Published in final edited form as:
Biol Blood Marrow Transplant. 2008 June ; 14(6): 641–650. doi:10.1016/j.bbmt.2008.03.005.

HLA-Haploidentical Bone Marrow Transplantation for Hematologic Malignancies Using Nonmyeloablative Conditioning and High-Dose, Posttransplantation Cyclophosphamide

Leo Luznik[1,*], **Paul V. O'Donnell**[2,3,*], **Heather J. Symons**[1], **Allen R. Chen**[1], **M. Susan Leffell**[1], **Marianna Zahurak**[1], **Ted A. Gooley**[2,3], **Steve Piantadosi**[1], **Michele Kaup**[1], **Richard F. Ambinder**[1], **Carol Ann Huff**[1], **William Matsui**[1], **Javier Bolaños-Meade**[1], **Ivan Borrello**[1], **Jonathan D. Powell**[1], **Elizabeth Harrington**[2], **Sandy Warnock**[2], **Mary Flowers**[2,3], **Robert A. Brodsky**[1], **Brenda M. Sandmaier**[2,3], **Rainer F. Storb**[2,3], **Richard J. Jones**[1], and **Ephraim J. Fuchs**[1]

[1]*Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, Maryland* [2]*Fred Hutchinson Cancer Research Center, Seattle, Washington* [3]*University of Washington School of Medicine Seattle, Washington*

Abstract

We evaluated the safety and efficacy of high-dose, posttransplantation cyclophosphamide (Cy) to prevent graft rejection and graft-versus-host disease (GVHD) after outpatient nonmyeloablative conditioning and T cell-replete bone marrow transplantation from partially HLA-mismatched (haploidentical) related donors. Patients with advanced hematologic malignancies (n = 67) or paroxysmal nocturnal hemoglobinuria (n = 1) received Cy 50 mg/kg i.v. on day 3 (n = 28) or on days 3 and 4 (n 5 40) after transplantation. The median times to neutrophil (>500/μL) and platelet recovery (>20,000/μL) were 15 and 24 days, respectively. Graft failure occurred in 9 of 66 (13%) evaluable patients, and was fatal in 1. The cumulative incidences of grades II–IV and grades III–IV acute (aGVHD) by day 200 were 34% and 6%, respectively. There was a trend toward a lower risk of extensive chronic GVHD (cGVHD) among recipients of 2 versus 1 dose of posttransplantation Cy (P = .05), the only difference between these groups. The cumulative incidences of nonrelapse mortality (NRM) and relapse at 1 year were 15% and 51%, respectively. Actuarial overall survival (OS) and event-free survival (EFS) at 2 years after transplantation were 36% and 26%, respectively. Patients with lymphoid malignancies had an improved EFS compared to those with myelogenous malignancies (P = .02). Nonmyeloablative HLA-haploidentical BMT with posttransplantation Cy is associated with acceptable rates of fatal graft failure and severe aGVHD or cGVHD.

Keywords

Bone marrow transplantation; Cyclophosphamide; Histocompatibility antigens; Conditioning regimens; Leukemia; Lymphoma

Correspondence and reprint requests: Ephraim J. Fuchs, MD, 488 Bunting-Blaustein Cancer Research Building, 1650 Orleans Street, Baltimore, MD 21231. (e-mail: fuchsep@jhmi.edu).
*These authors contributed equally to this work.

INTRODUCTION

Allogeneic blood or marrow transplantation (alloBMT), following either marrow-ablative or non-myeloablative conditioning, is a potentially curative treatment for a variety of hematologic malignancies and nonmalignant hematologic disorders [1]. Of all the potential sources of allografts, those from human leukocyte antigen (HLA)-matched siblings have generally produced the best overall and progression-free survival (OS, PFS) [2]. Unfortunately, only about a third of candidates for alloBMT have HLA-matched siblings. For patients who lack HLA-matched siblings, there are 3 alternative sources of stem cells for alloBMT: (1) volunteer unrelated donors, (2) umbilical cord blood, and (3) partially HLA-mismatched, or haploidentical, related donors [3]. Because any patient shares exactly one HLA haplotype with each biologic parent or child and half of siblings, an eligible HLA-haploidentical donor can be identified rapidly in nearly all cases. However, HLA-haploidentical BMT has been associated with significant risks of graft rejection and severe GVHD [4–6], which are manifestations of excessive alloreactivity by host [7] and donor T cells [8], respectively. The risk of severe GVHD may be reduced in intensively conditioned recipients of grafts that have been rigorously depleted of mature T cells or selectively depleted of alloreactive T cells, but the risks of serious infection and death from prolonged immune compromise in these patients remain high [9–14]. To reduce the toxicity of haploidentical BMT, methods to selectively inhibit alloreactivity while preserving immunity to infection and the malignancy are clearly required [15].

Cyclophosphamide (Cy) is a highly immunosuppressive antineoplastic agent that has an established role in conditioning for alloBMT. Typically, the drug is administered prior to transplantation to prevent graft rejection by suppressing the host immune system. However, pretransplantation conditioning with Cy increases the risk of GVHD following allogeneic T cell infusion in mouse models [16]. In contrast, administration of a properly timed, high dose of Cy *after* BMT inhibits both graft rejection and GVHD [17–20]. In light of this observation, we conducted a Phase I/II trial of high-dose, posttransplantation Cy after nonmyeloablative conditioning and transplantation of non-T cell-depleted, HLA-haploidentical marrow for patients with poor risk hematologic malignancies and nonmalignant hematologic disorders [21]. That trial demonstrated that partially HLA-mismatched bone marrow can engraft rapidly and stably after nonmyeloablative conditioning that includes posttransplantation Cy. However, the cumulative incidence of graft failure and severe GVHD remained high, approaching 60% at 6 months posttransplant. We now report on outcomes after nonmyeloablative transplantation from related, haploidentical donors resulting from a modified regimen of posttransplant immunosuppression.

PATIENTS AND METHODS

Patients

Sixty-eight consecutive patients were accrued to 2 similar clinical trials, J9966 at Johns Hopkins and 1667 at the Fred Hutchinson Cancer Research Center. The protocols were open between 1999 and 2006. Eligible patients were 0.5–70 years of age with high-risk hematologic malignancies or paroxysmal nocturnal hemoglobinuria (PNH) for whom standard allogeneic (HLA-matched, related, or unrelated) or autologous BMT was unavailable or inappropriate. Eligible hematologic malignancies included interferon-or imatinibrefractory chronic myelogenous leukemia (CML) in first chronic phase, CML in second chronic phase, poor-risk acute leukemia in first complete remission, acute leukemia beyond the first complete remission, advanced myelodysplastic syndrome (MDS), and lymphoma or multiple myeloma (MM) in resistant relapse (not responsive to conventional salvage therapy prior to transplantation) or in relapse after autologous transplantation. Poor-risk characteristics of acute leukemia in first remission included white blood cell count >100,000/μL at diagnosis, mixed lineage, delayed response to induction chemotherapy, or unfavorable cytogenetics [22], including 3 or more

chromosomal abnormalities, Philadelphia chromosome, −7, t(6;9), t(6;11), isolated trisomy 8, inv(3) or t(3;3), or t(11;19)(q23;p13.1). Patients were ineligible for the protocols if they were pregnant, HIV-seropositive, had active, serious infections, CNS disease refractory to intrathecal chemotherapy or radiation, Karnofsky performance status <60, or organ dysfunction defined by a left ventricular ejection fraction (LVEF) >35%, DLCO <35%, or total bilirubin >3 mg/dL. The respective protocols received approval by the institutional review boards (IRBs) of the Johns Hopkins Medical Institutions or the Fred Hutchinson Cancer Research Center. All patients signed consent forms approved by the respective IRBs.

