.6 x 10^6 cells/kg (0.3-6.6 x 10^6)</td>
</tr>
<tr>
<td>Diagnosis, n (disease status pretransplantation)</td>
<td>Non-Hodgkin lymphoma 18 (CR2, 2; CR3, 2; PR, 9; stable, 1; refractory, 4)</td>
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<td>Hodgkin lymphoma 5 (CR1, 1; PR, 2; refractory, 2)</td>
</tr>
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<td></td>
<td>Adult T cell leukemia/lymphoma AML 16 (CR1, 6; CR2, 5; CR3, 4; CR4, 1)</td>
</tr>
<tr>
<td></td>
<td>Acute lymphoblastic leukemia 5 (CR1, 1; CR2, 1; CR3, 3)</td>
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<tr>
<td></td>
<td>Chronic lymphocytic leukemia 6 (stable, 1; refractory, 5)</td>
</tr>
<tr>
<td></td>
<td>Multiple myeloma 7 (CR2, 1; PR, 3; stable, 2; refractory, 1)</td>
</tr>
<tr>
<td>MDS/AML</td>
<td>1 (CR1, 1)</td>
</tr>
<tr>
<td>HLA mismatch, n, total (related donor)</td>
<td>Both HVG and GVHD vectors 51 (4)</td>
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<td></td>
<td>GVHD vector 8 (1)</td>
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<tr>
<td></td>
<td>One HLA antigen alone 48 (5)</td>
</tr>
<tr>
<td></td>
<td>HLA-A/-B/-C 20 (5)/5/23</td>
</tr>
<tr>
<td></td>
<td>One HLA antigen + 1 allele 16 (10)</td>
</tr>
<tr>
<td></td>
<td>HLA-A/-B/-C (additional allele mismatch) 2/4/4</td>
</tr>
<tr>
<td></td>
<td>Two allele-level 1</td>
</tr>
</tbody>
</table>

HCT indicates hematopoietic stem transplantation; G-CSF, granulocyte colony-stimulating factor; MDS, myelodysplastic syndrome; AML, acute myelogenous leukemia; HVG, host-versus-graft; GVHD, graft-versus-host disease.

followed by NMA HSCT (auto-allo). The HSCT-specific comorbidity index (HSCT-CI) [19] score was available in 57 of the 59 patients; 12 patients had an HSCT-CI score of 0, 21 patients had a score of 1 or 2, and 24 patients had a score of 3 or higher.

HLA Typing and Matching

Final selection of unrelated donors was based on the results of high-resolution HLA typing for HLA-A, -B, -C, -DRB1, and -DQB1 alleles (Table 1). Seven unrelated pairs and 1 related pair were mismatched only in the GVHD vector and were not considered evaluable for the rejection endpoint. Fifty-four unrelated pairs were mismatched for at least 1 antigen (19 at HLA-A, 8 at HLA-B, and 26 at HLA-C), except 1 patient, who had 2 allele mismatches (HLA-A and HLA-B). All 5 related pairs were mismatched for 1 HLA-A antigen. Ten patients had, in addition to the class I antigen mismatch, 1 allelic mismatch (2 at HLA-A, 4 at HLA-B, and 4 at HLA-C).

Conditioning Regimen and GVHD Prophylaxis

Patients received Flu (30 mg/m^2/day) on days −4, −3, and −2 before HCT and 2 Gy TBI at a rate of 0.07 Gy/minute from a linear accelerator on day 0. Postgrafting immunosuppression with oral CsA was started at 5 mg/kg twice a day on day −3 and continued to day +180, then tapered to day +365. MMF was given orally at a dose of 15 mg/kg, based on adjusted body weight (routed to the nearest 250-mg increment), every 8 hours from the evening of day 0 (4-6 hours after HSCT infusion) up to day +100, and then tapered at a rate of approximately 11% per week for the next 8 weeks. The taper was completed by day +156 unless GVHD occurred. When significant nausea or vomiting occurred during MMF treatment, MMF was administrated i.v. at the same dose. CsA levels were measured by immunoassay, and doses were targeted to achieve trough levels of 500 ng/mL for the first 28 days and 150-450 ng/mL thereafter. CsA was given i.v. in patients not able to take it orally.

