1083-8791/03/0903-0007$30.00/0
doi: 10.1053/bbmt.2003.50012

Lowered-Intensity Preparative Regimen for Allogeneic Stem Cell Transplantation Delays Acute Graft-versus-Host Disease but Does Not Improve Outcome for Advanced Hematologic Malignancy

John E. Levine,[1] Joseph P. Uberti,[1] Lois Ayash,[1] Christopher Reynolds,[1] James L. M. Ferrara,[1] Samuel M. Silver,[1] Thomas Braun,[2] Gregory Yanik,[1] Raymond Hutchinson,[1] Voravit Ratanatharathorn[1]

[1]Blood and Marrow Stem Cell Transplantation Program, [2]Department of Biostatistics, School of Public Health, University of Michigan, Ann Arbor, Michigan

Correspondence and reprint requests: Dr. John E. Levine, Blood and Marrow Stem Cell Transplantation Program, B1-207 CCGC, 1500 E. Medical Center Drive, Ann Arbor, MI 48109-0914 (e-mail: jelevine@umich.edu).

Received June 20, 2002; accepted December 23, 2002

ABSTRACT

Reduced conditioning intensity has extended the option of allogeneic hematopoietic stem cell transplantation to patients who cannot tolerate fully myeloablative regimens. However, relapse and graft-versus-host disease (GVHD) continue to be major causes of morbidity and mortality. We prospectively tested whether a moderate reduction of the intensity of the preparative regimen would lead to significant reduction in regimen-related toxicity without compromising tumor control in a cohort of 44 patients ineligible for conventional hematopoietic stem cell transplantation. Patients were conditioned with fludarabine, busulfan, mycophenolate, and total lymphoid irradiation. Tacrolimus and methotrexate were given as prophylaxis for GVHD. Donors were 5 of 6 or 6 of 6 matched family members. The median age was 61 years. Eleven patients had comorbid conditions that precluded conventional myeloablative transplantation. Fatal regimen-related organ toxicity occurred in 3 patients. The cumulative incidence of grade 2 to 4 or grade 3 to 4 acute GVHD by day 100 was 38% (95% confidence interval [CI] = 25%, 55%) and 20% (95% CI = 10%, 39%), respectively, with a median time to onset of 66 days. For the entire cohort, 1-year overall survival, disease-free survival, and relapse rates were 54% (95% CI = 41%, 71%), 47% (95% CI = 35%, 65%), and 37% (95% CI = 19%, 51%), respectively. Outcomes differed based on stage of disease at time of transplantation, advanced (n = 19) versus nonadvanced (n = 25). Median survival times were 138 days and 685 days for subjects with advanced and nonadvanced disease, respectively (P = .005). After adjusting for age and comorbidity, disease stage continued to be significantly associated with overall survival (P = .005). In conclusion, a moderate reduction in conditioning dose intensity resulted in delayed onset of acute GVHD (compared with historical controls). A reduction in conditioning intensity is associated with poor survival for patients with advanced-stage disease, highlighting the importance of the conditioning regimen for tumor control.

KEY WORDS

Reduced-intensity regimen • Allogeneic hematopoietic stem cell transplantation

INTRODUCTION

Highly intense, myeloablative preparative regimens for allogeneic stem cell transplantation (SCT) are associated with substantial regimen-related toxicity, thus limiting use of this treatment strategy to younger patients, generally free of comorbid conditions other than their underlying hematologic disease. To extend the option of allogeneic SCT to older patients and/or those with comorbid conditions, several centers have piloted preparative regimens of varying intensity based on the observation that allogeneic lymphocytes can sometimes exert powerful anti-tumor effects [1-9]. Preparative regimens that dramatically reduce the intensity of conditioning have been complicated by high rates of relapse, underscoring the importance of the anti-tumor component of the preparative regimen [1,6]. Alternatively, in a cohort of young patients with standard-risk malignancies or nonmalignant disorders who would normally be candidates for conventional transplantation, Slavin et al. found that a moderate reduction in the intensity of the preparative

regimen was generally well tolerated and associated with a high 14-month survival rate of 77.5% [4]. We prospectively tested whether a similar moderate reduction of the intensity of the preparative regimen would reduce regimen-related toxicity without compromising tumor control in a cohort of 44 patients ineligible for conventional allogeneic hematopoietic stem cell transplantation (HSCT) with generally more advanced disease states. We modified the conditioning used by Slavin et al. by substituting oral mycophenolate (MMF) and a single 200 cGy fraction of total lymphoid irradiation (TLI) for intravenous anti-thymocyte globulin (ATG). Although we did not have comparative data, we believed that MMF/TLI would provide similar host immunosuppression to aid engraftment with easier administration and less toxicity than ATG.

PATIENTS AND METHODS

Eligibility Criteria

The University of Michigan institutional review board approved this study and all patients gave written informed consent before enrollment. Between March 1999 and December 2000, 44 patients were prospectively enrolled into the study. Patients were eligible if they were aged 55 years or older and diagnosed with any of the following conditions or high-risk factors: acute myeloid leukemia (AML) not in complete remission; acute lymphoblastic leukemia not in complete remission; Philadelphia chromosome, t(4;11), a presenting white blood cell count >30,000/μL, or took longer than 4 weeks to enter CR; chronic myeloid leukemia (CML) in chronic or accelerated phase; myelodysplastic syndrome (MDS) with international prognostic score of intermediate 1 and higher who were also transfusion-dependent; chronic lymphocytic leukemia (CLL) who progressed after first-line therapy; or patients with Hodgkin's or non-Hodgkin's lymphoma (NHL) who were not curable with conventional chemotherapy and did not have any tumor >5 cm in diameter. Patients younger than 55 years of age were eligible if they were diagnosed with a hematologic malignancy not curable with conventional chemotherapy and were ineligible for a conventional myeloablative SCT protocol because of compromised organ function. All patients had an ejection fraction of at least 30%, FEV1 and FVC of at least 60% of normal, bilirubin level <2.0, transaminases no greater than 3 times normal for age, and Karnofsky performance status of at least 60%. All patients had a willing and healthy related donor who was either 5 of 6 or 6 of 6 HLA-matched by molecular techniques.

