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Marrow Transplants From Unrelated Donors for Patients With Aplastic Anemia: Minimum Effective Dose of Total Body Irradiation

H. Joachim Deeg,[1] Michael D. Amylon,[2] Richard E. Harris,[3] Robert Collins,[4,5] Patrick G. Beatty,[6] Stephen Feig,[7] Norma Ramsay,[8] Mary Territo,[7] Shakila P. Khan,[9] Derwood Pamphilon,[10] José F. Leis,[11] Stefan Burdach,[12] Claudio Anasetti,[1] Robert Hackman,[1] Barry Storer,[1] Beth Mueller[13]

[1]Clinical Research Division, Fred Hutchinson Cancer Research Center, University of Washington School of Medicine, Seattle, Washington; [2]Division of Hematology/Oncology, Stanford University Medical Center, Stanford, California; [3]Children's Hospital Medical Center, Cincinnati, Ohio; [4]Baylor University, Dallas, Texas; [5]University of Texas Southwestern Medical Center, Dallas, Texas; [6]University of Utah, Salt Lake City, Utah; [7]UCLA, Los Angeles, California; [8]University of Minnesota, Minneapolis, Minnesota; [9]Texas Children's Cancer Center, Houston, Texas; [10]Royal Hospital for Sick Children, University of Bristol, Bristol, United Kingdom; [11]Oregon Health Sciences University, Portland, Oregon; [12]University of Düsseldorf, Düsseldorf, Germany; [13]Public Health Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, Washington

Correspondence and reprint requests: H. Joachim Deeg, Fred Hutchinson Cancer Research Center, 1100 Fairview Avenue N, D1-100, PO Box 19024, Seattle, WA 98109-1024 (e-mail: jdeeg@fhcrc.org).

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ABSTRACT

Patients with aplastic anemia who do not have suitably HLA-matched, related donors generally receive immunosuppressive treatment as first-line therapy and are considered for transplantation from an unrelated donor only if they fail to respond to immunosuppressive treatment. In this setting, rates of transplantation-related morbidity and mortality have been high. We conducted a prospective study to determine the minimal dose of total body irradiation (TBI) sufficient to achieve sustained engraftment when it is used in combination with 3 cycles of 30 mg/kg of antithymocyte globulin (ATG) and 4 cycles of 50 mg/kg of cyclophosphamide (CY). We also wanted to determine the tolerability and toxicity of the regimen. The starting dosage of TBI was 3 × 200 cGy given over 2 days following CY/ATG. The TBI dose was to be escalated in increments of 200 cGy if graft failure occurred in the absence of prohibitive toxicity, and de-escalated for toxicity in the absence of graft failure. Twenty-one female and 29 male patients aged 1.3 to 46.5 years (median age, 14.4 years) underwent transplantation at 14 medical centers. The time interval from diagnosis to transplantation was 2.8 to 264 months (median, 14.5 months). All patients had been transfused multiple times and all had received 1 to 11 courses (median, 4 courses) of immunosuppressive treatment and other modalities of treatment. In 38 cases, the donors were HLA-A, -B and -DR phenotypically matched with the patients, and, in 12 cases, the donor phenotype differed from that of the recipient by 1 HLA antigen. Recipients of mismatched transplants were considered separately for TBI dose modification, and this study is still ongoing. Seven patients did not tolerate ATG and were prepared with 6 × 200 cGy of TBI plus 120 mg/kg of CY. Of the HLA-matched recipients prepared with CY/ ATG/TBI, all 20 who received 3 × 200 or 2 × 200 cGy of TBI achieved engraftment, and 10 are alive. Of the 13 patients who received 1 × 200 cGy of TBI, 1 failed to engraft, and 8 are alive. Each of 10 patients who received an HLA-nonidentical transplant achieved engraftment, and 3 of 6 who were given 3 × 200 cGy of TBI, and 4 of 4 who were given 2 × 200 cGy are alive. Pulmonary toxicity occurred in 8 of 30 patients who were given 3 × 200 or 2 × 200 cGy of TBI concurrently with ATG and CY at 200 mg/kg, and in 2 of 13 patients who received 1 × 200 cGy of TBI, a pattern that suggests a decrease in toxicity with TBI dose de-escalation. Overall, the highest probability of survival (73%) was observed