Escalation of Alemtuzumab Dose

The initial study design incorporated a provision for the addition of low-dose alemtuzumab if more than 1 rejection was seen in either of the first 2 cohorts of 7 evaluable patients treated without alemtuzumab. Eleven patients were enrolled in the first cohort, of whom 7 were evaluable for the chimerism endpoint and engrafted. In the second cohort, only 1 of 7 patients rejected the graft. Therefore, the protocol was amended to allow continued accrual without alemtuzumab. A total of 59 patients underwent transplantation without alemtuzumab.

Collection of Hematopoietic Cells

All patients received G-CSF-mobilized PBSC grafts. All related donors received 16 μg/kg/day of G-CSF by s.c. injection for 5 consecutive days before PBSC collection on days −1 and 0. Collection of unrelated donor PBSCs was arranged through the National Marrow Donor Program (NMDP) or international donor centers. G-CSF 10 μg/kg was administered by s.c. injection starting 5 days before day 0 according to the NMDP protocol.

Supportive Care

All patients received standard prophylactic antibiotics with a third-generation cephalosporin or quinolone when absolute neutrophil counts (ANCs) declined to < 500 μL. All patients received fluconazole (400 mg/day) from the start of conditioning to at least day +75 as prophylaxis for yeast infection. Trimethoprim-sulfamethoxazole (TMP-SMZ) was used as frontline prophylaxis against Pneumocystis jiroveci, with dapsone (50 mg twice a day) as second-line prophylaxis until day +180 or until discontinuation of immunosuppressive therapy. The varicella zoster virus (VZV) prophylaxis (acyclovir 250 mg/24 h), followed by 800 mg orally or valacyclovir 500 mg orally twice
a day) was given until 1 year after HSCT or 6 months after discontinuation of all immunosuppressive therapy. Preemptive treatment with ganciclovir was started during the first 100 days after HSCT when cytomegalovirus (CMV) polymerase chain reaction or pp65 antigenemia for weekly CMV surveillance was positive. After day +100, surveillance and preemptive therapy, on a weekly or biweekly basis, were recommended for patients at intermediate or high risk for CMV.

**GVHD Grading and Treatment**

Diagnosis and clinical grading of aGVHD and cGVHD were done by local investigators according to established criteria [20,21]. In most cases, biopsy analysis confirmed the clinical diagnosis. Treatment of aGVHD typically involved prednisone 1-2 mg/kg/day and reintiation of CsA at full dose if it had been previously tapered or discontinued. Primary treatment of extensive cGVHD comprised of prednisone 1 mg/kg/day and CsA 5.0 mg/kg orally twice a day.

**Treatment of Persistent/Progressive or Relapsed Malignancies**

Substantial persistent disease at day +84 or disease progression at any time was considered an indication for therapeutic intervention. In the absence of GVHD, MMF was stopped and CsA was tapered over 2 weeks, or, at the attending physician’s discretion. If stopping immunosuppression provoked no response, then chemotherapy or radiation therapy was considered. Donor lymphocyte infusion (DLI) was not offered on this trial.

**Chimerism Analyses**

Chimerism analysis of peripheral blood T cell (CD3+), granulocyte (CD33+), and whole marrow were performed on days +28, +56 +84, + 180, and +365, and then yearly as described previously [7]. For the purposes of this study, full donor T cell chimerism was defined as > 95% donor CD3+ T cells, and graft rejection was defined as the inability to detect at least 5% donor CD3+ T cells (as a proportion of the total T cell population in the PB) after HSCT. Mixed or full donor chimerism was considered evidence of donor engraftment. Sustained engraftment was defined as continued evidence of donor engraftment up to evaluation on day +84 without subsequent loss at later evaluations.

**Immune Reconstitution**

Immune reconstitution was studied in 9 recipients of HLA-class I mismatched unrelated grafts at FHCRC. PB samples were obtained before conditioning, immediately before HSCT on day 0, and at 1, 3, 6, and 12 months after HSCT. Mononuclear cells (MNCs) were separated from blood specimens, stained with fluorochrome-conjugated mAbs, and analyzed by 3-color flow cytometry as described previously [22,23]. Naive B cells were represented by IgD+ B cells, because most of these cells lack somatic mutations [24-26]. Naive CD4+ T cells were defined as CD45RA+ CD4+ T cells, because this subset contains thymic emigrants [27-29]. Naive CD8+ T cells were defined as CD11a+ CD8+ T cells, because virtually all cord blood (CB) CD8+ T cells are CD11a+ and become CD11a+ after activation [30,31]. CD28+ T cells represent cells that can receive both the T cell receptor-mediated signal and the CD28-mediated costimulatory signal. Monocytes were defined as CD14+ MNCs. Natural killer (NK) cells were defined as MNCs expressing CD16 or CD56 and not expressing CD3 or CD14. Dendritic cells (DCs) were defined as HLA-DR+ MNCs not expressing CD3, CD14, CD16, CD20, CD34, or CD56.