Preparative Regimen

The preparative regimen consisted of fludarabine 25 mg/m^2 intravenously daily for 5 days (days -11 to -7) followed by busulfan 0.5 mg/kg orally every 6 hours for 16 doses (days -6 to –3). MMF 750 mg orally twice per day for actual weight ≤80 kg (1000 mg orally twice per day for weight >80 kg) was given from day -6 to day 0. TLI 2 Gy was given on day 0. For the calculation of the body surface area, the actual body weight was used. For the calculations of drug dosages based on body weight, the mathematic mean of the actual and ideal body weight was used. Patients with acute leukemia or high-grade lymphoma received intrathecal cytarabine of 30 mg/m^2 before transplantation. If tumor was seen on examination of the spinal fluid, the same cytarabine dose was repeated intrathecally every 2 to 3 days until clearance of tumor and then 1 additional dose was given.

Stem Cell Collection

Donors received granulocyte colony-stimulating factor 16 μg/kg divided twice per day for 4 days for stem cell mobilization. The target dose was a minimum of 2×10^6 CD34+ cells/kg of recipient weight.

Graft-versus-Host Disease Prophylaxis

Tacrolimus 0.06 mg/kg was given orally twice per day starting on day -6 until day 56. Dose adjustments were made as necessary to achieve a target level of 10 to 15 ng/mL. Provided that the patient was free from graft-versus-host disease (GVHD), starting on day 57 the tacrolimus dose was tapered by 20% every 4 weeks until it was discontinued on day 180. Methotrexate of 5 mg/m^2 was given on days 1, 3, 6, and 11 following transplantation.

Chimerism Studies

Bone marrow aspirates were obtained monthly for 6 months following transplantation to assess chimerism by differences in the variable number of tandem repeats between recipient and donor.

Definitions of Toxicity, GVHD, and Response

Toxicities were graded using the Bearman scale [10]. GVHD was staged and graded according to the International Bone Marrow Transplant Registry scale [11].

Patients were evaluated for response starting at day 28. Patients were evaluated by disease-specific guidelines. For leukemia, the absence of any histopathological, cytogenetic, or molecular evidence of disease (by polymerase chain reaction when applicable) was considered a CR. Persistent disease was defined as persistent evidence of disease by histopathological, cytogenetic, or molecular determination. Relapse was defined as the appearance of leukemia cells in the peripheral blood or marrow. For lymphomas, CR meant the radiographic (and, when appropriate, histological) disappearance of all tumor for a period of 1 month. Partial remission (PR) meant a ≥50% decrease in the sum of the products of the perpendicular diameters of all measured lesions without any evidence of progression of any lesion or the appearance of any new lesion for 1 month. Stable disease meant any change in measurable disease, which is less than the criteria for PR and without evidence for progression. Progressive disease was defined as a ≥50% increase in the biperpendicular product of any single lesion or the development of any new lesion. For myeloma, complete response was disappearance of serum M-protein and Bence Jones protein by immunofixation and no monoclonal plasma cells in the bone marrow. A partial response included >75% reduction in serum myeloma protein, >95% reduction in Bence Jones protein, and <5% bone marrow plasma cells. Progression was defined as an increase of 50% in serum myeloma protein over prior measurements that did not resolve within 2 months. All other persistent myeloma was considered stable disease.

Statistics

CML in chronic phase, refractory anemia, multiple myeloma, acute leukemia in CR, and lymphoma in PR or CR were considered nonadvanced disease for the purposes of analysis. Untreated refractory anemia with excess blasts with or without transformation and leukemia or lymphoma in relapse or refrac-

Table 1. *Patient Characteristics (n = 44)*

Age	**Median, 61 y (range, 29-71 y)**
Reason for eligibility	
Age > 55 y	**31**
Age > 55 y and comorbid condition	**7**
Comorbid condition alone	**6**
Comorbid conditions	
Performance status < 80	**5**
Renal disease*	**3**
Lung disease†	**2**
Congestive heart failure	**1**
Hemachromatosis	**1**
Aspergillus pneumonia under treatment	**1**

*Renal disease consisted of 2 patients with elevated creatinine levels (1.9 and 2.9) and 1 patient with a prior kidney transplantation.

†Lung disease consisted of 1 patient with emphysema and 1 patient with both restrictive and obstructive pulmonary disease (presumed from CLL).

tory to therapy were considered advanced disease for the purposes of analysis. Rates of overall survival and relapse were computed with Kaplan-Meier methods, and between-group comparisons of overall survival and relapse were made with a log-rank test. Due to the competing risk of death, GVHD rates were summarized with cumulative incidence rates and corresponding 95% confidence intervals (CIs). A Cox regression model of overall survival was used to assess the association of stage with overall survival adjusted for age, presence of renal comorbidity, and presence of other comorbidity. A similar Cox regression model was used for rate of relapse. Association of covariates to best response was assessed with Fisher exact test. All *P* values are two-sided.