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among patients who underwent transplantation within 1 year of diagnosis, compared with patients who underwent transplantation after a longer period of disease. In addition, younger patients (aged ≤20 years) were more likely to survive than older patients (aged >20 years). Thus, for patients with an HLA-matched, unrelated donor, a TBI dose of 200 cGy (in combination with CY/ATG) was sufficient to allow for engraftment without inducing prohibitive toxicity. As in previous studies, patient age and pretransplantation disease duration remain important prognostic factors.

KEY WORDS
Aplastic anemia • Volunteer donors • Disease duration • Total body irradiation • Pulmonary toxicity

INTRODUCTION

Hematopoietic stem cell transplantation from an allogeneic donor provides curative therapy for patients with severe aplastic anemia. However, only 25% to 30% of patients have a suitably HLA-matched, related donor and proceed to transplantation as first-line therapy [1-3]. Patients who do not have a suitably HLA-matched, related donor generally receive immunosuppressive therapy as the treatment of choice. This is a reasonable approach because acute toxicity is moderate and response rates as high as 80% have been reported with this therapy [4,5]. However, recurrence of aplasia is frequent, and 20% to 45% of patients receiving immunosuppressive therapy have been reported to develop a clonal hematopoietic disorder 5 to 7 years after treatment [6,7]. Nevertheless, these patients are generally considered for transplantation from an unrelated donor only when they fail to respond to immunosuppressive treatment (often after repeated courses). They usually do not undergo transplantation at the time of the initial diagnosis because the results of immunosuppressive therapy are good [4,5], transplantation-related mortality with unrelated donor transplants historically has been high, and probability of survival has been low [8,9]. Causes of transplantation failure include increased incidence of graft rejection, regimen-related toxicity due to intensified conditioning regimens aimed at preventing rejection, and high incidence of acute graft-versus-host disease (GVHD) [8-10].

In a pilot study of 5 patients who received transplants from unrelated donors, we used antithymocyte globulin (ATG) and cyclophosphamide (CY) treatment, a regimen used for patients who receive transplants from HLA-identical sibling donors [11]. In transplantations using HLA-identical sibling donors, this regimen allows for successful outcomes in more than 90% of patients. However, we observed at least 1 immunologic graft rejection and 2 graft failures in transplantations using unrelated donors; only 1 patient is currently surviving [12]. At the same time, previous reports by European groups and by the National Marrow Donor Program (NMDP) suggest that high-dose irradiation regimens, although effective in securing engraftment, will further increase toxicity without increasing the probability of survival [8,13,14].

Therefore, with support from the NMDP, we initiated a collaborative, multicenter, prospective, phase I study designed to define the minimum effective dose of total body irradiation (TBI) in conditioning regimens for patients with aplastic anemia, who, having failed to respond to immunosuppressive therapy, received transplants from unrelated donors [3]. Here, we present results on the first 50 patients enrolled in this study, indicating that a TBI dose of 200 cGy (in combination with ATG and CY) is sufficient to achieve engraftment of transplants from HLA-matched unrelated donors. The minimum effective dose of TBI for patients who receive grafts from HLA-nonidentical unrelated donors remains to be determined.

PATIENTS AND METHODS

The objective of this study was to determine the minimum effective dosage of TBI, in combination with ATG and CY, necessary to achieve engraftment in patients with severe aplastic anemia who received transplants from unrelated donors [3]. Patients included in the study were those with aplastic anemia who had failed to respond to the best available immunosuppressive treatment by 75 days after initiation of therapy, who did not have an HLA-identical family member, and who were aged 55 years or younger if an HLA-A, -B, -DR phenotypically matched unrelated donor was identified, or 35 years or younger if an HLA-nonidentical donor was identified.

