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CLINICAL RESEARCH

Conditioning with Treosulfan and Fludarabine followed by Allogeneic Hematopoietic Cell Transplantation for High-Risk Hematologic Malignancies

Eneida R. Nemecek,[1] Katherine A. Guthrie,[2,3] Mohamed L. Sorror,[2,3] Brent L. Wood,[2] Kristine C. Doney,[2,3] Ralf A. Hilger,[4] Bart L. Scott,[2,3] Tibor J. Kovacsovics,[1] Richard T. Maziarz,[1] Ann E. Woolfrey,[2,3] Antonio Bedalov,[2,3] Jean E. Sanders,[2,3] John M. Pagel,[2,3] Eileen J. Sickle,[2] Robert Witherspoon,[2,3] Mary E. Flowers,[2,3] Frederick R. Appelbaum,[2,3] H. Joachim Deeg[2,3]

In this prospective study 60 patients of median age 46 (range: 5-60 years), with acute myelogenous leukemia (AML; n = 44), acute lymphoblastic leukemia (ALL; n = 3), or myelodysplastic syndrome (MDS; n = 13) were conditioned for allogeneic hematopoietic cell transplantation with a treosulfan/fludarabine (Flu) combination. Most patients were considered at high risk for relapse or nonrelapse mortality (NRM). Patients received intravenous treosulfan, 12 g/m^2/day (n = 5) or 14 g/m^2/day (n = 55) on days −6 to −4, and Flu (30 mg/m^2/day) on days −6 to −2, followed by infusion of marrow (n = 7) or peripheral blood stem cells (n = 53) from HLA-identical siblings (n = 30) or unrelated donors (n = 30). All patients engrafted. NRM was 5% at day 100, and 8% at 2 years. With a median follow-up of 22 months, the 2-year relapse-free survival (RFS) for all patients was 58% and 88% for patients without high-risk cytogenetics. The 2-year cumulative incidence of relapse was 33% (15% for patients with MDS, 34% for AML in first remission, 50% for AML or ALL beyond first remission and 63% for AML in refractory relapse). Thus, a treosulfan/Flu regimen was well tolerated and yielded encouraging survival and disease control with minimal NRM. Further trials are warranted to compare treosulfan/Flu to other widely used regimens, and to study the impact of using this regimen in more narrowly defined groups of patients.

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INTRODUCTION

Allogeneic hematopoietic cell transplantation (HCT) is a potentially curative treatment modality for hematologic malignancies, including patients not responsive to initial chemotherapy. Conventional preparative regimens consisting of high-dose systemic chemotherapy with or without total body irradiation (TBI) are effective in eradicating leukemia and result in relapse-free survival (RFS) rates of 10% to 80%, depending on the patient's disease status at the time of HCT [1-4]. However, the probability of success with this strategy is tempered by morbidity and mortality because of regimen-related toxicity [5,6]. Reduced-intensity conditioning (RIC) regimens have been used with increasing frequency, particularly in older patients with hematologic malignancies and in patients considered at high risk for treatment-related toxicity and treatment-related mortality (TRM) associated with high-dose conventional transplant regimens [7-10]. RIC regimens consistently result in less acute toxicity and reduced nonrelapse mortality (NRM) compared to conventional regimens, but in many studies, they have been associated with higher cumulative incidence rates of relapse [11-14]. An ideal transplant regimen should generate sufficient disease control to allow for

From the [1]Oregon Health and Science University, Portland, Oregon; [2]Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, Washington; [3]the University of Washington School of Medicine, Seattle, Washington; and [4]University of Essen, Germany.

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Correspondence and reprint requests: Eneida R. Nemecek, MD, Oregon Health & Science University, 3181 SW Sam Jackson Park Road, Mailcode CDRCP, Portland, OR 97239 (e-mail: nemeceke@ohsu.edu).

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sustained remissions without inducing substantial toxicity and NRM.

Treosulfan (L-threitol-1,4-bis-methanesulfonate; dihydroxybusulfan; IND 72479) is an alkylating agent approved in European countries for the treatment of ovarian carcinoma [15]. As a single agent, the most common dose-limiting toxicities are mucositis, diarrhea, and myelosuppression [16]. Several European teams have, therefore, evaluated treosulfan as part of transplant conditioning regimens where bone marrow suppression would not be a major concern [17-22]. Treosulfan has several characteristics that make it attractive for use in HCT, including a highly predictable pharmacokinetic (PK) profile, adequate immunosuppressive activity to allow for engraftment of donor cells across histocompatibility barriers, and conceivably greater antileukemic activity than other currently used agents [23-27]. Preliminary results of European studies suggest low graft failure rates, reduced NRM, and improved RFS. Here, we present the results of the first trial conducted in the United States, which combined treosulfan with fludarabine (Flu) in a conditioning regimen for patients with acute leukemia or myelodysplastic syndrome (MDS) undergoing allogeneic HCT.