HLA Typing

HLA phenotyping was performed by the same methods and similar protocols at both institutions. Briefly, typing prior to alloBMT was performed using 2 different molecular test methods. Samples were PCR amplified with HLA locus-specific primers. Amplified samples were hybridized to panels of sequence specific oligonucleotide probes (SSOP) bound to plastic microspheres with a single tube hybridization for each HLA locus for each sample. The positive and negative probe reactions were captured by a Luminex Flow Analyzer and submitted to an HLA analysis program (One Lambda LabType) loaded with a library of the expected hybridization patterns of the known HLA alleles. High-resolution HLA typing to confirm allele level matching was performed by direct DNA sequencing of HLA locus-specific PCR amplified DNA, using dye terminator chemistry, with analysis on an ABI 3130xl Genetic Analyzer and Assign (Conexio Genomics, Australia) HLA sequencing analysis software. Potential family members were initially typed at the HLA-A, HLA-B, and HLA-DRB1 loci at an intermediate resolution level. Family members selected as donors were then further typed at the HLA-C locus at an intermediate resolution level. DRB1 and DQB1 alleles were typed at a high-resolution level. As needed, recipients and potential donors were typed at a high-resolution level for HLA-Cw alleles. Haplotypes were determined based on family studies whenever possible.

Conditioning Regimen and Postgrafting Immunosuppression (Figure 1)

All patients were intended to be treated as outpatients. Transplantation conditioning was modified from the regimen developed by Storb and colleagues [23], and consisted of Cy 14.5 mg/kg/day i.v. on days −6 and −5, fludarabine 30 mg/m^2/day i.v. on days −6 to −2, and 200 cGy of TBI on day −1 as reported previously [21]. On day 0, patients received donor marrow, which was obtained in a targeted collection of 4×10^8 nucleated cells/kg recipient weight and depleted of red blood cells by processing on a Gambro Spectra apheresis instrument. On day 3 (28 patients in Seattle) or on days 3 and 4 (40 patients in Baltimore), 50 mg/kg Cy was administered over 90 min together with Mesna (80% dose of Cy in 4 divided doses over 8 hours) by i.v. infusion. Pharmacologic prophylaxis of GVHD with tacrolimus and mycophenolate mofetil (MMF) was not initiated until the day following completion of posttransplantation Cy to avoid blocking Cy-induced tolerance [24]. All patients received tacrolimus (Prograf®; Astellas, Deerfield, IL), which was initiated at a dose of 1 mg i.v.. daily, adjusted to achieve a therapeutic level of 5–15 ng/mL, and then converted to oral form until discontinuation. If there was no active GVHD, tacrolimus was tapered off by day 180. All patients received MMF (Cellcept®, Roche Laboratories, Nutley, NJ) until day 35 at a dose of 15 mg/kg orally 3 times daily with a maximum daily dose of 3 g. Patients received filgrastim (Neupogen®, Amgen, Thousand Oaks, CA), 5 μg/kg/day by subcutaneous injection starting on day 4 and continuing until recovery of neutrophils to >1000/μL for 3 days.

Supportive Care

Antimicrobial prophylaxis was administered according to the practice guidelines of the respective institutions and included antibiotics for prophylaxis of *Pneumocystis carinii*,

Candida albicans, and herpes zoster/simplex. Standard broad-spectrum bacterial antibiotic prophylaxis with a third-generation cephalosporin or quinolone was commonly given to patients who became neutropenic. All blood products except for the allograft were irradiated with 25 Gy before transfusion. The thresholds of RBC and platelet transfusions were hematocrit <26% or platelet count < 10,000/μL. Cytomegalovirus (CMV)-seronegative patients were given transfusions from CMV-seronegative donors, or leuko-reduced blood products if CMV-negative products were unavailable. Patients were monitored for CMV reactivation by weekly measurement of CMV pp65 in mononuclear cell smears by immunofluorescent staining or CMV copy number by PCR of serum until day 100. Preemptive therapy with ganciclovir (5 mg/kg i.v. twice daily) was initiated when ≥1CMV antigen-positive cell or ≥600 copies of CMV/mL of serum (≥100 copies/mL if the patient was receiving ≥1 mg/kg steroids) were detected. The ganciclovir dose was reduced to 5 mg/kg/day after the viral load began to decline and was discontinued after 2 negative surveillance tests. Alternatively, foscarnet was used at 90 mg/kg twice daily for induction and then changed to once daily for maintenance.

Chimerism Analyses

At monthly intervals, nucleated cells were isolated from the marrow or peripheral blood, or T cells (CD3-positive) and granulocytes (CD33-positive) were sorted from peripheral blood by flow cytometry. Percentages of donor-host chimerism for recipients of sex-mismatched BMT were determined by fluorescein in situ hybridization (FISH) [25] using probes for X-and Y-chromosomes. For recipients of sex-matched BMT, chimerism was based on restriction fragment length polymorphisms [26] or PCR analysis of variable nucleotide tandem repeats [27] unique to donors or recipients [28].

GVHD Grading and Therapy

Acute GVHD (aGVHD) was graded according to the Keystone Criteria [29]. Chronic GVHD (cGVHD) was graded according to standard guidelines [30]. First-line therapy of clinically significant aGVHD consisted of methylprednisolone 1–2.5 mg/kg/day i.v. plus full-dose tacrolimus or full-dose tacrolimus plus resumption of MMF.

Study End Points

Patient outcomes are reported as of April 3, 2007. The major study end points were sustained donor engraftment, incidence and severity of GVHD, and nonrelapse mortality (NRM). Successful donor engraftment was defined as donor chimerism ≥ 50% on day ~60 and stable or improved thereafter. Graft failure was defined as the inability to detect or loss of detection of ≥5% donor cells after transplantation, not because of progressive hematologic malignancy. Patients were considered to have died of NRM if there was no evidence of disease relapse or progression before death. Time to recovery of neutrophils was defined as the interval between transplantation and the first of 3 consecutive days with an absolute neutrophil count (ANC) >500/μL. Platelet recovery was defined as the time interval between transplantation and the first day of a platelet count >20,000/ μL without a platelet transfusion in the preceding 7 days.