RESULTS

Patient Characteristics

Patient characteristics are summarized in Table 1. The median age was 61 years (range, 29-71 years). Thirty-eight of the 44 patients were older than 55 years. Thirteen patients (including 7 patients also eligible by age criteria) had comorbid conditions that precluded conventional myeloablative transplantation. The 6 patients who were eligible on the basis of comorbid conditions alone had a Karnofsky performance status between 60 and 80 (3), renal failure (1), combined severe restrictive and obstructive lung disease presumably from CLL (1), and aspergillus pneumonia still under therapy (1).

Engraftment

The median cell dose transplanted for the 44 patients was 5.4×10^6 CD34+ cells/kg (range, 1.1-29.3 $\times$ 10^6 CD34+ cells/kg). Forty-one of the 44 patients developed severe neutropenia (absolute neutrophil count $<500/\mu L$). Three patients died before engraftment (days 6, 10, and 16 from rapidly progressive lymphoma, sepsis, and acute respiratory distress syndrome (ARDS) associated with sepsis, respectively). All remaining patients achieved a minimum absolute neutrophil count of $500/\mu L$ at a median of 12 days. There was no difference in time to engraftment between those with advanced and nonadvanced disease.

Chimerism

Donor/recipient chimerism in the marrow was assessed at day 30 in 38 patients. Patients were not assessed for chimerism due to early death (4), persistent disease (1), and lack of genetic polymorphism between the donor and recipient for selected markers (1). Only 6 patients had evidence of >10% recipient cells at day 30. Two of these patients relapsed at day 40 and day 98, respectively. One patient with refractory anemia achieved cytogenetic remission at day 60 and was alive and disease-free 17 months after HSCT. Two patients with CLL had persistent disease at day 30 that slowly cleared without intervention at 3 and 7 months, respectively. These two patients were alive, disease-free, and with 100% donor cells at 13 and 17 months after HSCT, respectively. One patient with persistence of >10% recipient cells at day 30 was alive with persistent CLL at 13 months after HSCT.

Surveillance marrow examinations detected reappearance of recipient DNA bands (<5%) by variable number of tandem repeat analysis in 4 cases following prior 100% donor chimerism. In 2 cases, overt clinical relapse occurred 1 month later; in the other 2 cases, the recipient DNA bands disappeared without intervention on follow-up examination 1 month later.

Regimen-Related Organ Toxicities

Fatal regimen-related organ toxicity occurred in 3 patients. One patient with a history of prior renal transplantation died of sepsis and renal failure on day 10. A second patient died of sepsis and ARDS on day 16. Congestive heart failure present before transplantation contributed to the death of another patient of sepsis and ARDS on day 61. There were no cases of veno-occlusive disease nor were there any other grade 3 or 4 regimen-related toxicities. Renal toxicity, generally reversible, was common. Fourteen of the 44 (32%) patients developed a peak creatinine level between 1.5 and 1.9 mg/dL and 5 patients (11%) developed a peak creatinine level >2 mg/dL during the first 100 days. In many of these patients, the dose of tacrolimus was decreased to reduce further renal toxicity. However, there was no difference in the probability of death or acute GVHD in this group of patients compared with patients who did not have renal toxicity. One patient, with pretransplantation renal failure, was unable to tolerate tacrolimus and died of GVHD on day 181 despite treatment with other non-nephrotoxic immunosuppressive agents.

Infections

Infectious complications in the first 100 days were common. Thirty-one infectious episodes occurred in 23 different patients. Bacterial infections, accounting for 22 of the 31 infectious episodes, included 14 positive blood cultures, 3 bacterial urinary tract infections, 1 case of pneumonia, one case of cellulitis, one case of salmonella enteritis, and 2 cases of *Clostridium difficile* enteritis. Bacterial infections accounted for 2 deaths, both of vancomycin-resistant enterococcus sepsis in patients with persistent AML. Three fungal infections, all candidal, 2 invasive and 1 case of thrush also developed in the first 100 days. There were also 2 mycobacterial pneumonias, 1 each of *Mycobacterium kansasii* and *Mycobacterium tuberculosis* diagnosed from bronchial alveolar lavages. Five viral infections occurred in 4 patients, which included 1 patient with simultaneous cytomegalovirus and respiratory syncytial virus pneumonia, 1 patient with cyto-

megalovirus antigenemia, and 2 patients with viral enteritis from rotavirus infection and adenovirus. Finally, 1 patient died of presumed sepsis and adult respiratory distress syndrome, but no organism was identified.

After day 100, infectious complications were less common, more often viral or fungal, and generally developed while patients were under treatment for GVHD. Five bacterial infections were diagnosed: 3 episodes of bacteremia, including 1 death of sepsis, 1 case of *Nocardia pneumonia*, and 1 case of urosepsis. There were 5 cases of invasive aspergillus and 8 cases of viral infection (4 oropharyngeal herpes simplex, 1 nasopharyngeal rhinovirus, and 3 viral diarrhea: rotavirus, cytomegalovirus, and adenovirus).