PATIENTS, MATERIALS, AND METHODS

Patient Characteristics

Sixty patients, median age 46 years (range: 5-60 years) were enrolled in this prospective, opened-label, nonrandomized clinical trial conducted from September 2005 to December 2008 at the Fred Hutchinson Cancer Research Center (FHCRC) in Seattle, WA, and the Knight Cancer Institute at Oregon Health & Science University (OHSU) in Portland, OR. Patient characteristics are summarized in Table 1. Diagnoses included acute lymphoblastic leukemia (ALL), acute myelogenous leukemia (AML), and MDS.

The diagnosis was confirmed by internal review of BM samples obtained before referral and by repeat BM examination before initiation of the transplant conditioning regimen. BM evaluations included morphology, flow cytometry, and cytogenetic analysis. MDS was classified according to WHO criteria and the International Prognostic Scoring System (IPSS) [28,29]. Disease subtypes for ALL and AML were classified using the French-American-British (FAB) system [30,31]. Patients with leukemia were considered in morphologic complete remission (CR) if they had <5% blasts in a normocellular BM. Minimal residual disease (MRD) was defined as the presence of detectable disease by flow cytometry, cytogenetic analysis, or fluorescein in situ hybridization (FISH) in patients with <5% marrow blasts by morphology.

Table 1. Pretransplant Characteristics of 60 Patients Conditioned with Treosulfan and Fludarabine

Characteristic	No. of Patients
Age at transplantation (years)*	
◆ <21	10
◆ 21-50	31
◆ 50-60	19
Sex (female:male)	36:24
Diagnosis and disease status at transplantation	
◆ ALL, second/third remission†	3
◆ AML	44
First remission†	26
Second or greater remission†	10
Relapsed or primary refractory	8
◆ MDS‡	13
RA	6
RAEB/RAEBT	7
Disease risk group (low:standard:high)§	26:22:12
Cytogenetic risk group (good:intermediate:poor)¶	16:8:36
HCT comorbidity index (0; 1-2; ≥3)	13:19:28
Pretransplant comorbidities and risk factors	
◆ Previous malignancy	12
◆ Previous myeloablative HCT	10
◆ Underlying health conditions*	25
Donor type	
◆ HLA-identical sibling	30
◆ HLA-matched unrelated donor	30
Stem cell source	
◆ Bone marrow	7
◆ Filgrastim-mobilized PBPC	53

ALL indicates acute lymphoblastic leukemia; AML, acute myelogenous leukemia; HCT, hematopoietic cell transplantation; HLA, human leukocyte antigen; IPSS, International Prognosis Scoring System; MDS, myelodysplastic syndrome; PBPC, peripheral blood stem cells; RA, refractory anemia; RAEB/T, refractory anemia with excess blasts/in transformation.
*Patients were 5-60 years (median 46 years) old.
†Minimal residual disease was present in 28% of the 39 patients with ALL or AML in morphologic remission.
‡By IPSS criteria, 4 patients had intermediate-1, 5 intermediate-2, and 4 high-risk disease.
§Low-risk disease: AML or ALL in first remission, MDS with IPSS = 0; standard risk: ALL or AML in second or greater remission, MDS with IPSS 0.5-2; high risk: relapsed/refractory ALL or AML, MDS with IPSS ≥2.5.
¶Good risk cytogenetics: t(8;21), t(15;17), or inversion 16 for AML, hyperdiploidy for ALL, -Y, del(5q), del(20q), or normal for MDS; poor risk: 11q23 abnormalities, monosomy 7, monosomy 5, deletion 5q, or abnormalities of 3q for AML, t(9;22) or extreme hypodiploidy for ALL, chromosome 7 abnormalities in MDS, ≥3 chromosome abnormalities for any disease type; Intermediate risk: all others.

Marrow cytogenetic studies at diagnosis and at pretransplant relapse were available for all patients. Cytogenetic risk was assigned following the combined guidelines from cooperative groups [29,32-36]. Cytogenetic abnormalities were classified as good risk if t(8;21), t(15;17), or inversion 16 was present in patients with AML, hyperdiploidy in patients with ALL and -Y, del(5q), del(20q), or normal karyotype in patients with MDS. Poor-risk cytogenetics were 11q23 abnormalities or monosomy 7, monosomy 5, deletion 5q or abnormalities of 3q for AML, t(9;22) or extreme hypodiploidy for ALL, chromosome 7 abnormalities in MDS patients, and complex abnormalities in ≥3 chromosomes for any disease type. All other abnormalities were classified as intermediate risk.