Statistical Methods

Probabilities of OS and EFS were estimated using the Kaplan-Meier method [31]. Probabilities of aGVHD and cGVHD, relapse, and NRM were summarized using cumulative incidence estimates [32]. Death without engraftment was considered a competing risk for engraftment; death without relapse was a competing risk for relapse; relapse was a competing risk for NRM; graft failure, relapse, or death without GVHD were considered competing risks for GVHD. The hazard of failure for each of these endpoints was compared using Cox regression [33].

RESULTS

Patient and Graft Characteristics

Characteristics of the patient population are listed in Table 1. All study subjects had poor risk hematologic malignancies, as defined in the Materials and Methods, except for 1 patient with PNH. Twenty-one patients (31%) had failed at least 1 autologous stem cell transplantation (SCT), including 12 of 13 patients with Hodgkin lymphoma (HL), 4 of 10 patients with non-Hodgkin lymphoma (NHL), 3 of 27 patients with acute myelogenous leukemia (AML), and 1 patient each with chronic lymphocytic leukemia (CLL) and MM. Twenty-five percent of patients were from ethnic minority groups.

Characteristics of the donors and the grafts are listed in Table 2. For the entire group, about half (33 of 68) of the donors were siblings of patients, and about a quarter each of the donors were either parents or children of patients (19 of 68 and 16 of 68, respectively). The numbers of HLA allele mismatches in the host-versus-graft (HVG) or graft-versus-host (GVH) directions are listed. Donors differed from their recipients at a median of 4 HLA loci in both the HVG and GVH directions. Greater than 70% of donor-recipient pairs were mismatched for at least 4 HLA loci.

Engraftment and Chimerism

The median time to neutrophil recovery (Figure 2A) was 15 days, and the median time to platelet recovery (Figure 2B) was 24 days.

Graft rejection occurred in 9 of 66 evaluable patients (13%). All but 1 patient with graft failure experienced recovery of autologous hematopoiesis with median times to neutrophil and platelet engraftment of 15 days (range: 11–42 days) and 28 days (range: 0–395 days), respectively.

Achievement of full donor chimerism was rapid after transplantation from HLA-haploidentical donors (Figure 2C). Analysis of peripheral blood that was either (1) unfractionated or (2) separated by cell sorting into T cell (CD3-positive) or granulocyte (CD33-positive) fractions showed that with few exceptions, donor chimerism in patients with sustained engraftment was virtually complete (>95%) by 2 months after transplantation.

Hospitalizations, Transfusions, and Infections

All patients received their initial treatment in the outpatient department and were discharged to their referring oncologist between 60 and 90 days after transplantation, unless complications requiring admission to the hospital supervened. The median number of hospitalizations prior to day 60 was 1 (range: 0–4), and the median length of stay was 4 days (range: 0–66). Neutropenic fever accounted for 51% of the admissions, nonneutropenic infections accounted for 22%, aGVHD accounted for 9%, and other causes were the reason for the remaining 19% of admissions. A total of 22 patients (32%) did not require hospitalization within the first 60 days of transplantation.

The median number of red blood cell transfusions per patient was 6 (range: 0–34), and the median number of times patients received platelet transfusions was 4 (range: 0–44). Two and 5 patients, respectively, did not receive red blood cell or platelet transfusions.

Patients who are seropositive for CMV are known to be at high-risk for reactivating CMV after transplantation, regardless of the serologic status of the donor [34]. In this study, CMV reactivation was observed in 17 of 45 (38%) high-risk patients with a median time to reactivation of 34 days (Table 3). aGVHD was present in 7 patients on or about the time of

CMV reactivation. There were no cases of CMV pneumonia and there was no CMV-associated mortality.

Despite effective antifungal prophylaxis, invasive mold infections, especially *Aspergillus*, remain an important problem after alloBMT [35]. However, with the advent of antimold agents such as voriconazole, survival of patients with invasive mold infections has improved significantly [36]. Proved or probable invasive mold infections posttransplant, all caused by *Aspergillus* sp, were observed in 5 of 68 (7%) patients. Two patients died from *Aspergillus* infection: 1 while persistently neutropenic following graft failure, and 1 with fungal sinusitis.

aGVHD and cGVHD

The probabilities of grades II–IV and III–IV aGVHD by day 200 were 34% and 6%, respectively (Figure 3A). There was no statistically significant difference in the probability of aGVHD between patients who received 1 versus 2 doses of posttransplantation Cy (data not shown). However, Figure 3B shows that the incidence of extensive cGVHD at 1 year in the group of patients who received 2 doses of posttransplantation Cy (5%) was suggestively lower than the incidence of extensive cGVHD in the group of patients who received 1 dose of posttransplantation Cy (25%; hazard ratio [HR] 0.21; 95% confidence interval [CI] 0.04–1.01; $P = .05$).

NRM and Relapse

The probabilities of NRM at 100 days and at 1 year after transplantation were 4% and 15%, respectively, and the probabilities of relapse at 1 and 2 years after transplantation were 51% and 58%, respectively (Figure 4A). There was no statistically significant effect of the dose of posttransplantation Cy on either NRM or relapse (data not shown). Patients with lymphoid malignancies had a significantly lower risk of relapse than patients with myeloid malignancies (HR 0.54, 95% CI 0.30–0.97, $P = .04$).

OS and EFS

At a median follow-up among survivors of 745 days (range: 112–1483 days), the actuarial OS at 1 and at 2 years was 46% and 36%, respectively (Figure 4B). The actuarial EFS at 1 and at 2 years was 34% and 26%, respectively (Figure 4B). OS and EFS were not statistically significantly different between groups (data not shown). However, compared to patients with myelogenous malignancies, patients with lymphoid malignancies had a significantly improved EFS (HR 5 0.50; 95% CI 0.29–0.87; $P = .02$) (Figure 4C).

Table 4 lists the causes of death among transplanted patients. Of 42 deaths, 31 occurred in patients with relapsed or progressive disease. GVHD accounted for 2 deaths (3%). Only 4 patients died of infection without GVHD or disease progression (6%).

DISCUSSION

The current report builds upon our initial experience in the use of high-dose, posttransplantation Cy for the prevention of graft rejection and aGVHD after nonmyeloablative conditioning and HLA-haploidentical bone marrow transplantation for advanced hematologic malignancies [21]. Several conclusions may be drawn. First and foremost, posttransplantation immunosuppression with high-dose Cy, tacrolimus, and thrice daily MMF was associated with an acceptably low incidence of fatal graft rejection, severe aGVHD, and extensive cGVHD, while allowing prompt engraftment. The incidence of GVHD in our study is similar to that reported with reduced intensity HLA-matched sibling and unrelated donor transplantation [37–40]. Second, in addition to control of HLA-haploidentical alloreactivity, there was a suggestion of effective clinical immune reconstitution as demonstrated by the low incidence

of severe opportunistic infections. Third, relapse was the major cause of treatment failure in this population of patients with poor-risk hematologic malignancies. Finally, the transplantation regimen is truly nonmyeloablative, as autologous hematopoiesis recovered in 8 of 9 patients who rejected their grafts.