**Statistical Analysis**

Survival curves were estimated by the Kaplan-Meier method. Cumulative incidence curves for aGVHD and cGVHD and relapse treated death as a competing risk. Cumulative incidence curves for nonrelapse mortality (NRM) treated relapse as a competing risk. Comparative analyses of mortality and competing risk endpoints were performed via Cox regression; all P values reflect likelihood ratio statistics from these models and are 2-sided.

**RESULTS**

**Engraftment, Chimerism, and Graft Rejection**

The median numbers of CD34+ and CD3+ cells in the grafts were 7.0 x 10^6 (range, 2.1-37.7 x 10^6) and 2.6 x 10^6 (range, 0.3-6.6 x 10^6) cells/kg recipient body weight, respectively. The median neutrophil nadir was 100 cells/μL (range, 0-860 cells/μL). The median duration of neutropenia (ANC <0.5 x 10^9/μL) was 9 days (range, 0-33 days), and 9% of patients did not experience neutropenia. The median platelet nadir was 23 x 10^3/μL (range, 4-110 x 10^3/μL). The median duration of thrombocytopenia (platelet count < 20 x 10^3/μL) was 0 days (range, 0-23 days), and 62% of patients did not develop thrombocytopenia.

The rates of full donor T cell chimerism in the evaluable patients were 76% at day +28, 84% at day +56, and 81% at day +84. Sixteen patients were excluded for evaluation of the graft rejection outcome: 4 died early (less than 30 days after HSCT), 8 received transplants from donors mismatched only in the GVHD vector, and 4 received planned tandem auto-allotransplants. Four patients died, at days +8, +16, +23, and +29, because of NRM in 2 patients and disease progression in 2 patients. The patient who died at day 8 after HSCT did not exhibit hematopoietic recovery.
Among the 43 patients eligible for evaluation of engraftment outcome, 77% achieved full donor T cell chimerism at day +28, and 92% did so at day +56. Sustained engraftment was observed in 95% of the evaluable patients.

Two patients (one with follicular lymphoma and the other with AML) experienced early graft rejection (on day +56 and day +84, respectively), and no late graft rejections occurred. Both patients with early graft rejection underwent successful retransplantation, one from the same donor with conditioning consisting of Flu and 4 Gy TBI, and the other from an alternative HLA class I-mismatched donor with Flu, 4 Gy TBI, and alemtuzumab (total dose, 10 mg).

**GVHD**

Forty-four of 57 patients (70%; excluding the 2 patients who experienced rejection) developed aGVHD (grade I, n = 3; grade II, n = 25; grade III, n = 8; grade IV, n = 8). The cumulative incidences of grade II-IV and grade III-IV aGVHD were 69% and 26%, respectively (Figure 1A). Twenty-one patients developed cGVHD; the 3-year cumulative incidence of extensive cGVHD was 41% (Figure 1B).

**Toxicity and NRM**

Thirty-five patients (59%) experienced at least one nonhematopoietic grade III toxicity, and 20 (34%) had at least one nonhematopoietic grade IV toxicity. The most common grade III and IV nonhematopoietic toxicities were cardiovascular and pulmonary complications (Table 2). Grade IV cardiac toxicities included atrial fibrillation/flutter (n = 3), elevated cardiac enzymes suggestive of ischemic events (n = 3), pulmonary embolism (n = 1), septic shock with kidney failure requiring dialysis (n = 1), acute myocardial infarction (n = 1), severe congestive heart failure (n = 1), and acute vascular leak syndrome (n = 1). Grade IV pulmonary toxicities included hypoxia/apnea requiring intubation or pressure support (n = 7) and acute respiratory distress syndrome (ARDS) (n = 2). Grade IV gastrointestinal toxicity consisted of diarrhea or colitis associated with GVHD (n = 6) and small bowel perfusion of unknown etiology (n = 1).