GVHD

There were 13 cases of significant (grades 2 to 4) acute GVHD: 9 grade 2, 3 grade 3, and 1 grade 4. The patient with grade 4 GVHD died. GVHD of the skin was the most common manifestation (11 patients), followed by intestinal GVHD (5 patients), and liver GVHD (2 patients). The cumulative incidence of grade 2 to 4 or grade 3 to 4 acute GVHD by day 100 was 38% (95% CI = 25%, 55%) and 20% (95% CI = 10%, 39%), respectively, with a median time to onset of 66 days. Two additional patients developed biologically acute GVHD that occurred after day 100. In one case, biopsy-proven GVHD of the intestines and liver developed on day 129 and progressed rapidly to death on day 148. In another case, a maculopapular rash characteristic of acute GVHD developed on day 136. The patient remained alive with chronic GVHD of the mouth, eyes, and lungs. If the 2 late-onset cases are included, the median time to onset of grade 2 to 4 GVHD was 75 days and acute GVHD was the proximate cause of death for 3 patients. Chronic GVHD occurred in 24 of 33 patients (limited 6 and extensive 18) who survived to at least day 100. The last occurrence of GVHD was day 220, at which point the cumulative incidence of chronic GVHD was 68% (95% CI = 54%, 82%). No cases of biologically chronic GVHD occurred before day 100. The most common manifestation was skin involvement (18 cases), followed by oral (16), liver (6), ocular (6), intestinal (3), and pulmonary (3). Skin and/or mouth involvement was more likely than liver, ocular, intestinal, or pulmonary involvement ($P < .003$). Chronic GVHD was considered the cause of death for 3 patients. One patient died of opportunistic infection at day 181 while on immunosuppression for GVHD and 2 patients died on days 347 and 435 from liver and pulmonary GVHD, respectively.

Disease Responses

Patient responses are listed in Table 2 and Table 3. Ten patients were not evaluable for disease response due to death before day 28 from nonrelapse cause (3) or continuous CR for acute leukemia (7). The remaining patients, with disease present at time of transplantation, were evaluable for response. Fourteen of 16 patients with MDS, AML, or CML achieved CR by 60 days posttransplantation, including cytogenetic or molecular remission when assessable. The 2 patients who did not respond had MDS (refractory anemia with excess blasts in transformation) were refractory to additional chemotherapy after transplantation, and donor leukocyte infusions (DLIs) were not performed.

Slower times to maximal response were seen for patients with CLL and multiple myeloma. Three of the four patients with CLL achieved a CR, but the responses took more than 3 months to achieve. One patient with CLL remained alive, but never achieved a CR. Three of the 5 patients with multiple myeloma responded, 1 CR and 2 PR. The other 2 patients have stable disease, ongoing now for 13 and 18 months, respectively. Among the responders, the myeloma response was slow to develop with the maximal decrease in the monoclonal gammopathy occurring 4 to 12 months posttransplantation. Finally, 5 of the 8 patients with NHL responded, 2 CRs and 3 PRs.

The likelihood of achieving a response did not differ between patients with advanced and nonadvanced stage (P = not significant), but the lack of significance may simply reflect the size of the study population or the heterogeneous diseases treated. A relationship between GVHD and the likelihood of achieving a response could not be demonstrated, but the size of the study is not large enough to make any definitive statements about GVHD and likelihood of response.

Outcomes

For the entire cohort, with a median follow-up of 24 months, the 1-year overall survival rate was 54% (95% CI = 41%, 71%) (Figure 1). The 1-year disease-free survival and relapse rates were 47% (95% CI = 35%, 65%) and 37% (95% CI = 19%, 51%), respectively. Outcomes differed based on the presence of advanced (n = 19) or nonadvanced (n = 25) disease. Median survival times (Figure 2) were 685 days and 138 days for subjects with nonadvanced and advanced disease, respectively ($P = .005$). After adjusting for age and comorbidity, disease stage continued to be significantly associated with overall survival ($P = .005$). Although relapse rates were lower for patients with nonadvanced disease compared with advanced disease, this difference was not statistically significant even after adjustment for age and comorbidity. Neither relapse nor survival was associated with the occurrence of acute GVHD, but this may be simply a reflection of sample size. Cell dose had no significant relationship with overall survival whether treating cell dose as a continuous variable or categorizing cell dose into quartiles.

DISCUSSION

Reducing the intensity of preparative regimens to minimize regimen-related morbidity and mortality has been the focus of much research over the past few years [1-9]. The intensity of these regimens range from nonablative [6,7,12] to moderately ablative [4]. The reduced-intensity regimen used in this study was moderately ablative and similar to that used by Slavin et al. in a study of younger, generally healthy patients [4]. Because we did not observe any primary graft failures, we believe that the substitution of MMF/TLI for ATG is adequate for the purpose of preventing rejection. Toxicity from the conditioning regimen in this study was significant, but rarely fatal, and similar to that seen in other studies of moderately reduced–intensity conditioning [1,4,5,8]. Although a direct comparison of these groups is not possible, we suspect that the overall regimen-related toxicity we observed is probably less than would be seen in this older and sicker patient population with a conventional conditioning regimen.