Patients

The study population consisted of 50 patients who presented with severe aplastic anemia (excluding patients with Fanconi anemia) and who received bone marrow transplants between February 1994 and June 1999 at 1 of 14 centers in the United States, Germany, or the United Kingdom. Forty-five donors were recruited through the NMDP (and were registered with the NMDP), and 5 through other registries. All patients were registered with the study coordinator at the Fred Hutchinson Cancer Research Center in Seattle for TBI dosage assignment. Patient and transplant characteristics are summarized in Table 1. Patients were aged between 1.3 and 46 years (median, 14 years). All had been transfused with either red blood cells or platelet preparations, or both, and all had received immunosuppressive therapy, growth factors, and other modalities of treatment with 1 to 11 agents (median, 4). The protocol required that patients had received at least 1 course of conventional immunosuppressive therapy with either no hematologic response or a response followed by recurrence of marrow aplasia. Following failure of immunosuppressive therapy, patients were eligible for this protocol even if hematologic parameters did not satisfy the criteria for severe aplastic anemia at the time of recurrence.

Table 1. *Characteristics of 50 Patients With Aplastic Anemia and Their Bone Marrow Donors**

Characteristic		Number of Patients
Age, y		
Mean (range)	**14 (1.3-46)**	
≤20 y		**29**
>20 y		**21**
Sex (M/F)		**29/21**
Race/ethnicity		
White		**36**
Hispanic		**9**
African American		**3**
Asian		**2**
Disease duration, mo		
Mean (range)	**14.5 (2.8-264)**	
Pretransplantation therapy		
Transfusions		**50**
Immunosuppression		**50**
No. of courses of therapy, mean (range)	**4 (1-11)**	
Donor age, y		
Mean (range)	**37 (22-54)**	
Donor sex (M/F)		**28/20***
Donor/recipient sex		
F/F		**14**
F/M		**6**
M/M		**21**
M/F		**7**
Donor/recipient HLA compatibility		
Phenotypically matched		**38**
1-antigen–mismatched		**12**

*Data was not available for 2 patients.

Donor Selection, Marrow Collection, and Processing

The donors were 20 women and 28 men (sex information was unavailable for 2 donors) who were unrelated to the patients. They were aged between 18 and 54 years (median, 37 years), and were selected on the basis of HLA typing available at the time of enrollment into the study. In 38 cases, the donor was HLA-matched with the patient for HLA-A, -B and -C as determined by serologic typing, and in some cases, by additional high-resolution oligonucleotide typing [15,16]. In 12 cases, the donor phenotype differed from that of the recipient by 1 HLA antigen. The 2 patient groups were considered separately for TBI dosage determination. Bone marrow donations were collected at 37 unique collection centers, each of which harvested marrow from 1 to 6 donors. Marrows were harvested according to the policies of the NMDP and brought to the transplantation center by a courier.

Transplantation Regimen

The protocol prescribed 4 doses of intravenous CY, 50 mg/kg per day on 4 consecutive days, and, evenly spaced between these, 3 infusions of horse ATG (ATGAM; Upjohn, Kalamazoo, MI) at doses of 30 mg/kg. CY and ATG were followed by TBI at a starting dosage of 3×200 cGy (2 doses on 1 day, and 1 on the next day), followed by the infusion of donor marrow [3]. As described in the statistical section, dependent upon toxicity and efficacy, the TBI dose was to be escalated or de-escalated in steps of 200 cGy in sequential groups of patients. Any organ system toxicity of grade IV (by the Bearman criteria) [17] that was thought to be related to the conditioning regimen was considered a prohibitive toxicity. In addition, stomatitis, cardiac, pulmonary, or hepatic toxicity of grade III severity based on the Bearman criteria [17] was considered unacceptable toxicity. Patients who could not complete the ATG regimen, because of either a prior anaphylactic reaction or a severe adverse reaction to the initial dose of ATG (n = 7), alternatively received CY at 60 mg/kg per day for 2 consecutive days (if the first CY dose of 50 mg/kg had been given [1 patient], the second CY dose was 70 mg/kg), followed by TBI (200 cGy twice a day for 3 consecutive days for a total of 1200 cGy), followed by donor marrow infusion.