An extensive body of preclinical literature supports the hypothesis that Cy is more effective at suppressing HVG reactions when given after rather than before allogeneic solid organ or stem cell transplantation [17,19,20,41–43]. Posttransplantation Cy was also found to reduce the incidence and severity of GVHD following alloBMT in rodents [20,44,45], but not in a canine model [46]. However, a randomized clinical trial demonstrated that a lower dose of Cy (7.5 mg/ kg i.v. on days 1, 3, 5, 7, and 9 and then weekly there-after) was inferior to cyclosporine A in the prophylaxis of aGVHD after HLA-matched sibling alloBMT [47]. Subsequent studies in the mouse demonstrated that tolerance to minor histocompatibility antigens could be induced only if a single dose of ≥150 mg/kg Cy was given precisely between 48 and 72 hours after alloantigen exposure; tolerance was not induced if the same dose of Cy was given 24 or 96 hours after transplantation [48]. Thus, in the early clinical trial of post-transplantation Cy [47], the drug may have been given at the wrong time or at too low a dose to be maximally effective at suppressing GVHD. The selection of a low, intermittent dose of Cy in that trial was motivated by concerns that higher doses of the drug might kill or impair donor stem cells, leading to graft failure or delayed engraftment. However, lympho-hematopoietic stem cells are relatively quiescent [49] and express high levels of aldehyde dehydrogenase, which likely confer cellular resistance to cyclophosphamide [50]. These findings may explain why high dose Cy is not marrow ablative and provided a rationale for its use in this study and our previous study [21].

The dose, timing, and sequence of administering Cy, tacrolimus, and MMF were selected based upon 2 lessons learned from the mouse studies. First, a high dose of Cy must be given in a narrow time window [48]. Therefore, we gave Cy 50 mg/kg i.v. on day 3 or on days 3 and 4 after transplantation. Second, because cyclosporine A administration blocks Cy-induced tolerance in the mouse [24], initiation of both tacrolimus and MMF was delayed until after the last dose of posttransplantation Cy. The exquisite timing of Cy-induced tolerance and its sensitivity to blockade by calcineurin inhibitors suggest that the drug induces tolerance only if the target population of T cells has been recently and synchronously activated. These stringent requirements for Cy-induced tolerance may exempt populations of T cells that are resting, that have been activated but not recently, or that have been activated recently but not in synchrony. Thus, Cy-induced tolerance may afford the opportunity to selectively target alloreactive T cells while sparing immunity to infection. In this regard, we recently reported that 50 mg/kg of Cy on days 3 and 4 after myeloablative transplantation using HLA-matched sibling or unrelated donors was effective as a single agent for GVHD prophylaxis without the addition of a calcineurin inhibitor [51].

In this report, modifications were made to our original protocol [21] in an attempt to reduce the rates of graft rejection and GVHD reported previously. One modification was to increase the dose of posttransplant Cy in the group of patients transplanted in Baltimore. The second modification was to increase the frequency of dosing of MMF in both groups to achieve a higher steady-state concentration, which has been shown to reduce the rate of rejection in nonmyeloablative transplants from HLA-matched, unrelated donors [52]. The effect of increasing the dose of posttransplant Cy was to decrease significantly the cumulative incidence of severe cGVHD, a major cause of posttransplant morbidity. However, we cannot rule out the possibility of a center effect contributing to this difference. Increasing the dose of posttransplant Cy did not affect other transplant outcomes significantly. Thus, thrice daily MMF and 2 doses of posttransplant Cy appear to be an improved regimen of posttransplant immunosuppression in our nonmyeloablative transplant protocol using haploidentical donors.

The ability to effectively utilize haploidentical family members as donors potentially provides rapid access to allogeneic BMT for virtually all patients in need of one. However, HLA-haploidentical allogeneic transplantation has generally been unsuccessful because of unacceptably high rates of severe GVHD and opportunistic infections [53,54]. In most studies, depletion of host and donor T cells in vivo was accomplished using polycolonal or monoclonal antibodies (mAb) specific for lymphocytes. Relatively high rates of relapse, severe GVHD, and NRM, and low rates of donor chimerism were observed by Spitzer et al. [53,54]. Improved engraftment and reduced rates of aGVHD and cGVHD were observed in the study of Rizzieri et al. [55], but NRM was 31% mostly because of infection. In our patients, mortality from infection was low (>5%). Notably, CMV and invasive mold infection, which are currently the principal causes of infectious deaths after myeloablative or nonmyeloablative BMT, accounted for only 2 deaths on this study. In contrast, mortality from CMV disease was 14%in a recent study of nonmyeloablative transplantation using HLA-haploidentical donors [55]. The incidence of invasive mold infections in our patients (7%) also was lower than expected. Fukuda et al. [56] reported an incidence of 15% invasive mold infections in nonmyeloablative transplants from HLA-matched related or unrelated donors with a mortality rate of 56%. The low incidence of infection in this study is encouraging and warrants further investigation.

Certainly one reason for the high risk of relapse in this study was that eligibility for this trial was mostly restricted to patients with poor-risk hematologic malignancies, almost a third of whom had failed prior autologous transplants. Alternatively, it is possible that the GVL effect may have been diminished to some extent by the Cy-mediated deletion or inactivation of tumor-specific T cells. Some patients may have relapsed or progressed before a GVT effect of transplantation could manifest itself. Another possible explanation for the high rate of relapse seen in this trial and other trials of nonmyeloablative conditioning may be that the transplantation conditioning was not intense enough to achieve sufficient tumor cytoreduction or to augment a GVH reaction through epithelial tissue damage. Further study is clearly required to determine why patients continue to relapse despite acquisition of full donor hematopoietic chimerism.

For adult hematologic malignancies patients who lack suitably matched related or unrelated donors, encouraging outcomes have also been obtained with transplantation of HLA-haploidentical related stem cells after myeloablative conditioning [10,57] or with transplantation of 1 or 2 umbilical cord blood units after either myeloablative [58–60] or reduced intensity conditioning (RIC) [61–64]. Both HLA-haploidentical related and umbilical cord unrelated grafts can be obtained rapidly for >90% of patients lacking an HLA-matched donor. Therefore, the choice between these graft sources and the intensity of conditioning must balance other considerations, including the patient's age, weight, medical condition, the biologic characteristics of the cancer being treated, and the relative quality and compatibility of the graft. Carefully controlled clinical trials will ultimately be required to determine the best graft source for adult patients requiring alternative donor hematopoietic stem cell transplantation.

ACKNOWLEDGMENTS

This research was supported by grants CA15396, CA18029, HL36444, CA78902, and CA15704 from the NIH, and from Astellas Pharma, Inc. E.J.F. is a Clinical Research Scholar of the Leukemia-Lymphoma Society of America.