Table 2. *Outcomes for Advanced-Stage Patients*

Disease	Pre-BMT Status	Day of Onset of GVHD	Maximum Acute GVHD Stage	Chronic GVHD	Best Response	Day Best Response Achieved	Outcome
MDS → AML	1st relapse	—	0	−	Cytogenetic remission	29	Relapsed d 91, died d 138
MDS → AML	Refractory	—	0	−	Cytogenetic remission	30	Relapsed d 105, died d 168
AML	1st relapse	—	0	NE	NE	—	Died of sepsis d 16
CLL	Refractory	—	0	−	NR	—	Alive with stable disease, d 394
CLL	Refractory	—	0	+	CR	100	Alive in remission, d 336
CLL	Refractory	42	2	+	Cytogenetic remission	212	Alive in remission, d 493
CMML	1st relapse	—	0	NE	CR	31	Died of sepsis, d 69
CMML	1st relapse	—	0	+	Cytogenetic remission	34	Relapsed d 93, immunosuppression stopped, developed GVHD, and now alive in remission, d 306
NHL*	Refractory	73	3	NE	PR	65	Died of tuberculosis, d 101
NHL†	Refractory	36	4	NE	PR	12	Died of GVHD, d 51
NHL‡	Refractory	—	NE	NE	PD	—	Died of disease, d 6
NHL†	Refractory	98	2	+	CR	177	Alive in remission, d 366
NHL§	Refractory	169	0	+	CR	29	Died in remission of acetaminophen overdose, d 561
RAEB	Refractory	75	3	−	NR	—	Died of GVHD, d 93
RAEB-T	Untreated	—	NE	NE	NE	—	Died of ARDS, d 10
RAEB-T	Untreated	—	0	NE	NR	—	Died of disease, d 63
RAEB-T	Untreated	—	0	−	NR	—	Died of disease, d 108
RAEB-T	Untreated	27	2	+	Cytogenetic remission	36	Relapsed d 261, died d 314
RAEB-T	Untreated	90	2	+	Cytogenetic remission	34	Died in remission of pneumoccocus, d 336

BMT indicates bone marrow transplantation; MDS → AML, myelodysplastic syndrome transformed into acute myeloid leukemia; RAEB-T, refractory anemia with excess blasts in transformation; NE, not evaluable; NR, no response; PD, progressive disease.

*Cutaneous T-cell lymphoma.

†Mantle cell lymphoma.

‡Cutaneous B-cell lymphoma with central nervous system metastases.

§Follicular, mixed NHL.

Marrow Chimerism Did Not Predict Relapse

In the present study, neutrophil engraftment in this study was prompt. The intensity of the regimen was sufficient to ensure near-complete donor engraftment in most patients by day 30. We considered the possibility that the biological significance of mixed chimerism present after a moderately ablative regimen such as the one used in this study may be different compared with fully myeloablative conditioning. In this study, a small number of patients had low levels of mixed chimerism (<10% recipient cells) at day 30, which was not predictive of outcome. The level of recipient chimerism increased before relapse in 3 patients, but in 2 patients reappearance of recipient DNA did not herald relapse. In myeloablative transplantation, reappearance or an increasing percentage of recipient DNA can be predictive of relapse [13-15]. In the present study, analysis for bone marrow chimerism at day 30 did not predict outcome, however, this finding should be interpreted cautiously given the small numbers of patients involved. Perhaps, in the setting of reduced conditioning intensity, the presence of mixed chimerism at day 30 may reflect an ongoing graft-versus-leukemia process and remission may yet be achieved. Furthermore, because the graft-versus-malignancy effect is largely a lymphocyte-mediated event, perhaps chimerism of donor T cells, which was not examined here, would prove more informative [6,16].

GVHD Was Common but Delayed in Onset

The incidence of GVHD in this study (38%) is similar to the 35% to 44% reported in conventional HSCT [17,18] and other moderately ablative intensity trials [4,6,8]. The median time to onset of acute GVHD in this study was 66 days compared with 16 to 20 days for conventional HSCT recipients [17,18], all of whom received tacrolimus for a similar length of time. Similarly, in an analysis of more than 7000 HLA-identical sibling non–T-cell-depleted transplantations for early- or intermediate-stage leukemia, all of whom were given cyclosporine plus methotrexate, >90% of subjects who experienced GVHD

Table 3. *Outcomes for Patients with Nonadvanced Disease*

Disease	Pre-BMT Status*	Day of Onset of GVHD	Maximum Acute GVHD Stage	Chronic GVHD	Best Response	Day Best Response Achieved	Outcome
ALL	CR1	21	2	+	CCR	27	Relapsed d 174, died d 207
ALL-Ph+	CR1	143	0	+	CCR	31	Alive in remission, d 486
CMML → AML	CR1	—	0	−	CCR	30	Relapsed d 98, died d 128
MDS → AML	CR1	23	1	NE	NE	—	Died from sepsis d 26
MDS → AML	CR1	—	0	−	CCR	29	Alive in remission, d 210
MDS → AML	+8, +11	98	0	+	Cytogenetic remission	29	Alive in remission, d 638
AML	CR1	210	0	+	CCR	30	Relapsed d 336, died d 414
AML	CR2	—	0	−	CCR	30	Relapsed d 122, died d 179
AML	CR2	177	0	+	CCR	31	Alive in remission, d 408
CLL	PR	85	2	+	CR	116	Alive in remission, d 471
CML	CP	93	1	+	Molecular remission	58	Alive in remission, d 770
CML	CP	136	0	+	Molecular remission	29	Alive in remission, d 587
MM	3B	76	2	+	CR	136	Alive in remission, d 874
MM	3A	122	0	+	NR	—	Alive with stable disease, d 554
MM	3B	—	0	−	NR	—	Alive with stable disease, d 409
MM	3A	220	0	+	PR	364	Died of GVHD d 435
MM	3A	112	0	+	PR	211	Alive in partial remission, d 462
NHL†	PR	18	2	NE	NR	—	Relapsed d 59, died d 61
NHL‡	PR	23	1	+	PR	110	Alive in partial remission, d 486
NHL§	PR	60	3	+	Mixed response	29	Relapsed d 145, immunosuppression stopped, died of GVHD d 181
RA	+8	200	0	+	Cytogenetic remission	31	Alive in remission, d 773
RA	Complex	—	0	−	Cytogenetic remission	62	Alive in remission, d 351
RA	5q-, 13q-	129	0	+	Cytogenetic remission	35	Died of GVHD d 148
RA	5q-, der11	197	0	+	Cytogenetic remission	61	Alive in remission, d 252
RAEB-T	−5, −7, der11	92	2	+	CR	31	Alive in remission, d 322