Engraftment

The day of engraftment was defined as the first of 3 consecutive days on which the neutrophil count exceeded 0.5×10^9/L [18]. Therefore, patients who did not have a neutrophil count of $>0.5 \times 10^9$/L for 3 consecutive days at any time posttransplantation were considered to have primary graft failure. Patients who survived for at least 21 days and achieved initial engraftment but subsequently were found to have a severely hypocellular marrow or an absolute neutrophil count of $<0.5 \times 10^9$/L were considered to have secondary graft failure. Patients who survived fewer than 21 days were considered evaluable for engraftment only if they had developed GVHD before death. Marrow and peripheral blood cells were tested sequentially for chimerism using Y chromosome–specific complementary DNA probes in patients with a donor of opposite sex or probes specific for polymorphic DNA sequences (eg, variable number tandem repeats).

Graft-Versus-Host Disease Prophylaxis

For GVHD prophylaxis, patients received a regimen as described [19,20], including methotrexate at doses of 10 mg/m^2 on days 1, 3, 6, and 11 after the marrow infusion, and cyclosporine (Sandimmune, Sandoz, Basel, Switzerland), starting on day –1 at a dosage of 5 mg/kg per day as a continuous infusion, de-escalated to 3 mg/kg per day on day 4, and switched to the oral form at 12 mg/kg per day in 2 divided doses once the patient was able to take oral medications. Cyclosporine was continued daily, and it was given at gradually reduced doses from day 50 through day 180. Acute GVHD was graded based on findings in the skin, liver, and intestinal tract according to the Glucksberg criteria as recently modified [21,22]. Chronic GVHD was assessed as limited (mild skin involvement only) or extensive, as described previously [23].

Data Collection

Data were collected in forms designed specifically for this study, in addition to the standardized forms provided by the NMDP. Data collection included pretransplantation information, peritransplantation events, and follow-up at 6- to 12-month intervals. The median length of follow-up was 39 months (range, 12 to 76 months). Data were analyzed as of July 1, 2000.

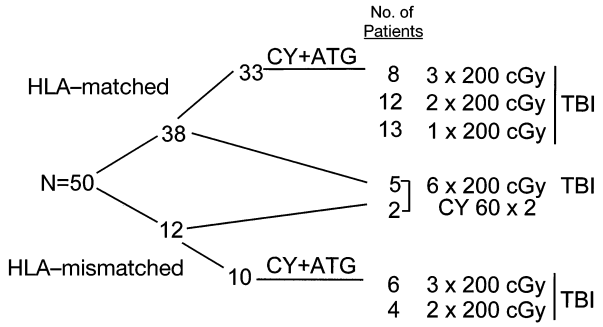

Figure 1. Conditioning regimen given to 50 patients who underwent transplantation. Of the 50 patients, 43 (33 with grafts from HLA-matched donors and 10 with grafts from HLA-mismatched donors) were conditioned with CY + ATG + TBI at the dosages shown. Seven patients (5 who received HLA-matched grafts and 2 who received HLA-mismatched grafts) were unable to receive ATG and were conditioned with CY and TBI only.

Study Design

This study was designed to develop and evaluate conditioning regimens for patients with severe aplastic anemia who received marrow transplants from unrelated donors. The goal was to achieve sustained engraftment without inducing prohibitive toxicity. The probability of graft failure (and possibly treatment-related toxicity) was expected to be different between patients receiving fully HLA-matched grafts and patients receiving partially HLA-mismatched grafts, and dosage determination was pursued separately for the 2 groups.