REFERENCES

1. Thomas' Hematopoietic Cell Transplantation. Vol. 3rd ed. Malden, MA: Blackwell Publishing; 2004.
2. Szydlo R, Goldman JM, Klein JP, et al. Results of allogeneic bone marrow transplants for leukemia using donors other than HLA-identical siblings. J Clin Oncol 1997;15:1767–1777. [PubMed: 9164184]

3. Zuckerman T, Rowe JM. Alternative donor transplantation in acute myeloid leukemia: which source and when? Curr Opin Hematol 2007;14:152–161. [PubMed: 17255793]
4. Anasetti C, Amos D, Beatty PG, et al. Effect of HLA compatibility on engraftment of bone marrow transplants in patients with leukemia or lymphoma. N Engl J Med 1989;320:197–204. [PubMed: 2643045]
5. Anasetti C, Beatty PG, Storb R, et al. Effect of HLA incompatibility on graft-versus-host disease, relapse, and survival after marrow transplantation for patients with leukemia or lymphoma. Hum Immunol 1990;29:79–91. [PubMed: 2249952]
6. Kanda Y, Chiba S, Hirai H, et al. Allogeneic hematopoietic stem cell transplantation from family members other than HLA-identical siblings over the last decade (1991–2000). Blood 2003;102:1541–1547. [PubMed: 12714500]
7. Kernan NA, Flomenberg N, Dupont B, O'Reilly RJ. Graft rejection in recipients of T-cell-depleted HLA-nonidentical marrow transplants for leukemia. Identification of host-derived antidonor allocytotoxic T lymphocytes. Transplantation 1987;43:842–847. [PubMed: 3296349]
8. Kernan NA, Collins NH, Juliano L, Cartagena T, Dupont B, O'Reilly RJ. Clonable T lymphocytes in T cell-depleted bone marrow transplants correlate with development of graft-v-host disease. Blood 1986;68:770–773. [PubMed: 3527302]
9. Aversa F, Tabilio A, Velardi A, et al. Treatment of high-risk acute leukemia with T-cell-depleted stem cells from related donors with one fully mismatched HLA haplotype. N Engl J Med 1998;339:1186–1193. [PubMed: 9780338]
10. Aversa F, Terenzi A, Tabilio A, et al. Full haplotype-mismatched hematopoietic stem-cell transplantation: a phase II study in patients with acute leukemia at high risk of relapse. J Clin Oncol 2005;23:3447–3454. [PubMed: 15753458]
11. Mehta J, Singhal S, Gee AP, et al. Bone marrow transplantation from partially HLA-mismatched family donors for acute leukemia: single-center experience of 201 patients. Bone Marrow Transplant 2004;33:389–396. [PubMed: 14716338]
12. Lang P, Greil J, Bader P, et al. Long-term outcome after haploid-dentical stem cell transplantation in children. Blood Cells Mol Dis 2004;33:281–287. [PubMed: 15528145]
13. Waller EK, Giver CR, Rosenthal H, et al. Facilitating T-cell immune reconstitution after haploidentical transplantation in adults. Blood Cells Mol Dis 2004;33:233–237. [PubMed: 15528137]
14. Guinan EC, Boussiotis VA, Neuberg D, et al. Transplantation of anergic histoincompatible bone marrow allografts. N Engl J Med 1999;340:1704–1714. [PubMed: 10352162]
15. Dey BR, Spitzer TR. Current status of haploidentical stem cell transplantation. Br J Haematol 2006;135:423–437. [PubMed: 16984391]
16. Lehnert S, Rybka WB. Amplification of the graft-versus-host reaction by cyclophosphamide: dependence on timing of drug administration. Bone Marrow Transplant 1994;13:473–477. [PubMed: 8019473]
17. Mayumi H, Umesue M, Nomoto K. Cyclophosphamide-induced immunological tolerance: an overview. Immunobiology 1996;195:129–139. [PubMed: 8877390]
18. Mayumi H, Himeno K, Tanaka K, Tokuda N, Fan JL, Nomoto K. Drug-induced tolerance to allografts in mice. XII. The relationships between tolerance, chimerism, and graft-versus-host disease. Transplantation 1987;44:286–290. [PubMed: 3307052]
19. Luznik L, Jalla S, Engstrom LW, Iannone R, Fuchs EJ. Durable engraftment of major histocompatibility complex-incompatible cells after nonmyeloablative conditioning with fludarabine, low-dose total body irradiation, and posttransplantation cyclophosphamide. Blood 2001;98:3456–3464. [PubMed: 11719388]
20. Luznik L, Engstrom LW, Iannone R, Fuchs EJ. Posttransplantation cyclophosphamide facilitates engraftment of major histocompatibility complex-identical allogeneic marrow in mice conditioned with low-dose total body irradiation. Biol Blood Marrow Transplant 2002;8:131–138. [PubMed: 11939602]
21. O'Donnell PV, Luznik L, Jones RJ, et al. Nonmyeloablative bone marrow transplantation from partially HLA-mismatched related donors using posttransplantation cyclophosphamide. Biol Blood Marrow Transplant 2002;8:377–386. [PubMed: 12171484]