ALL indicates acute lymphoblastic leukemia; ALL-Ph+, acute lymphoblastic leukemia, Philadelphia chromosome–positive; CMML → AML, chronic myelomonocytic leukemia transformed into acute myeloid leukemia; MM, multiple myeloma; RA, refractory anemia; CR1, first complete remission; CR2, second complete remission; CP, chronic phase; CCR, continuous complete remission; NR, no response.

*If the patient was in morphological remission but cytogenetic evidence of persistent disease was present at BMT, the cytogenetic abnormalities are noted.

†Lymphoblastic lymphoma.

‡Mantle cell lymphoma.

§Monocytoid B-cell lymphoma.

did so by day 60 (personal communication, Mary Horowitz, MD, International Bone Marrow Transplant Registry, June 6, 2002), compared with less than half of the patients in the present study. The delay in onset of acute GVHD in reduced-intensity transplantation suggests that perhaps time of onset should no longer be part of the definition of acute or chronic GVHD. We can not rule out the possibilities that differences in GVHD prophylaxis or the generally older patient population may account for the later onset of GVHD in this study, but we think that those are not likely explanations. The main difference

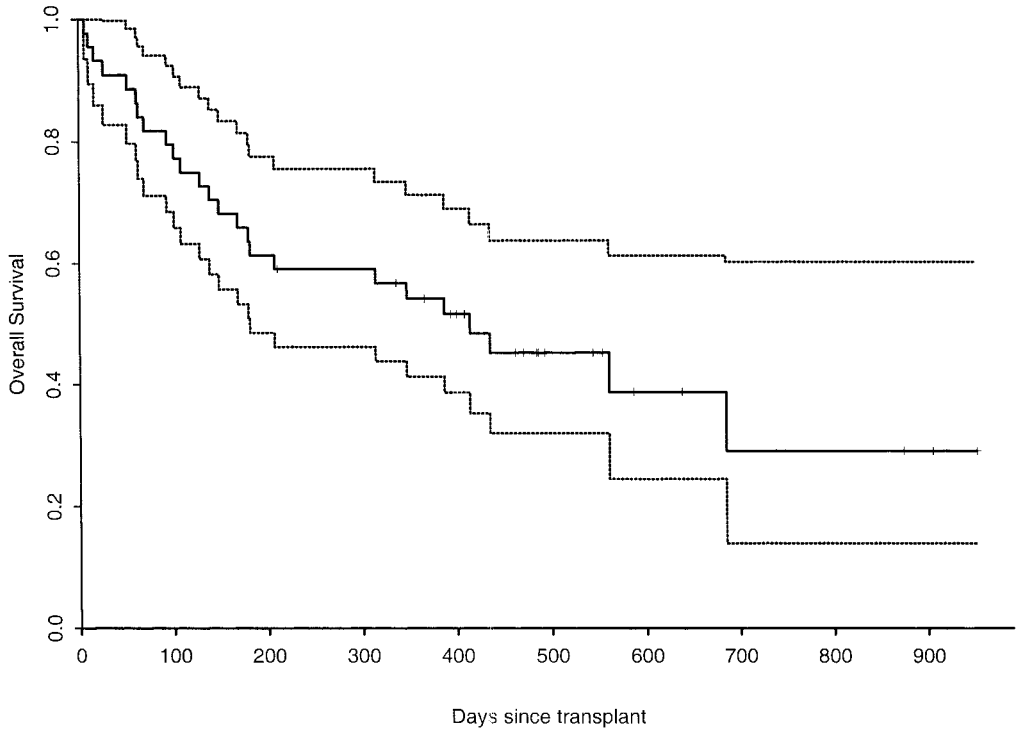

Figure 1. Kaplan-Meier plot of overall survival (solid line) with 95% confidence limits (dashed lines) for all patients (N = 44).

between this study and the earlier onset of GVHD observed in other studies using tacrolimus or cyclosporine to prevent GVHD in the context of related donor transplantation is the intensity of the conditioning regimen. We know that cytokine release from tissue damage is critical in the pathogenesis of GVHD [19], and it is possible that a reduction in conditioning intensity results in less cytokine release. This hypothesis needs to be confirmed by laboratory testing.