Patients were to be treated in groups of 6, with the first group receiving 3 × 200 cGy. If more than 1 patient was registered at a given time and endpoints of toxicity or efficacy were not yet evaluable in preceding patients, the actual number of patients in a dosage cohort could be greater than 6. Possible dosages for subsequent patients were 4 × 200, 5 × 200, 6 × 200, or alternatively, 2 × 200 or 1 × 200 cGy. Guidelines were as follows: if 2 or more patients suffered unacceptable toxicity at a given dose, the next lower dose would be chosen as the final dosage for testing. If 1 patient suffered unacceptable toxicity, the next 6 patients would be treated at the same dosage level. This dosage was to be considered the final dosage if no further patients experienced prohibitive toxicity and at least 11 of 12 achieved engraftment. If, however, 2 or more of the 12 patients had unacceptable toxicity, the next lower dosage was to be the final dosage for testing. If no patients suffered unacceptable toxicity and at least 1 patient failed to engraft, the dosage was to be escalated to the next higher level. If all patients achieved engraftment, the next group of patients was to be treated at the same dosage level. The final dosage was to be the dosage at which 11 of 12 patients achieved engraftment and no more than 1 of 12 suffered prohibitive toxicity. The properties of this TBI dosage determination method were evaluated by Monte Carlo simulation using various assumptions for the probabilities of prohibitive toxicity and engraftment failure. For the scenarios evaluated, this design had a high probability of choosing an acceptable dosage level. Survival probabilities were estimated by the Kaplan-Meier method. Likelihood ratio tests from proportion hazards regression models were used to compare survival among groups of patients.

RESULTS

Identification of Donors

Thirty-eight patients received transplants from phenotypically HLA-matched unrelated donors and 12 from HLA 1-antigen–mismatched donors (Table 1). The median time from initiation of the formal donor search to transplantation was 4 months (range, 2 to 65 months). Donor age and sex are given in Table 1.

Conditioning Regimen

The conditioning regimens given to patients, dependent upon tolerability of ATG and toxicity and efficacy of the TBI schedule, are summarized in Figure 1.

Outcome

Engraftment. Transplantation outcomes are summarized in Table 2. Patients were followed for a median of 39 months (range, 12 to 76 months). The median follow-up period for surviving patients was 32 months. Three patients died before day 21, one from cardiac toxicity, one from anoxic encephalopathy due to airway clot, and one from pulmonary damage. Of the 47 patients evaluable for engraftment (ie, those who survived ≥21 days), 1 patient who was conditioned with a regimen containing the lowest TBI dose used (200 cGy) and who received a graft from a fully matched donor failed to achieve sustained engraftment. The remaining 46 patients, who received transplants from either

Table 2. *Outcome of 50 Patients With Aplastic Anemia Who Received Bone Marrow Transplants From Unrelated Donors**

Parameter		No. of Patients Affected/No. of Evaluable Patients (%)
Follow-up, mo		
All patients, mean (range)	**34 (6-70)**	
Surviving patients, mean (range)	**27 (6-70)**	
Early deaths		**3/50 (6)**
Sustained engraftment		**46/47 (98)**
GVHD		
Acute, grades II-IV		**28/46 (61)**
Chronic		**11/30 (37)**
Survival		**29/50 (58)**
CY + ATG + TBI regimen		
HLA-identical donor		
3 × 200 cGy		**4/8 (50)**
2 × 200 cGy		**6/12 (50)**
1 × 200 cGy		**8/13 (59)**
HLA-nonidentical		
3 × 200 cGy		**3/6 (50)**
2 × 200 cGy		**4/4 (100)**
CY + TBI (6 × 200 cGy) regimen		
HLA-identical		**4/5 (80)**
HLA-nonidentical		**0/2 (0)**

*GVHD indicates graft-versus-host disease; CY, cyclophosphamide; ATG, antithymocyte globulin; TBI, total body irradiation.