22. Byrd JC, Mrozek K, Dodge RK, et al. Pretreatment cytogenetic abnormalities are predictive of induction success, cumulative incidence of relapse, and overall survival in adult patients with de novo acute myeloid leukemia: results from Cancer and Leukemia Group B (CALGB 8461). Blood 2002;100:4325–4336. [PubMed: 12393746]
23. Niederwieser D, Maris M, Shizuru JA, et al. Low-dose total body irradiation (TBI) and fludarabine followed by hematopoietic cell transplantation (HCT) from HLA-matched or mismatched unrelated donors and postgrafting immunosuppression with cyclosporine and mycophenolate mofetil (MMF) can induce durable complete chimerism and sustained remissions in patients with hematological diseases. Blood 2003;101:1620–1629. [PubMed: 12393457]
24. Nomoto K, Eto M, Yanaga K, Nishimura Y, Maeda T. Interference with cyclophosphamide-induced skin allograft tolerance by cyclosporin A. J Immunol 1992;149:2668–2674. [PubMed: 1401906]
25. Crescenzi B, Fizzotti M, Piattoni S, et al. Interphase FISH for Y chromosome, VNTR polymorphisms, and RT-PCR for BCRABL in the monitoring of HLA-matched and mismatched transplants. Cancer Genet Cytogenet 2000;120:25–29. [PubMed: 10913673]
26. Aaltonen LA, Peltomaki P, Leach FS, et al. Clues to the pathogenesis of familial colorectal cancer. Science 1993;260:812–816. [PubMed: 8484121]
27. Sreenan JJ, Pettay JD, Tbakhi A, et al. The use of amplified variable number of tandem repeats (VNTR) in the detection of chimerism following bone marrow transplantation. A comparison with restriction fragment length polymorphism (RFLP) by Southern blotting. Am J Clin Pathol 1997;107:292–298. [PubMed: 9052379]
28. Van Deerlin VM, Leonard DG. Bone marrow engraftment analysis after allogeneic bone marrow transplantation. Clin Lab Med 2000;20:197–225. [PubMed: 10702903]
29. Przepiorka D, Weisdorf D, Martin P, et al. 1994 Consensus conference on acute GVHD grading. Bone Marrow Transplant 1995;15:825–828. [PubMed: 7581076]
30. Sullivan KM, Agura E, Anasetti C, et al. Chronic graft-versus-host disease and other late complications of bone marrow transplantation. Semin Hematol 1991;28:250–259. [PubMed: 1887253]
31. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. J Am Stat Assoc 1958;53:457–480.
32. Gooley TA, Leisenring W, Crowley J, Storer BE. Estimation of failure probabilities in the presence of competing risks: new representations of old estimators. Stat Med 1999;18:695–706. [PubMed: 10204198]
33. Cox DR. Regression models and life-tables (with discussion). JR Stat Soc (B) 1972;34:187–220.
34. Junghanss C, Boeckh M, Carter RA, et al. Incidence and outcome of cytomegalovirus infections following nonmyeloablative compared with myeloablative allogeneic stem cell transplantation, a matched control study. Blood 2002;99:1978–1985. [PubMed: 11877269]
35. Marr KA, Carter RA, Crippa F, Wald A, Corey L. Epidemiology and outcome of mould infections in hematopoietic stem cell transplant recipients. Clin Infect Dis 2002;34:909–917. [PubMed: 11880955]
36. Upton A, Kirby KA, Carpenter P, Boeckh M, Marr KA. Invasive aspergillosis following hematopoietic cell transplantation: outcomes and prognostic factors associated with mortality. Clin Infect Dis 2007;44:531–540. [PubMed: 17243056]
37. Mielcarek M, Martin PJ, Leisenring W, et al. Graft-versus-host disease after nonmyeloablative versus conventional hematopoietic stem cell transplantation. Blood 2003;102:756–762. [PubMed: 12663454]
38. Crawley C, Lalancette M, Szydlo R, et al. Outcomes for reduced intensity allogeneic transplantation for multiple myeloma: an analysis of prognostic factors from the chronic leukemia working party of the EBMT. Blood 2005:2004–2006. [PubMed: 16269616]
39. Bruno B, Rotta M, Patriarca F, et al. A Comparison of allografting with autografting for newly diagnosed myeloma. N Engl J Med 2007;356:1110–1120. [PubMed: 17360989]
40. Giralt S, Logan B, Rizzo D, et al. Reduced-intensity conditioning for unrelated donor progenitor cell transplantation: long-term follow-up of the first 285 reported to the national marrow donor program. Biol Blood Marrow Transplant 2007;13:844–852. [PubMed: 17580263]

41. Santos GW, Owens AH Jr. A comparison of the effects of selected cytotoxic agents on allogeneic skin graft survival in rats. Bull Johns Hopkins Hosp 1965;116:327–340. [PubMed: 14284413]
42. Berenbaum MC. Prolongation of homograft survival in mice with single doses of cyclophosphamide. Nature 200;84:1963.
43. Colson YL, Wren SM, Schuchert MJ, et al. A nonlethal conditioning approach to achieve durable multilineage mixed chimerism and tolerance across major, minor, and hematopoietic histocompatibility barriers. J Immunol 1995;155:4179–4188. [PubMed: 7594573]
44. Owens AH Jr, Santos GW. The effect of cytotoxic drugs on graft-versus-host disease in mice. Transplantation 1971;11:378–382. [PubMed: 4934348]
45. Glucksberg H, Fefer A. Chemotherapy of established graft-versus-host disease in mice. Transplantation 1972;13:300–305. [PubMed: 5021439]
46. Storb R, Graham TC, Shiurba R, Thomas ED. Treatment of canine graft-versus-host disease with methotrexate and cyclophosphamide following bone marrow transplantation from histoincompatible donors. Transplantation 1970;10:165–172. [PubMed: 4393683]
47. Santos GW, Tutschka PJ, Brookmeyer R, et al. Cyclosporine plus methylprednisolone versus cyclophosphamide plus methylprednisolone as prophylaxis for graft-versus-host disease: a randomized, double-blind study in patients undergoing allogeneic marrow transplantation. Clin Transplant 1987;1:21–28.
48. Mayumi H, Himeno K, Tokuda N, Nomoto K. Drug-induced tolerance to allografts in mice. VII. Optimal protocol and mechanism of cyclophosphamide-induced tolerance in an H-2 haplotype-identical strain combination. Transplant Proc 1986;18:363–369. [PubMed: 3515673]
49. Cheshier SH, Morrison SJ, Liao X, Weissman IL. In vivo proliferation and cell cycle kinetics of long-term self-renewing hematopoietic stem cells. Proc Natl Acad Sci USA 1999;96:3120–3125. [PubMed: 10077647]
50. Jones RJ, Barber JP, Vala MS, et al. Assessment of aldehyde dehydrogenase in viable cells. Blood 1995;85:2742–2746. [PubMed: 7742535]
51. Luznik L, Fuchs EJ, Chen AR, et al. Post-transplantation high-dose cyclophosphamide (Cy) is effective single agent GVHD prophylaxis that permits prompt immune reconstitution after myeloablative HLA matched related and unrelated bone marrow transplantation (BMT). Biol Blood Marrow Transplant 2007;13:4.
52. Giaccone L, McCune JS, Maris MB, et al. Pharmacodynamics of mycophenolate mofetil after nonmyeloablative conditioning and unrelated donor hematopoietic cell transplantation. Blood 2005;106:4381–4388. [PubMed: 16144801]
53. Sykes M, Preffer F, McAfee S, et al. Mixed lymphohaemopoietic chimerism and graft-versus-lymphoma effects after non-myeloablative therapy and HLA-mismatched bone-marrow transplantation. Lancet 1999;353:1755–1759. [PubMed: 10347989]
54. Spitzer TR, McAfee SL, Dey BR, et al. Nonmyeloablative haploidentical stem-cell transplantation using anti-CD2 monoclonal antibody (MEDI-507)-based conditioning for refractory hematologic malignancies. Transplantation 2003;75:1748–1751. [PubMed: 12777868]
55. Rizzieri DA, Koh LP, Long GD, et al. Partially matched, non-myeloablative allogeneic transplantation: clinical outcomes and immune reconstitution. J Clin Oncol 2007;25:690–697. [PubMed: 17228020]
56. Fukuda T, Boeckh M, Carter RA, et al. Risks and outcomes of invasive fungal infections in recipients of allogeneic hematopoietic stem cell transplants after nonmyeloablative conditioning. Blood 2003;102:827–833. [PubMed: 12689933]
57. Lu DP, Dong L, Wu T, et al. Conditioning including antithymocyte globulin followed by unmanipulated HLA-mismatched/ haploidentical blood and marrow transplantation can achieve comparable outcomes with HLA-identical sibling transplantation. Blood 2006;107:3065–3073. [PubMed: 16380454]
58. Laughlin MJ, Eapen M, Rubinstein P, et al. Outcomes after transplantation of cord blood or bone marrow from unrelated donors in adults with leukemia. N Engl J Med 2004;351:2265–2275. [PubMed: 15564543]
59. Arcese W, Rocha V, Labopin M, et al. Unrelated cord blood transplants in adults with hematologic malignancies. Haematologica 2006;91:223–230. [PubMed: 16461307]