Disease risk was determined at the time of transplantation. Low risk included patients with AML or ALL in first CR (CR1) or MDS with IPSS score 0, standard risk patients with ALL or AML in second or greater CR or MDS with IPSS risk score 0.5-2; high-risk patients had relapsed or refractory leukemia or MDS with IPSS scores >2. Seventy-five percent of patients were considered at high risk for treatment with conventional transplant regimens, because of the secondary nature of AML or MDS, previous HCT, or comorbid conditions; 28 patients had transplant-specific comorbidity index (HCT-CI) scores of 3 or greater [37].

Patients with low general performance scores (ie, Karnofsky or Lansky Play-Performance Scale score <50% on pretransplant evaluation), HIV seropositivity, uncontrolled systemic infections, active central nervous system leukemia, or extramedullary disease or evidence of major organ dysfunction were excluded. Major organ dysfunction was defined as any of the following: cardiac ejection fraction <35% or cardiac insufficiency requiring treatment or symptomatic coronary artery disease; impaired pulmonary function as evidenced by need for continuous supplemental oxygen or diffusing capacity of the lung for carbon monoxide (DLCO) <60% of predicted; impaired renal function as evidenced by creatinine clearance <50% corrected for body surface area or serum creatinine >2 times the upper limit of normal for age or dialysis-dependence; hepatic dysfunction defined as total bilirubin or liver transaminase (AST/ALT) values >2 times the upper limit of normal.

Patients, and legal guardians for patients younger than 18 years, were informed of the investigational nature of the study and signed consent forms approved by the institutional review boards of the FHCRC or OHSU in accordance with the Declaration of Helsinki.

Donor Selection

Related donors were matched by intermediate resolution molecular typing for HLA-A, -B, -C, and -DQB1 and by high-resolution typing for DRB1. Unrelated donors were allele matched for HLA-A, -B, -C, and -DRB1 by high-resolution typing and DQB1 by intermediate resolution typing. A single allele disparity with the patient for HLA-A, -B, or -C was allowed. The preferred hematopoietic stem cell source was filgrastim-mobilized peripheral blood progenitor cells. However, 7 patients received unmanipulated BM cells either because of small donor size or donor refusal to be treated with filgrastim.

Preparative Regimen

The preparative regimen was administered in the outpatient setting for most adult patients. Patients younger than 18 years received the preparative regimen electively as inpatients. Treosulfan was given intravenously on days −6 to −4 at doses of 12 g/m^2/day (total dose 36 g/m^2/day) for the first 5 patients and was then escalated to 14 g/m^2/day (total dose 42 g/m^2/day) for the subsequent 55 patients after no toxicities were observed with the lower dose. The latter dose was set as the maximum tolerated dose based on results of previous studies in Europe where dose-limiting toxicities were observed with treosulfan doses above 42 g/m^2/day [16]. Flu was given intravenously at doses of 30 mg/m^2/day on days −6 to −2 (for a total of 150 mg/m^2). Ideal body weight was used for dosage calculations in patients for whom actual body weight exceeded 125% of ideal body weight. Graft-versus-host disease (GVHD) prophylaxis in most patients consisted of tacrolimus (n = 55) or cyclosporine (CsA; n = 3) starting on day −1, combined with methotrexate (MTX) 15 mg/m^2 administered intravenously on day +1, and 10 mg/ m^2/dose on days +3, +6, and +11 after transplantation. Two patients deemed at high risk for toxicity from MTX received CsA starting on day −3 in combination with mycophenolate mofetil (MMF) starting on day 0. Tacrolimus or CsA was maintained at therapeutic plasma levels (per institutional standard practice procedures) until at least day +50 and tapered through day +180 if there were no signs of GVHD. If active GVHD was present, treatment with tacrolimus or cyclosporine was generally continued. MMF was discontinued after day +28 if the patient was engrafted and free of GVHD.

PK Studies

Blood and urine samples were collected from the first 16 patients in the study (12 adults and 4 children) for treosulfan PK analysis. Samples were batched and analyzed at the University of Essen, Germany (R.H.), using methods previously described [16]. Blood samples were collected at 0, 2, 4, 5, 6, and 24 hours after each treosulfan dose and plasma was separated by centrifugation at 4°C preceded by microfiltration. Urine aliquots were collected at 4-hour intervals starting at the end of administration of the first treosulfan dose and for the entire period of treosulfan treatment (72 hours), with sample pH adjusted to 5.5 with the addition of citrate to prevent ex vivo degradation of treosulfan. Urine samples were centrifuged at 4°C and directly analyzed. Analysis involved a validated reverse-phase high-performance liquid chromatography method and quantification by refractometrical detection. Individual PK parameters were evaluated by 2-compartment disposition modeling using TOPFIT software, version 2.0 (G. Fischer Verlag/VCH Publishers, Stuttgart, Germany).