Table 3. *Toxicity by Pretransplantation Conditioning Regimens**

Conditioning regimen	No. Patients					
	With Toxicity/Total (%)	Pulmonary	Renal	Oral Mucosal	Hepatic	CNS
ATG/CY (4 × 50) plus						
3 × 200 cGy TBI	**6/14 (36)**	**3**	**4**	**2**	**0**	**0**
2 × 200 cGy TBI	**5/16 (31)**	**5**	**2**	**0**	**0**	**0**
1 × 200 cGy TBI	**2/13 (15)**	**2**	**1**	**0**	**1**	**0**
CY (2 × 60) plus						
6 × 200 cGy TBI	**2/7 (29)**	**2**	**1**	**1**	**1**	**1**
All patients	**15/50 (30)**	**12**	**8**	**3**	**2**	**1**

*Severity of grade IV in any organ system or ≥grade III for pulmonary, oral mucosal (stomatitis), or hepatic toxicity [17]. ATG indicates antithymocyte globulin; CY, cyclophosphamide; TBI, total body irradiation; CNS, central nervous system.

an HLA-matched or an HLA 1-antigen–mismatched donor, achieved sustained engraftment. There was no apparent difference in the tempo of hematologic recovery between patients who had received different dosages of TBI.

Toxicity. Major toxicities by conditioning regimen are summarized in Table 3, and causes of death are listed in Table 4. Clinically significant toxicity occurred in 15 (30%) of the 50 patients enrolled. There was no apparent difference between patients given HLA-matched and HLA-mismatched transplants. Although the number of patients per regimen was small, the lowest incidence of toxicity, 15%, was seen in patients conditioned with the lowest dose of TBI, 200 cGy. The lung was the most frequently affected organ, and the most striking finding was that of diffuse alveolar damage generally manifesting at about 3 weeks posttransplantation. This type of toxicity at the low dosages of TBI administered was unexpected, and the mechanism is not clear. One of us (R.H.) reviewed the autopsy tissue that was available in 7 cases, and found that the histology was remarkable in that hyaline membranes were formed most prominently in the terminal broncheoli. The kidney was the site of the next highest incidence of toxicity. No severe gastrointestinal or bladder toxicity was observed.

Graft-Versus-Host Disease. Although grades II to IV acute GVHD occurred in 61% of patients, an expected incidence with transplants from unrelated donors, clinical manifestations were often mild to moderate. However, 2 patients died with acute GVHD and infection, and 1 patient who received a transplant from an HLA-DQ–mismatched donor and was treated for extended periods of time with various immunosuppressive agents for persistent GVHD, died with a posttransplantation lymphoproliferative disorder involving primarily the intestinal tract. Chronic GVHD requiring therapy developed in 11 (37%) of 30 evaluable patients.

Survival. As of July 2000, 29 (58%) of 50 patients are surviving. Survival by dosage level of conditioning is shown in Table 2. For both recipients of HLA-matched and HLA-mismatched transplants, there appeared to be a slight increase in survival probability with progressively de-escalating TBI doses, however, this trend was not significant. Survival of patients who received HLA 1-antigen–mismatched transplants was comparable to that of patients who received fully matched transplants. The most significant factor for long-term survival was the duration of the interval from diagnosis to transplantation (Figure 2). Among patients who underwent transplantation within 1 year of diagnosis, 73% survived, compared to 53% of patients who underwent transplantation at 1 to 3 years after diagnosis, and 39% of patients who underwent transplantation more than 3 years after diagnosis (P = .32). Also, younger patients (aged ≤20 years) had a higher probability of survival than older patients (aged >20 years; P = .06) (Figure 3). In fact, 8 (89%) of 9 patients aged ≤20 years who underwent transplantation within 1 year of diagnosis are surviving, compared with 58% of patients aged ≤20 years who underwent transplantation more than 1 year after diagnosis, and 43% of patients aged >20 years at the time of transplantation (P = .06). Finally, 4 of the 7 patients are currently surviving who could not complete the regimen because of ATG toxicity and were conditioned with lower dosages of CY and 6 × 200 cGy of TBI.

DISCUSSION

This prospective trial confirms results from previous retrospective analyses which showed that bone marrow transplantation with grafts from unrelated volunteer donors offers curative therapy for patients with aplastic anemia [3,8,10,24-26]. The best outcome was observed in younger patients and in those who underwent transplantation within the shortest time period from diagnosis [3,27,28].