Infection rates were calculated based on infections within the first 100 days posttransplantation or until death before day +100. The rates of documented viral (including CMV reactivation), fungal, and bacterial infection were 1.5, 0.3, and 2.1 per 100 patient-days, respectively, with an overall infection rate of 3.9 per 100 patient-days.

Overall, 26 of 59 patients died from nonrelapse causes. The cumulative probabilities of NRM were 22% at day 100, 36% at 1 year, and 47% at 2 years (Figure 2A). The cumulative incidence of NRM at 2 years was 55% for patients in CR and 37% for those not in CR at the time of transplantation. The cumulative incidence of NRM for the patients with AML and MDS/AML was 56% at 2 years. Twenty patients died of nonrelapse causes within 1 year after HSCT. Causes of NRM within 1 year after HSCT included infections associated with GVHD (n = 6), multiorgan failure (n = 3), infections without GVHD (n = 2), GVHD (n = 2), secondary AML (n = 1), ARDS (n = 1), bronchiolitis obliterans (n = 1), diffuse alveolar damage (n = 1), myocardial infarction (n = 1), leukoencephalopathy (n = 1), and congestive heart failure (n = 1). Six patients died of NRM more than 1 year after HCT. Causes of late NRM included infections associated with GVHD (n = 2), infections without GVHD (n = 2), cardiac and respiratory failure (n = 1), and bronchiolitis

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**Table 2. Incidence of Grade III and IV Toxicities (n = 59)**

| Number of Episodes/Number of Patients (% of Patients) |
|-----------------|-----------------|-----------------|-----------------|
| Grade III       | Grade IV        | Grade III       | Grade IV        |
| Cardiovascular  | 16/16 (27)      | 11/7 (12)       | 9/8 (14)        |
| Pulmonary       | 12/10 (17)      | 9/8 (14)        | 5/5 (8)         |
| Hepatic         | 10/10 (17)      | 7/7 (12)        | 2/2 (3)         |
| Renal/genitourinary | 6/6 (10)   | 3/2 (3)         | 2/2 (3)         |
| Gastrointestinal| 2/2 (3)         | 2/2 (3)         | 0/0 (0)         |
| Neurologic      | 2/2 (3)         | 2/2 (3)         | 0/0 (0)         |
| Hemorrhage      | 3/3 (5)         | 0/0 (0)         | 0/0 (0)         |
obliterans organizing pneumonia (n = 1). In 3 of the 6 cases, cGVHD contributed to the late death.

Relapse and Progression-Free Survival

The cumulative probability of relapse/progression was 26% at 2 years (Figure 2A). Progression-free survival (PFS) was 28% at 2 years (Figure 2B). The cumulative incidence of relapse/progression at 2 years was 14% for patients in CR and 38% for patients not in CR at the time of HSCT. The cumulative incidence of relapse for the patients with AML and MDS/AML (all of whom underwent transplantation while in CR) was 18% at 2 years. The 2-year probability of PFS was 26% in high-risk disease and 28% in standard/low-risk disease (P = .54).

Survival

Nineteen of 59 patients were alive a median of 24 months (range, 2-79 months) after HSCT. Of these 19, 14 were in CR, 2 were in PR, 2 had SD, and 1 had relapsed. The 2-year overall survival (OS) was 29% (Figure 2B). Grade III-IV aGVHD adversely affected OS as a time-dependent covariate in a Cox regression model (hazard ratio = 8.35; 95% confidence interval = 3.4-21; P < .0001).

The 2-year Kaplan-Meier probability of OS was 26% in patients receiving a graft from one HLA class I antigen-mismatched donor (n = 48) and 42% in those with donors mismatched for 2 loci (n = 11) (P = .35). The 2-year probability of OS was 33% in high-risk disease and 28% in standard/low-risk disease (P = .92). Additionally, 2-year OS was 46% in the patients with an HCT-CI score of 0, 26% in those with a score of 1 or 2, and 20% in those with a score of 3 or higher (P = .46) (Figure 2C).