Engraftment and GVHD

Time to neutrophil engraftment was defined as the first of 3 consecutive days with an absolute neutrophil

count (ANC) of 0.5 × 10^9/L or greater, and platelet engraftment was defined as the first of 3 consecutive days with a platelet count >20 × 10^9/L and without platelet transfusions. Acute and chronic GVHD (aGVHD, cGVHD) were diagnosed, graded, and treated as previously described [37,38]. Patients were categorized according to the recently developed NIH Consensus Criteria for diagnosis and staging of cGVHD [38].

Supportive Care

All patients had central venous access lines placed before HCT. Infection prophylaxis was given according to standard institutional practices including broad-spectrum antibiotics during the neutropenic period, fluconazole, or other azole compounds for fungal prophylaxis, trimethoprim-sulfamethoxazole for prevention of *Pneumocystis* pneumonia, and acyclovir for viral prophylaxis. Surveillance for cytomegalovirus (CMV) reactivation or acquisition was carried out weekly by polymerase chain reaction (PCR) testing of plasma samples until day 100, and preemptive anti-CMV therapy was initiated in patients who showed an increase in viral copy numbers. Patients remained hospitalized from the day of transplant (adults) or from the time of initiation of conditioning (children <18 years) until achievement of engraftment and resolution of acute transplant-related complications.

Study Design and Statistical Methods

This opened-label, nonrandomized study was conducted in 2 stages. The primary objective of phase I was to determine the best of 2 doses of treosulfan that could be used in combination with Flu. The best dose of treosulfan was defined as the dose associated with acceptable rates of regimen-related toxicity (RRT; <25%) and graft failure (<5%). Regimen-related toxicity was defined as organ toxicity observed within the first 28 days posttransplant, of grade 3 or greater by National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE version 3.0; http://ctep.cancer.gov/reporting/ctc.html), and not attributable to primary or preexisting disease, GVHD, or infection. Graft failure was defined as failure to achieve an ANC of 0.5 × 10^9/L or greater by day 35 posttransplant or an ANC <0.5 × 10^9/L after initial engraftment without subsequent recovery and in the absence of other contributing causes such as disease relapse, medication effects, or infection.

As the lower treosulfan dose was well tolerated in the first 5 patients, the dose was escalated to 14 g/m^2/day for 3 doses, and was given to the remaining 55 patients in phase II of the study. In addition to further evaluating safety of the conditioning regimen (ie, RRT and graft failure) in a larger group of patients, the objective of the second phase was to determine if the drug combination proved sufficiently attractive for further study, as evidenced by an incidence of NRM of <25% at 1 year.

Estimates of overall survival (OS) and RFS were calculated using the method of Kaplan and Meier. Relapse or death, whichever occurred first, was considered as failure for the endpoint of RFS. Cumulative incidence curves were used to estimate the probabilities of GVHD, relapse, and NRM. Death was treated as a competing risk for GVHD and relapse. Relapse was considered a competing risk for NRM. Simple proportions were used to summarize patient pretransplant characteristics and to estimate the probabilities of RRT. The statistical significance of differences in event rates was evaluated with the Cox regression model. Explanatory variables examined for the regression models included patient age, disease status, donor type, and HCT-CI [39]. All reported 2-sided *P*-values from regression models were derived from the Wald test. The statistical analysis was performed on SAS software version 9 (SAS Institute, Inc., Cary, NC). Results were analyzed as of June 30, 2009.

RESULTS

Treosulfan Dose Escalation and PKs

Five patients received treosulfan at 12 g/m^2/dose. After no limiting toxicities were observed at that dose, the remaining 55 patients received treosulfan at 14 g/m^2/dose. Analysis of treosulfan PK was conducted in 16 patients treated consecutively, including 4 of the 5 patients receiving 12 g/m^2 (all adults) and 12 patients receiving 14 g/m^2 (4 children, 12 adults). Table 2 shows the PK parameters observed by dose group.

Engraftment and Donor Cell Chimerism

One patient died on day 10 from pulmonary aspergillosis (present pretransplant) and was not evaluable for engraftment. The remaining 59 patients achieved

Table 2. Pharmacokinetic Parameters of Treosulfan*

Parameter	Treosulfan 12 g/m^2	Treosulfan 14 g/m^2*
N	4	12
Age in years (median, range)	34 (18-47)	34 (5-55)
AUC (mg/L*h)	1365 ± 293	1309 ± 262
C_{max} (mg/L)	461 ± 102	409 ± 84
Half-life (hours)	1.73 ± 0.10	1.83 ± 0.30
V_{ss} (L)	16.9 ± 4.3	22.1 ± 3.8
Cl_{tot} (mL/min)	154 ± 35	185 ± 37
Urine excretion (%)	31 ± 2	27 ± 4

N indicates number of patients; AUC, area under the curve; C_{max}, maximum plasma concentration; V_{ss}, volume of distribution; Cl_{tot}, total systemic clearance.
Results presented as mean ± standard deviation unless otherwise noted.
*Four children ages 5-17 years treated at the 14 g/m^2 dose underwent pharmacokinetic testing.