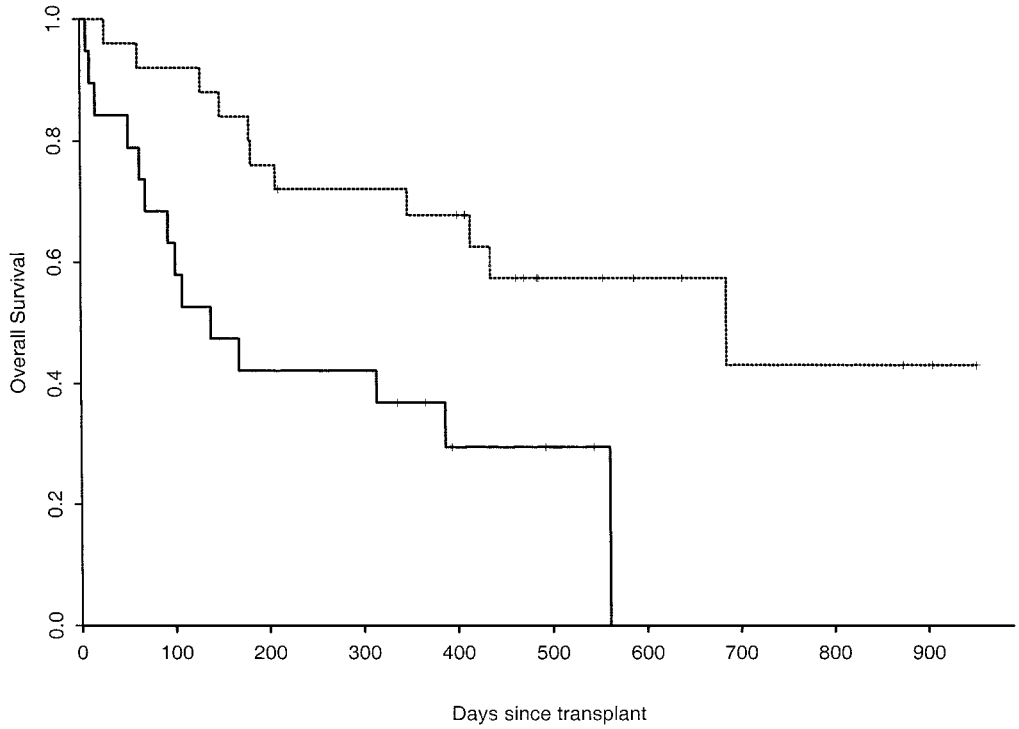

Figure 2. Kaplan-Meier plot of overall survival for patients with advanced-stage disease (n =19, solid line) and patients with non-advanced disease (n = 25, dashed line).

Reduced-Intensity Conditioning Did Not Cure Most Patients with Advanced-Stage Disease

In this study, durable progression-free survival was not achieved for most patients with advanced-stage disease, even when disease responses were achieved early after transplantation. For example, patients with leukemia or MDS were likely to be in remission at day 30. But remission duration was much shorter for patients with advanced-stage disease compared with nonadvanced disease. Median follow-up is 24 months and more relapses may yet occur. For patients with advanced-stage disease, treatment-related toxicity and GVHD also contributed to the high mortality rate. Our finding that pretransplantation disease stage is prognostically important is consistent with a retrospective analysis of 92 patients treated with a variety of reduced-intensity regimens [20]. In that study, with a median follow-up of 8 months, multivariate analysis found that patients with advanced-stage disease were more than twice as likely to die as patients with nonadvanced stage disease. Thus, although allogeneic HSCT can be offered to older and sicker patients, the benefit of this approach for such patients with advanced-stage disease is yet to be established. Reducing treatment-related morbidity and mortality is desirable, but further reduction in conditioning intensity without other measures to control cancer is unlikely to result in long-term disease control for patients with advanced-stage disease. Because relapse is also a major cause of poor outcome for patients who undergo intensive conditioning, it is not realistic to expect fewer relapses with reduced-intensity conditioning unless other mechanisms of enhancing an antitumor effect are developed. Strategies that protect against regimen-related toxicity, such as amifostine [21], may permit more intense regimens for this group of patients. Another approach is the use of DLIs to prevent relapse. In a report of 21 patients with refractory hematologic malignancies, Spitzer et al. observed complete responses in 5 of 9 patients (56%) who received prophylactic DLI compared with only 3 of 11 patients (27%) of those who did not receive prophylactic DLI [22]. In this relatively young group of patients, acute GVHD developed in 5 of 9 (56%) patients who received a prophylactic DLI, highlighting the potential toxicity such an approach may have in an older, less healthy population. Clearly, strategies to preserve the graft versus leukemia (GVL) effect while protecting against GVHD are needed. If the delayed onset of GVHD observed in this study reflects reduced cytokine release from tissue damage, then strategies that protect against inflammatory cytokines may prove to be useful. Experimental models of keratinocyte growth factor as a cytokine shield or blockade of lipopolysaccharide, an important mediator of cytokine release, have shown protection against GVHD while preserving GVL [23,24]. In this study, the potential advantages of reduced-intensity conditioning in decreasing regimen-related mortality for older, sicker patients with advanced-stage malignancies were offset by the high rates of relapse and other complications. By comparison, the patients with nonadvanced disease, who would not otherwise have been eligible for allogeneic transplantation, fared relatively well. The successful development of strategies to enhance cancer control, such as adoptive immunotherapy combined with agents that protect against GVHD while preserving GVL, will permit this approach to be extended to higher-risk patients.

ACKNOWLEDGMENTS

This work was supported in part by grant CA88930 awarded by the National Institutes of Health, Bethesda, Maryland.