60. Barker JN, Weisdorf DJ, Defor TE, et al. Transplantation of 2 partially HLA-matched umbilical cord blood units to enhance engraftment in adults with hematologic malignancy. Blood 2005;105:1343–1347. [PubMed: 15466923]

61. Miyakoshi S, Yuji K, Kami M, et al. Successful engraftment after reduced-intensity umbilical cord blood transplantation for adult patients with advanced hematological diseases. Clin Cancer Res 2004;10:3586–3592. [PubMed: 15173064]

62. Brunstein CG, Barker JN, Weisdorf DJ, et al. Umbilical cord blood transplantation after nonmyeloablative conditioning: impact on transplantation outcomes in 110 adults with hematologic disease. Blood 2007;110:3064–3070. [PubMed: 17569820]

63. Barker JN, Weisdorf DJ, Defor TE, Blazar BR, Miller JS, Wagner JE. Rapid and complete donor chimerism in adult recipients of unrelated donor umbilical cord blood transplantation after reduced-intensity conditioning. Blood 2003;102:1915–1919. [PubMed: 12738676]

64. Ballen KK, Spitzer TR, Yeap BY, et al. Double unrelated reduced-intensity umbilical cord blood transplantation in adults. Biol Blood Marrow Transplant 2007;13:82–89. [PubMed: 17222756]

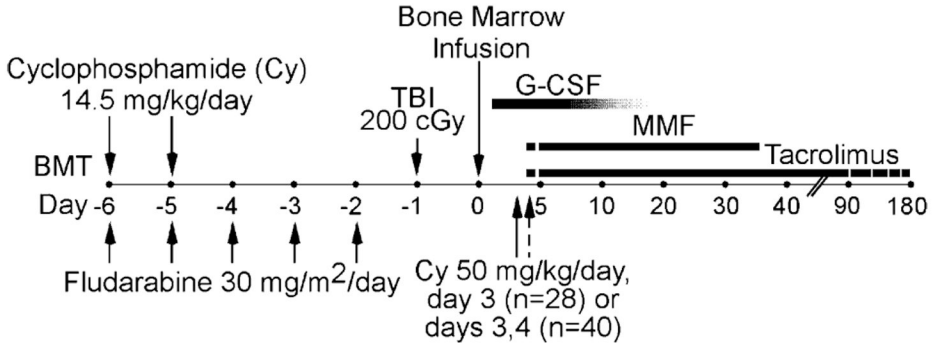

Figure 1.
Nonmyeloablative haploidentical BMT conditioning and postgrafting immunosuppresive regimen.

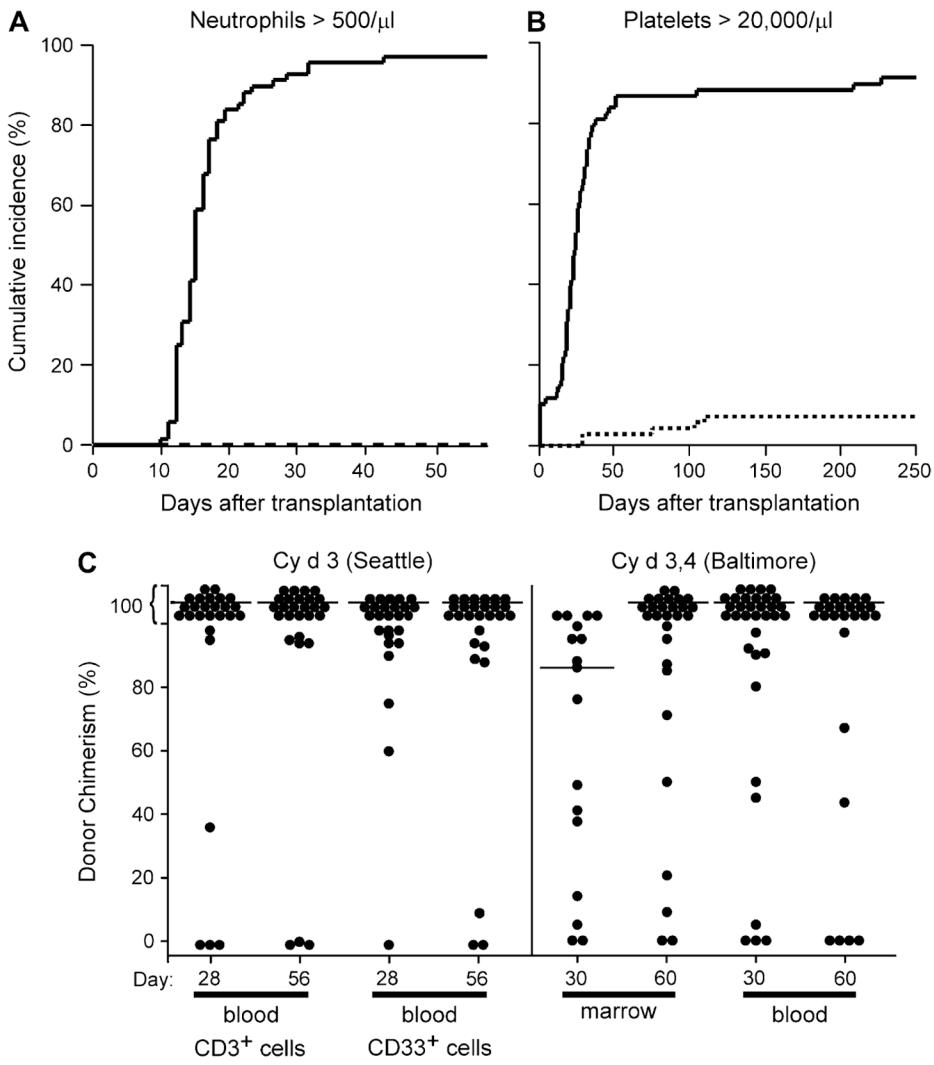

Figure 2.

Engraftment and chimerism. Cumulative incidence of (A) neutrophil and (B) platelet engraftment. Dashed line represents death without engraftment (competing risk). (C) Percentage of donor chimerism at days 28–30 and 56–60. In Seattle, donor chimerism was analyzed from the $CD3^+$ and $CD33^+$ fractions of peripheral blood, whereas in Baltimore donor chimerism was analyzed from whole peripheral blood or bone marrow. Bone marrow samples were not obtained from all evaluable patients.