Table 3. Nonhematologic Toxicities in 60 Patients

	Grade (No. of Patients)*			
Organ System	1	2	3	4
Cardiac	2	2	1	0
Bladder	5	1	0	0
Hepatic	23	13	0	0
Mucosal/gastrointestinal	18	13	6	1
Neurologic	0	0	0	0
Pulmonary	5	8	2	0
Renal	17	9	1	0
Skin/dermatitis	4	7	0	0

*Grading according to National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE), and not attributable to primary or preexisting disease, graft-versus-host disease (GVHD), or infection.

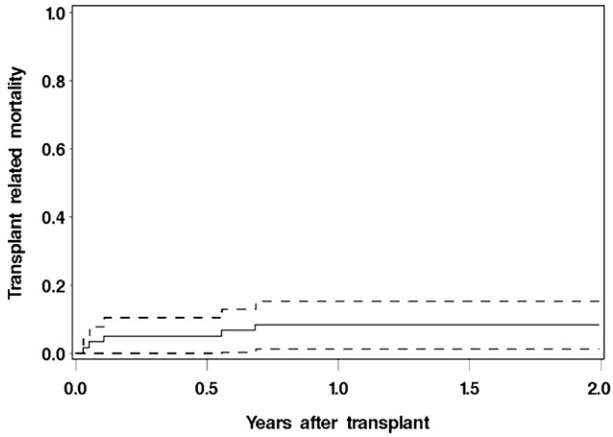

Figure 1. NRM. Estimates (solid line) and 95% confidence intervals (dotted lines) are shown.

neutrophil engraftment at a median of 18 (range: 11-26) days. Platelet engraftment occurred at a median of 16 (range: 9-92) days. Complete donor T cell chimerism (ie, >95% $CD3^+$ cells of donor origin) was achieved by day 28 in 82% (49/60) and by day 100 in 85% (40/47) of patients evaluated. Complete donor myelogenous cell chimerism (ie, >95% CD33 cells of donor origin) was achieved by day 28 in 97% (58/60) and by day 100 in 97% (46/47) of patients evaluated. The 7 patients with mixed chimerism at day 100 showed 100% donor cell chimerism at 1 year.

RRT and TRM

Observed toxicities are summarized in Table 3. Mild-to-moderate mucositis (NCI grades 1-2) was observed in 52% of patients during the neutropenic phase. A temporary mild-to-moderate elevation of hepatic transaminases was seen in 60% of patients, typically during the days when MTX was administered. No sinusoidal obstruction syndrome of the liver occurred. Dermatitis of grades 1-2, consisting of a self-limited skin rash, was observed in 18% of patients. Grade 4 RRT was noted in 1 patient (1.5%), and consisted of severe mucositis requiring temporary intubation (n = 1). Day 100 NRM was 5.0% (95% confidence interval [CI] 0-10.5] (Figure 1). The estimated NRM at 2 years was 8.3% (95% CI 1.3-15.3). Causes of death included cGVHD (n = 2), fungal infection (n = 2), and intracranial hemorrhage (n = 1). All 5 patients who died from nonrelapse causes had high comorbidity scores (4-5) prior to transplant, and 3 were recipients of second allogeneic HCT. NRM was observed in 1 of 10 patients younger than 21 years and 4 of 19 patients older than 50 years.

GVHD

Thirty-six of 58 evaluable patients experienced aGVHD, which was grade I in 4, grade II in 27, and grade III in 5 patients, for a cumulative incidence of grades II-III of 55.2% (95% CI 42.4-68.0) at 90 days after HCT. No grade IV GVHD was observed. cGVHD developed in 31 of 48 evaluable patients (65%). Resolution of cGVHD, evidenced by discontinuation of immunosuppressive therapy, was achieved in 21 of these 31 patients (68%) at a median of 18 (range: 8-26) months after HCT.