REFERENCES

1. Giralt S, Estey E, Albitar M, et al. Engraftment of allogeneic hematopoietic progenitor cells with purine analog-containing chemotherapy: harnessing graft-versus-leukemia without myeloablative therapy. *Blood*. 1997;89:4531-4536.
2. Storb R, Yu C, Sandmaier B, et al. Mixed hematopoietic chimerismafter hematopoietic stem cell allografts. *Transplant Proc*. 1999;31: 677-678.
3. Storb R, Yu C, Sandmaier BM, et al. Mixed hematopoietic chimerism after marrow allografts – transplantation in the ambulatory care setting. *Ann N Y Acad Sci*. 1999;872:372-376.
4. Slavin S, Nagler A, Naparstek E, et al. Nonmyeloablative stem cell transplantation and cell therapy as an alternative to conventional bone marrow transplantation with lethal cytoreduction for the treatment of malignant and nonmalignant hematologic diseases. *Blood*. 1998;91:756-763.
5. Khouri IF, Keating M, Korbling M, et al. Transplant-lite: induction of graft-versus-malignancy using fludarabine-based nonablative chemotherapy and allogeneic blood progenitor-cell transplantation as treatment for lymphoid malignancies. *J Clin Oncol*. 1998;16:2817-2824
6. McSweeney PA, Niederwieser D, Shizuru JA, et al. Hematopoietic cell transplantation in older patients with hematologic malignancies: replacing high-dose cytotoxic therapy with graft-versus-tumor effects. *Blood*. 2001;97:3390-3400.
7. Sykes M, Preffer F, McAfee S, et al. Mixed lymphohaemopoietic chimerism and graft-versus-lymphoma effects after nonmyeloablative therapy and HLA-mismatched bone-marrow transplantation. *Lancet*. 1999;353:1755-1759.
8. Giralt S, Thall PF, Khouri I, et al. Melphalan and purine analog-containing preparative regimens: reduced-intensity conditioning for patients with hematologic malignancies undergoing allogeneic progenitor cell transplantation. *Blood*. 2001;97:631-637.
9. Carella AM, Lerma E, Dejana A, et al. Engraftment of HLA-matched sibling hematopoietic stem cells after immunosuppressive conditioning regimen in patients with hematologic neoplasias. *Haematologica*. 1998;83:904-909
10. Bearman SI, Appelbaum FR, Buckner CD, et al. Regimen-related toxicity in patients undergoing bone marrow transplantation. *J Clin Oncol*. 1988;6:1562-1568.
11. Rowlings PA, Przepiorka D, Klein JP, et al. IBMTR severity index for grading acute graft-versus-host disease: retrospective comparison with Glucksberg grade. *Br J Haematol*. 1997;97:855-864.
12. Childs R, Clave E, Contenin N, Bahceci E, Barrett AJ. Full donor chimerism predicts for graft-versus-host disease, graft-versus-marrow and graft-versus-malignancy effects following a non-myeloablative preparative regimen. *Blood*. 1999;94:3234-3241.
13. Tamura S, Saheki K, Takatsuka H, et al. Early detection of relapse and evaluation of treatment for mixed chimerism using fluorescence in situ hybridization following allogeneic hematopoietic cell transplant for hematological malignancies. *Ann Hematol*. 2000;79:622-626.
14. Wang LJ, Chou P, Gonzalez-Ryan L, Huang W, Haut PR, Kletzel M. Evaluation of mixed hematopoietic chimerism in pediatric patients with leukemia after allogeneic stem cell transplantation by quantitative PCR analysis of variable number of tandem repeat and testis determination gene. *Bone Marrow Transplant*. 2002;29:51-56.

15. Bader P, Beck J, Frey A, et al. Serial and quantitative analysis of mixed hematopoietic chimerism by PCR in patients with acute leukemias allows the prediction of relapse after allogeneic BMT. *Bone Marrow Transplant*. 1998;21:487-495.
16. Childs R, Clave E, Contentin N, et al. Engraftment kinetics after nonmyeloablative allogeneic peripheral blood stem cell transplantation: full donor T-cell chimerism precedes alloimmune responses. *Blood*. 1999;94:3234-3241.
17. Nash RA, Pepe MS, Storb R, et al. Acute graft-versus-host disease: analysis of risk factors after allogeneic marrow transplantation and prophylaxis with cyclosporine and methotrexate. *Blood*. 1992;80: 1838-1845.
18. Ratanatharathorn V, Nash RA, Przepiorka D, et al. Phase III study comparing methotrexate and tacrolimus (Prograf, FK506) with methotrexate and cyclosporine for graft-versus-host-disease prophylaxis after HLA-identical sibling bone marrow transplantation. *Blood*. 1998;92:2303-2314.
19. Antin JH, Ferrara JLM. Cytokine dysregulation and acute graft-versus-host disease. *Blood*. 1992;80:2964-2968.
20. Michallet M, Bilger K, Garban F, et al. Allogeneic hematopoietic stem-cell transplantation after nonmyeloablative preparative regimens: impact of pretransplantation and posttransplantation factors on outcome. *J Clin Oncol*. 2001;19:3340-3349.
21. Martens AC, Hagenbeek A. Amifostine (WR2721) for dose escalation in marrow-ablative treatment of leukaemia. *Eur J Cancer*. 1999; 35:634-640.
22. Spitzer TR, McAfee S, Sackstein R, et al. Intentional induction of mixed chimerism and achievement of antitumor responses after nonmyeloablative conditioning therapy and HLA-matched donor bone marrow transplantation for refractory hematologic malignancies. *Biol Blood Marrow Transplant*. 2000;6:309-320.
23. Krijanovski OI, Hill GR, Cooke KR, et al. Keratinocyte growth factor separates graft-versus-leukemia effects from graft-versus-host disease. *Blood*. 1999;94:825-831.
24. Cooke KR, Gerbitz A, Crawford JM, et al. LPS antagonism reduces graft-versus-host disease and preserves graft-versus-leukemia activity after experimental bone marrow transplantation. *J Clin Invest*. 2001;107:1581-1589.