The 2-year 57% survival rate in the present trial was superior to the 37% survival rate observed in our recent retrospective analysis of outcome in patients undergoing transplantation

Table 4. *Causes of Death in Patients With Aplastic Anemia Who Received Bone Marrow Transplants From Unrelated Donors**

Cause of Death	No. of Patients
Diffuse alveolar damage	**6**
Other respiratory failure	**2**
Systemic infections	**3**
Organ failure†	**5**
PTLD‡	**1**
Acute GVHD and associated problems	**2**
Asphyxia due to aspiration	**1**
Complications from second transplantation	**1**

*PTLD indicates posttransplantation lymphoproliferative disorder; GVHD, graft-versus-host disease.

† See Table 3

‡ After extensive treatment for acute GVHD.

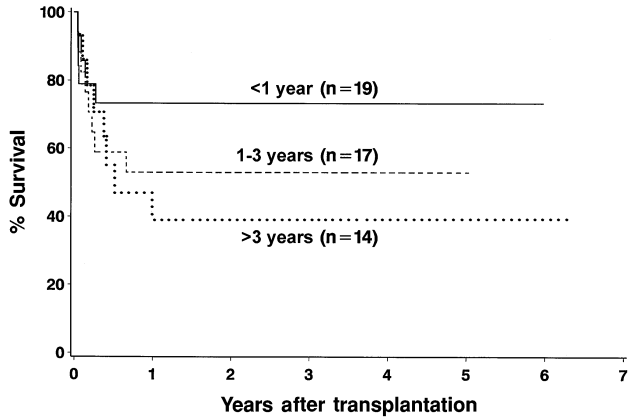

Figure 2. Survival by pretransplantation disease duration. The survival rate was 73% with a pretransplantation disease duration of <1 year (———), 53% for 1 to 3 years (- - - - -), and 39% for >3 years (·····) (P = .32).

under the auspices of the NMDP [3] and in other earlier reports [8,10,26]. This survival figure suggests that the primary objective of the prospective study, reduction of regimen-related toxicity and mortality without jeopardizing engraftment, was met. In fact, engraftment was achieved with the lowest TBI dosage reached by dosage de-escalation, 1 × 200 cGy for patients receiving HLA-matched transplants. However, although decreased TBI dosages were associated with a lower incidence of severe organ toxicity, the impact on survival was not as conclusive as was anticipated, and differences were not significant, probably due in part to the small number of patients in each group. To substantiate this data, a Phase II study using a TBI dose of 200 cGy is currently underway. Dosage de-escalation for recipients of HLA-nonidentical transplants on this protocol is still in progress, and results to date suggest that this strategy will be beneficial for these patients as well.

Earlier trials showed evidence that patients with aplastic anemia have a poor tolerance for high-dosage TBI [29], and it was this notion that provided the rationale for the present trial. Of 7 patients who did not tolerate ATG and were given higher dosages of TBI (6 × 200 cGy) with lower dosages of CY (2 × 60 mg/kg), 4 are surviving (4 of 5 given HLA-identical transplants). This outcome appears to be in contrast to data from earlier studies that showed a 10% survival rate among patients with aplastic anemia who were conditioned with CY/TBI and received a transplant from an HLA-identical sibling [29]. However, the numbers are too small to draw any firm conclusions. Furthermore, there were numerous additional differences in the regimens used in the present study, compared with earlier studies.