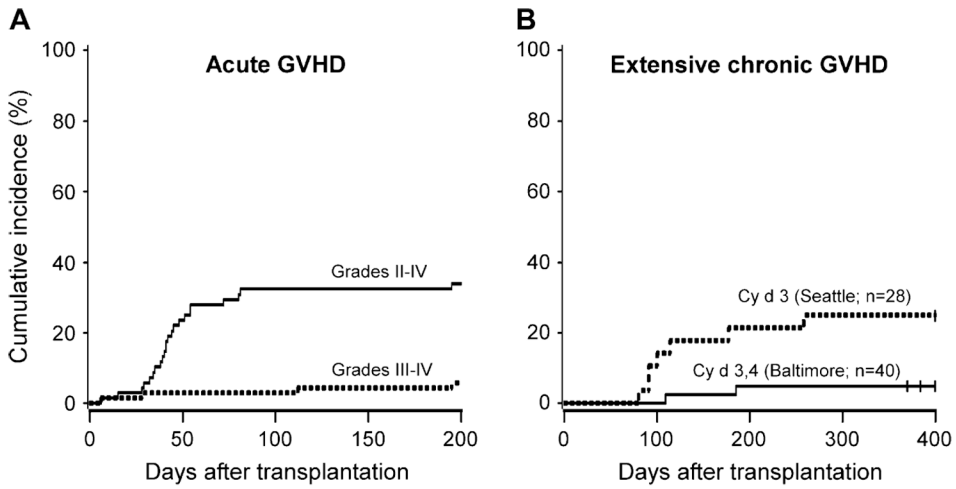

Figure 3.

Cumulative incidence of aGVHD and cGVHD. (A) Cumulative incidence of aGVHD grades II–IV and III–IV. (B) Cumulative incidence of extensive cGVHD for patients who received 1 (Seattle) versus 2 (Baltimore) doses of posttransplant Cy.

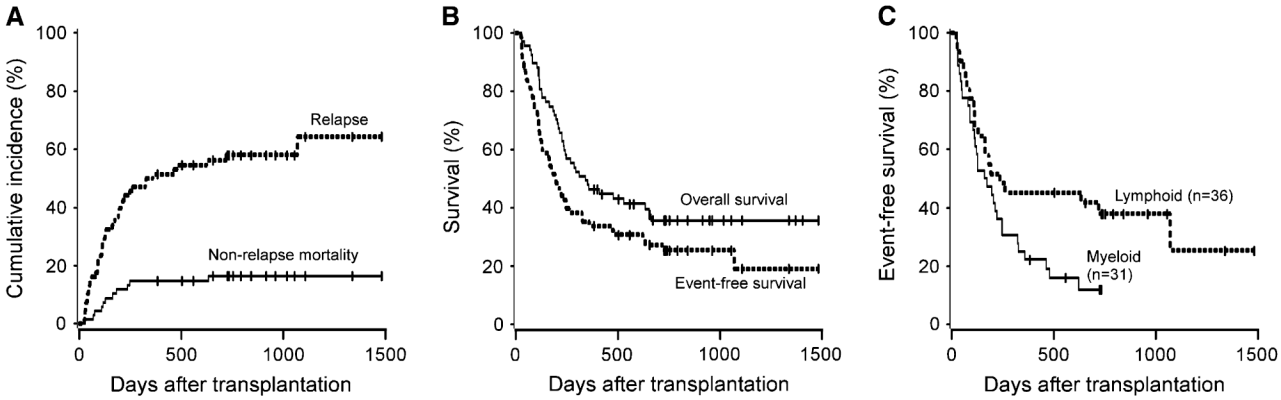

Figure 4.

Outcomes among nonmyeloablative haploidentical BMT recipients. (A) Cumulative incidence of NRM and relapse. (B) OS and EFS. (C) EFS, according to disease category (myeloid versus lymphoid).

Table 1

Patient Characteristics

No. Patients	68
Median age, years (range)	46 (1–71)
Sex: No. (%)	
Male	42 (62%)
Female	26 (38%)
Ethnicity: No. (%)	
White	51 (75%)
African American	12 (18%)
Asian	2 (3%)
Hispanic	2 (3%)
Native American	1 (1%)
Diagnosis (No.)	
AML	27 (40%)
CR1/CR>1/Not in CR	12/13/2
ALL	4 (6%)
CR1/CR>1/Not in CR	2/1/1
MDS	1 (1%)
CML/CMML	6 (9%)
CLL	3 (4%)
HL	13 (19%)
NHL	10 (15%)
MM/plasmacytoma	3 (4%)
PNH	1 (1%)
No. prior treatment regimens (range)	4 (0–10)
No. sensitive to prior treatment (%)*	49 (77%)
No. prior autologous transplant (%)	21 (31%)

AML indicates acute myelogenous leukemia; ALL, acute lymphocytic leukemia; MDS, myelodysplastic syndrome; CML, chronic myelogenous leukemia; CMML, chronic myelomonocytic leukemia; CLL, chronic lymphocytic leukemia; HL, Hodgkin lymphoma; NHL, non-Hodgkin lymphoma; MM, multiple myeloma; PNH, paroxysmal nocturnal hemoglobinuria.

*Not available for 4 patients.

Table 2

Donor and Graft Characteristics

Median age, years (range)		44 (21–69)
Sex: No. (%)		
Male		30 (44%)
Female		38 (56%)
Relationship: No. (%)		
Parent		19 (28%)
Sibling		33 (49%)
Child		16 (24%)
$CD3^+$ cells/kg × 10^{-7} Mean (SD)		4.2 (1.5)
$CD34^+$ cells/kg × 10^{-6} Mean (SD)		4.8 (2.2)
Infused MNC/kg × 10^{-8} Mean (SD)		1.6 (0.5)
HLA mismatches: No. (%)	HvG direction	GvH direction
0	1 (1%)	1 (1%)
1	1 (1%)	0 (0%)
2	5 (7%)	4 (6%)
3	12 (18%)	14 (21%)
4	18 (27%)	24 (35%)
5	31 (46%)	25 (37%)
Median (range)	4 (0–5)	4 (0–5)

Table 3

CMV Reactivation and Invasive Mold Infection

No. of patients at high-risk for CMV reactivation	45
No. of high-risk patients with CMV reactivation (%)	17 (38%)
No. of high-risk patients with CMV disease	0
Median days to onset (range)	34 (17–80)
No. of patients with invasive mold infection (%)	5 (7%)

Table 4

Causes of Death

Cause of Death	No. (%)
Relapse	31 (46%)
GVHD	2 (3%)
Infection	4 (6%)
CNS hemorrhage	2 (3%)
Secondary AML *	1 (1%)
Unknown	2 (3%)

AML indicates acute myelogeneous leukemia; GVHD, graft-versus-hose disease; CNS, central nervous system.

* Recipient origin.