Immune Reconstitution

Counts of innate immune cells (ie, NK cells, neutrophils, and monocytes) recovered within 1 month posttransplantation (Figure 3A; data on neutrophils and monocytes not shown). Recovery of dendritic cells (DCs) was slower than that of NK cells, neutrophils, and monocytes, and more so for the plasmacytoid subset than for the conventional (“myeloid”) subset. T cells recovered slowly over several months. B cell counts were typically still low at 12 months; this was true for both naive (surface IgD+) and memory (surface IgD-) B cells (data not shown). CD8+ T cell counts reached the normal range between 6 and 12 months, whereas CD4+ T cell counts were still low at 12 months. Both naive and memory/effector T cells recovered slowly (Figure 3B). CD28- T cells recovered faster than CD28+ T cells (Figure 3B).

DISCUSSION

Studies of patients receiving MA conditioning and unrelated marrow grafts have shown a higher rate of graft rejection in patients given grafts mismatched for 1 or more class I HLA antigens [5]. We sought to determine whether graft rejection was increased when HLA class I-mismatched grafts were transplanted after NMA conditioning, and found sustained donor engraftment in 95% of recipients. Although this trial was initially designed as a dose-escalation trial of alemtuzumab according to the number of patients experiencing graft rejection, the dose-escalation rule was...
never activated. Thus the combination of Flu and 2 Gy TBI, followed by a posttransplantation combination of MMF given 3 times a day and CsA, was sufficient to ensure a high rate of sustained full donor T-cell chimerism and engraftment even with HLA class I-mismatched grafts.

We previously used MMF 15 mg/kg twice a day administered from day 0 to day +40, followed by taper through day +96, in combination with CsA as GVHD prophylaxis after NMA HLA-MUD HSCT. A high graft rejection rate of 21% was found, however. By increasing the MMF dose to 15 mg/kg 3 times a day (because of the short half-life [only 3 hours] of the active metabolite of MMF) [7] and using only G-CSF mobilized PBSCs as the stem cell source, we successfully reduced the rejection rate to 5% in NMA HSCT from HLA-MUD [32]. Thus, in the current study we used MMF 15 mg/kg 3 times a day and extended the

![Graphs showing recovery of lymphocyte subsets](image)

**Figure 3.** Recovery of lymphocyte subsets. Recovery of NK, B cells, dendritic cell precursors, CD4+ and CD8+ T cells after transplantation (A). Recovery of naive, memory/effecter, CD28+ or CD28–, CD4+ and CD8+ T cells after transplantation (B). The y-axis shows the number of cells per microliter of blood. Patient medians (diamonds) and 25th-75th percentiles (error bars) are displayed. Normal medians (of 104 normal adults) are indicated by the dashed horizontal lines; the thick horizontal lines denote the normal 10th-90th percentiles. Pretransplantation studies are arbitrarily shown as performed using blood collected at 1-month pretransplantation, despite the fact that blood was drawn at any time between 1 month pretransplantation and the morning of starting conditioning. Day 0 studies were performed using blood drawn immediately before graft infusion. Nine patient blood samples were analyzed before transplantation, 7 were analyzed on day 0, 6 were analyzed at 1 month posttransplantation, 8 were analyzed at 3 months posttransplantation, 6 were analyzed at 6 months posttransplantation, and 5 were analyzed at 12 months posttransplantation.
duration of MMF administration up to day +100 in the HLA-mismatched setting. We found sustained engraftment in 95% of the evaluable patients without the use of alemtuzumab.

We detected no enhanced graft-versus-tumor effects with the use of HLA-mismatched unrelated grafts. Observed cumulative probabilities of relapse/progression of 22% at 1 year and 36% at 2 years were very similar to those in our earlier HLA-matched unrelated HSCT study (26% at 1 year and 31% at 2 years) [32]. When limited to patients not in CR at HSCT, the CR rate in the present study was lower than that in our previous study (27% vs 48%).