Survival and Disease Recurrence

Thirty-five patients were alive and disease-free at a median follow-up of 22 (range: 14-41) months. The estimated OS at 2 years was 65% (95% CI 51.5-75.6), and RFS 58% (95% CI 44.9-69.6) as illustrated in Figure 2. Source of stem cells, comorbidity score, disease risk, and cytogenetic risk categories were the strongest predictors of RFS in a multivariable model (Table 4a). Figure 3 shows the unadjusted RFS survival in subsets defined by disease risk (Figure 3A) and cytogenetic risk categories (Figure 3B). Estimates of RFS at 2 years were 68%, 54%, and 44% for patients with low, standard, and high risk by disease category, respectively ($P < .001$ for linear trend in an adjusted model). Patients with high-risk cytogenetics had lower RFS than patients with standard or intermediate cytogenetic risk (39% versus 88% at 2 years, $P < .001$ in an adjusted model). **Transplantation from unrelated donors and the presence of MRD at the time of HCT were** associated with inferior RFS in univariable models; however, after adjustment for cell source, disease risk, and cytogenetic risk, these factors were no longer significant predictors of RFS. Four of 9 patients receiving second transplantation are alive and leukemia-free with median follow-up of 19 (range: 15-31) months. Transplant number was a significant predictor of event-free survival (EFS) in an unadjusted model (HR = 3.2, 95% CI 1.3-7.6, $P = .01$), but no longer statistically significant after adjustment for cell source, disease risk, and cytogenetic risk ($P = .15$). Age was not a significant predictor of OS or RFS on multivariate analysis.

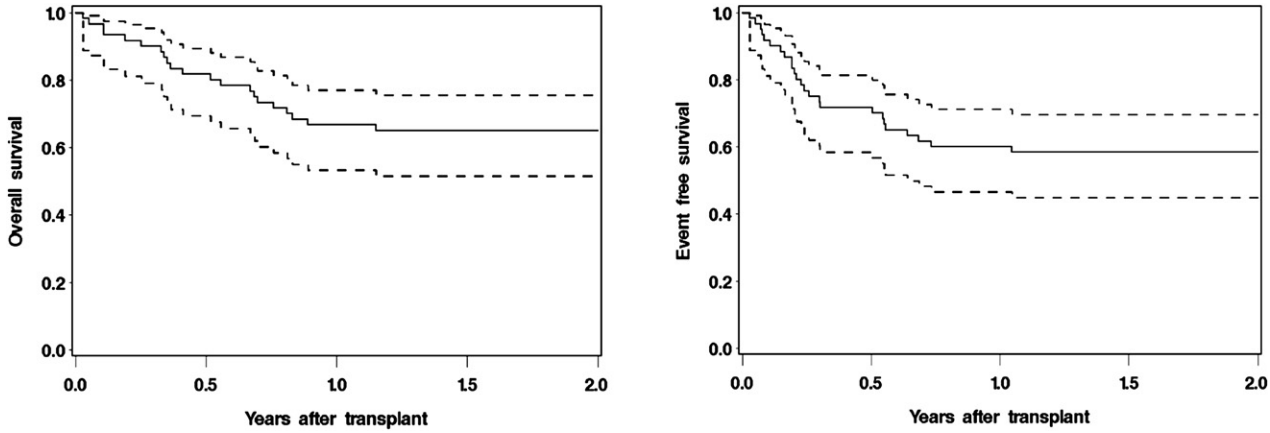

Figure 2. OS and RFS. Estimates of OS (left) and RFS (right) and 95% confidence intervals (dotted lines) for all patients.

The 2-year cumulative incidence of relapse among all patients was 33% (95% CI 21.4-45.3). Patient age, cell source, disease risk, and cytogenetic risk categories were the strongest predictors of relapse in a multivariable model (Table 4b). The 2-year estimates of relapse were 35% (95% CI 16.3-52.9) for patients with AML in CR1, 50% (95% CI 15.4-84.6) for AML or ALL beyond CR1, 63% (95% CI 29.0-96.0) for AML not in remission, and 15% (95% CI 0-35.0) for patients with MDS (Figure 4).

Table 4. Multivariable Analyses for Relapse-Free Survival and Relapse

a) Relapse-free survival by clinical characteristics

	HR (95% CI)	*P*-Value
Comorbidity score*	1.3 (1.0-1.7)	.02
Cell source		
• PBPC	1.0	
• Bone marrow	7.2 (2.0-26.2)	.003
Disease risk		
• Low	1.0	
• Intermediate	3.8 (1.3-11.1)	.01
• High	7.9 (2.7-23.3)	<.001
Cytogenetic risk		
• Standard or intermediate	1.0	
• High	15.5 (4.3-56.7)	<.001

b) Relapse by clinical characteristics

	HR (95% CI)	*P*-Value
Age†	0.7 (0.5-1.0)	.02
Cell source		
• PBPC	1.0	
• Bone marrow	9.6 (2.4-37.6)	.001
Disease risk		
• Low or intermediate	1.0	
• High	7.0 (2.1-22.9)	.001
Cytogenetic risk		
• Standard or intermediate	1.0	
• High	8.0 (2.2-29.1)	.002

CI indicates confidence interval; HR, hazard ratio; PBPC, peripheral blood progenitor cells.
*Continuous variable with truncation at score of 5 (ie, 1, 2, 3, 4, ≥5).
†HR corresponds to difference of 10 years in age.