The same caution must be applied to any comparison of outcome in patients prepared with 6 × 200 cGy of TBI and 2 × 60 mg/kg of CY compared with that in patients given much lower dosages of TBI (1 to 3 × 200 cGy) but in combination with higher dosages of CY (4 × 50 mg/kg) and ATG. The observed toxicities could be related to high-dosage CY or interactions between high-dosage CY and TBI, or conceivably, between TBI and ATG. The latter appears unlikely because ATG is well tolerated with TBI (plus CY at 2 × 60 mg/kg) in patients undergoing transplantation for malignant disorders [30]. Another explanation for the high incidence of organ toxicity, in particular, pulmonary damage in patients with aplastic anemia, might be the presence of an unusual cytokine profile. Patients with aplastic anemia express high levels of proinflammatory cytokines such as interferon-γ and tumor necrosis factor (TNF)-α [31]. TNF-α levels have been shown to correlate with transplantation-related toxicity and transplantation outcome [32]. Further upregulation by high-dosage conditioning might lead to irreversible toxicity and mortality. Also, Dirksen et al. [33] recently reported high levels of interleukin (IL)-10 in a subgroup of patients with aplastic anemia, and others [34] have observed a correlation between a posttransplantation rise in plasma levels of IL-10 (presumably related to macrophage activation) and death from complications, in particular, acute respiratory failure. Ten of 11 deaths in that study occurred in patients who received transplants from unrelated or HLA-nonidentical donors. Further investigations into this question appear warranted.

The probability of developing GVHD has been related to the intensity of the conditioning regimen, at least after transplantation of grafts from HLA-identical sibling donors [19]. The 61% incidence of grades II to IV acute GVHD and the 37% incidence of chronic GVHD in the present trial were lower than those generally reported after unrelated donor transplantations [15,35], and GVHD was a major factor contributing to death in only 3 patients. Conceivably, therefore, lowering the dosage of TBI was beneficial in attenuating the development of GVHD.

In many respects, the present results confirm previous observations [3], although the lack of a significant impact of HLA disparity on transplantation outcome was surprising. However, considering the small number of patients involved, these results must be interpreted cautiously. Nevertheless, if the reduced dosage of TBI alleviated tissue damage and augmentation of proinflammatory cytokine levels as discussed above, then this reduction may have modified clinical

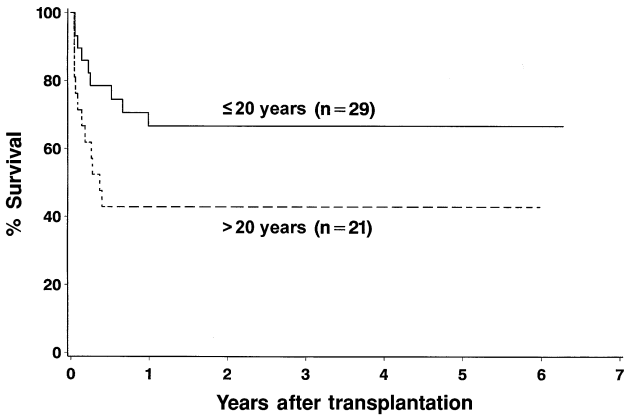

Figure 3. The impact of patient age at the time of transplantation on survival rates. The 2-year survival rate for 29 patients who were ≤20 years of age (———) at the time of transplantation was 67%, compared to 43% for 21 patients who were >20 years of age (- - - - -) at the time of transplantation (P = .06).

manifestations of allogeneic interactions even after transplantation from HLA-mismatched donors. The lowest TBI dosage used here was identical to that currently employed in so-called nonmyeloablative conditioning regimens. These regimens generally incorporate agents other than CY, eg, fludarabine, and are well tolerated regarding acute toxicity [36-38]. Some of those regimens also avoid the use of methotrexate, another agent known to have mucosal and pulmonary toxicity. Although GVHD occurs in a substantial proportion of patients treated in this way, the manifestations are often only mild to moderate [38]. Although the current trial is still enrolling patients, one approach in follow-up protocols for patients with aplastic anemia who are receiving transplants from unrelated donors might be a substantial dosage reduction of CY, or even replacement of CY by other agents such as fludarabine. In addition, such a trial might use mycophenolate mofetil (rather than methotrexate) or monoclonal antibodies for GVHD prophylaxis.

In conclusion, the results from our study are encouraging for patients with aplastic anemia who do not have a suitably HLA-matched related donor. TBI dosage reduction was effective in reducing transplantation-related toxicity without jeopardizing engraftment. The data suggest that in some patients (especially young patients), transplantation with grafts from unrelated donors should be carried out early in the disease course, possibly before the patient has been exposed to repeat courses of immunosuppressive therapy.

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