An association between HLA class I mismatches and a high incidence of GVHD has been reported in recipients of MA conditioning regimens followed by unrelated donor HSCT [33-35]. Previous studies reported that the cumulative incidence of grade II-IV aGVHD varied from 43% to 74% after MA HSCT with HLA-matched unrelated marrow and from 63% to 80% after MA HSCT with 1 HLA antigen-mismatched unrelated marrow [36-39]. cGVHD was observed in >55% of patients after MA HSCT with HLA-matched unrelated marrow and in 70% of patients after MA HSCT with 1 HLA antigen-mismatched unrelated marrow [21,36-39]. In a previous study using the same NMA conditioning regimen (ie, Flu and 2 Gy TBI), 103 patients received either 10/10 HLA-matched (n = 93) or single HLA class I allele-level-mismatched (single each; n = 10) unrelated donors.
donor HSCT. In that study, we reported a cumulative incidence of grade II-IV aGVHD and extensive cGVHD of 53% and 56%, respectively [32]. Interestingly, the 41% incidence of extensive cGVHD found in the current study is comparable to or lower than that observed in the HLA-matched unrelated setting. The prolonged CsA and MMF administration in this study may have contributed to the similar incidence of cGVHD. On the other hand, the observed 69% inci-
dence of grade II-IV aGVHD in this study is higher than that seen in the HLA-matched unrelated setting [32]. The 47% cumulative probability of NRM at 2 years in the current study is also higher than that seen in the HLA-matched unrelated setting. In addition, in the current study, aGVHD contributed to death in 9 of 26 nonrelapse deaths (35%), and 6 of 7 (86%) grade IV gastrointestinal episodes were associated with gut GVHD that directly or indirectly caused mortality.

As might be expected, there was a concern that the prolonged CsA and MMF administration in the present protocol might have increased the risk of infection. Indeed, the incidence of infection was higher than that in our earlier study on unrelated HSCT (documented rates of viral, fungal, and bacterial infection of 0.86, 0.26, and 1.05 per 100 patient-days, respectively [32]). In 12 of 26 patients (46%), NRM was associated with infection.

Because excessive immunosuppression can lead to high rates of relapse and infection due to delayed immune reconstitution, an optimal prophylactic regimen for GVHD in HLA-mismatched or unrelated HSCT has been explored by other investigators. Alemtuzumab (total 20-100 mg/person, or 1.2 mg/kg) has been applied in HLA-haploidentical related and HLA-matched and -mismatched unrelated HSCT [40-43]. Some investigators have reported that alemtuzumab-containing regimens were highly effective in preventing both cGVHD and aGVHD without increased risk of relapse [40,41]; however, high relapse rates, particularly in patients with active disease at HSCT (5 of 8 patients [63%]) [42], and high infection rates (serious infections complication 62.2%) [43] because of delayed immune reconstitution also have been reported. Furthermore, in a recent publication, we reported that HLA-haploidentical NMA related HSCT with high-dose posttransplantation cyclophosphamide showed an acceptable sustained engraftment rate of > 95% and improved control of aGVHD (34% for grade II-IV and 6% for grade III-IV) with no increased risk of severe infection, but with a relatively high relapse rate of 51% at 1 year [44]. In our earlier retrospective study, extensive cGVHD, but not grade II-IV aGVHD, was significantly associated with decreased risk of relapse or progression without increased NRM [45]. These data indicate that intensified prophylaxis for aGVHD, but not cGVHD, with an additional immunosuppressive agent, such as low-dose alemtuzumab [46] or sirolimus [47], may be a reasonable strategy to improve outcome.

The observed OS in all 3 groups stratified by HSCT-CI score was inferior to that in recipients of HLA-matched related and/or unrelated HSCT. These data suggest that HLA-class I disparity might be another independent outcome factor in the nonmyeloablative setting.

Immune reconstitution was similar to that after HLA-matched unrelated donor NMA HSCT [48], with the exception of slower recovery of CD8+ T cells and B cells. Both naive and memory/effector CD8+ T cells recovered slowly, suggesting reduced thymopoiesis and peripheral expansion, possibly because many of our patients had GVHD (clinical or subclinical) and were treated with prolonged immunosuppressive therapy. The slow recovery of B cells also may result from the fact that many patients had GVHD, because GVHD and/or its treatment hamper B lymphopoiesis [49,50]. The slow recovery of CD8+ T cells and B cells might have contributed to the relatively high infection rates detected.

In conclusion, this study demonstrates the feasibility of NMA HSCT from HLA class I-mismatched donors using Flu and low-dose TBI conditioning. Although almost 30% of the patients achieved long-term survival with this approach, there was a high incidence of aGVHD, and NRM decreased survival compared with that in fully HLA-matched unrelated patients. Future studies of more intense early prophylaxis of GVHD in this HLA class I-mismatched setting may decrease severe aGVHD and improve survival.

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