DISCUSSION

The present study shows that a combination of treosulfan and Flu provides effective conditioning for allogeneic HCT from related and unrelated donors in patients with hematologic malignancies. Engraftment was achieved in all evaluable patients. NRM was low, 5% at day 100, and 8% at 2 years, in a cohort that included 22 patients who had either secondary leukemia or MDS, or had previously received a transplant using conventional high-dose conditioning regimens. In addition, 47% of patients had transplant comorbidity scores of 3 or higher.

Analysis of survival, based on widely accepted risk factors, showed a clear separation of results between patients with high risk compared to intermediate- or good-risk cytogenetics and a similar separation of patients with respect to disease risk. Dependent upon disease category, the cumulative incidence rates of relapse ranged from 15% to 63%, and RFS at 2 years from 39% to 88%. Taken together, these results suggest that a combination of treosulfan and Flu would be an excellent conditioning regimen for patients with low- or standard-risk cytogenetics. In high-risk patients, however, the observed relapse rate was high. As the toxicity profile of the regimen was excellent, it may be possible to add other components, for example, low-dose TBI [40] to this combination to increase antileukemic efficacy without unacceptably increasing toxicity. Of interest in this context is a recent preclinical study that provides evidence for radiation sensitization by treosulfan [41].

The current results are in good agreement with reports by others in regard to both toxicity and efficacy. Beelen and colleagues [42] reported results in 18 patients, 19 to 64 years of age with various hematologic malignancies, who were conditioned with treosulfan (36-42 g/m^2 over 3 days) and cyclophosphamide (Cy; 120 mg/kg over 2 days) and transplanted from HLA-identical siblings. The 1-year RFS was 56%, and 22% of patients died from nonrelapse causes. The major

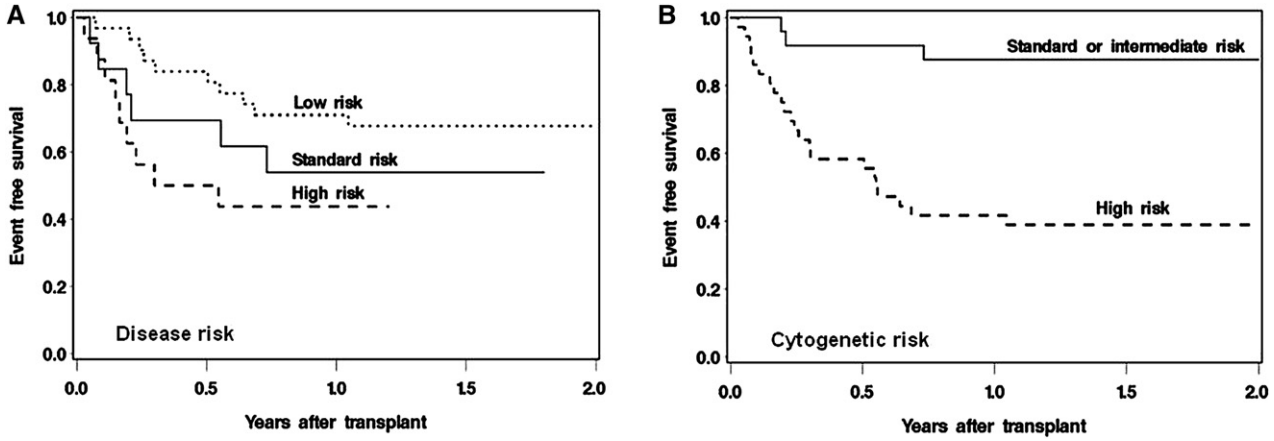

Figure 3. RFS by disease risk (A) and cytogenetic risk (B). (A) Disease risk was classified using CIBMTR criteria. Low risk: ALL or AML in first remission or MDS-RA; standard risk: AML or ALL in second or greater remission; high risk: relapsed or primary refractory ALL or AML, RAEB and RAEBT. (B) Cytogenetic abnormalities were classified as favorable if t(8;21), t(15;17) or inversion 16 for AML or hyperdiploidy for ALL were present; high risk included 11q23 abnormalities, monosomy 7, monosomy 5, deletion 5q or abnormalities of 3q, t(9;22), extreme hypodiploid for ALL or complex abnormalities (>3 chromosomes); intermediate risk included all other abnormalities.

toxicities were mucositis and transaminase elevations. Casper and colleagues [17] reported results in 56 patients, 18 to 66 years of age, with various hematologic malignancies, who were conditioned with treosulfan (30, 36, or 42 g/m^2 over 3 days) and Flu (150 mg/m^2 over 5 days), and transplanted from HLA-identical related or unrelated donors. The source of stem cells was marrow or mobilized peripheral blood progenitor cells, and unrelated transplant recipients were also given antithymocyte globulin (ATG). The 1-year RFS (median follow-up 21 months) was 53%. The major toxicities were mucositis/enteritis and transaminase elevations; NRM was 20%.

Although no controlled prospective data are available, these results with treosulfan in combination with Flu are comparable to those obtained with targeted busulfan (Bu) combined with Flu or Cy [43,44]. The incidence of GVHD in our study was also comparable to that observed in earlier Bu trials [45], indicating that the reduced overall toxicity was not associated with a change in the incidence of GVHD. Some investigators have incorporated serotherapy with ATG or alemtuzumab into treosulfan-containing regimens, thereby possibly lowering the frequency of GVHD when using unrelated donor sources [17,18,46]. Such a strategy has also been successful with Bu-containing regimens [47].

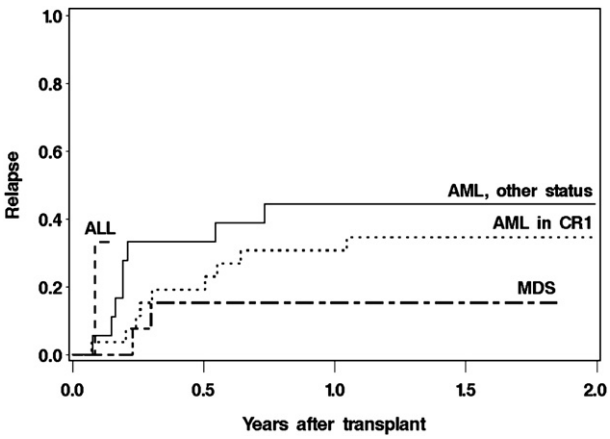

Figure 4. Cumulative incidence of relapse by diagnosis and disease status at transplantation.

Most encouraging in the current trial were the low rates of severe toxicity and NRM of 8% at 2 years. Mild mucositis and reversible transaminase elevations were the most frequent complications. Although Bu, similarly, has not been associated with severe mucositis, hepatotoxicity has been a concern, particularly with the oral preparation, and when followed by the administration of Cy [48]. Treosulfan is spontaneously "activated" into an epoxy compound that crosslinks DNA. Because enzymatic activation is not required, hepatic metabolic pathways are bypassed. The half-life of treosulfan is similar to that of Bu (approximately 1.7-2 hours), although cumulative renal excretion is higher (50% versus 20%) [23]. It is possible that these differences in hepatic metabolism contribute to the better clinical tolerability observed with treosulfan to date.

Although NRM was low, relapse and associated mortality were considerable problems. The 2-year cumulative incidence of relapse was 33%. In patients with relapsed or refractory AML, the incidence was as high as 62%, compared to 15% in patients with MDS of any risk category. Although the numbers in these subcohorts of patients were too small to draw firm conclusions, the data suggest that a treosulfan/Flu regimen might be most effective and acceptable in patients with relatively low-risk disease, because of the remarkably low NRM, the very low incidence of relapse, and a 2-year RFS of 88%. The encouraging RFS of 44% in patients undergoing second transplantation also suggests that this regimen may be of

promise for this group of patients at very high risk for treatment failure.

The excellent tolerability of the treosulfan/Flu combination may allow for "intensification" of this regimen with the aim of achieving greater antileukemic efficacy. An escalation of the treosulfan dose is not attractive based on earlier data reported by European groups, which suggest that 14 g/m^2 is the maximum dose that can be given without inducing significant gastrointestinal symptoms (ie, enteritis and diarrhea) [16]. In fact, PK studies included in the present trial, along with previously published data, indicate that maximum plasma concentrations as well as AUC values for treosulfan at the lower dose of 12 g/m^2 are comparable to those achieved at 14 g/m^2. A further dose increase would be unlikely to provide greater efficacy. Given the possible radiosensitizing effects of treosulfan [41], appropriate sequencing of administration of radiation and treosulfan might enhance the antileukemic effect, although such a regimen would also likely increase the risk of mucositis or gastrointestinal complications. Incorporation of different modalities, such as antibody conjugates or posttransplant interventions, may also be attractive [49]. It may also be of interest to compare treosulfan-containing regimens to other protocols currently in use, such as Flu/melphalan combinations [14]. An ongoing prospective randomized study at European centers is comparing a treosulfan/Flu combination with a Bu/Flu regimen in adult patients with myelogenous malignancies.

Although the definition of what constitutes an RIC regimen varies, there is general consensus that the goal of such a regimen must be to achieve maximum disease control with minimal toxicity [50,51]. A treosulfan/Flu combination represents 1 such effort toward this goal. Determining which patient and disease categories are likely to derive the greatest benefit from this regimen requires further investigation.

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