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Early (Day −7) versus Conventional (Day −1) Inception of Cyclosporine-A for Graft-versus-Host Disease Prophylaxis after Unrelated Donor Hematopoietic Stem Cell Transplantation in Children. Long-Term Results of an AIEOP Prospective, Randomized Study

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We carried out a randomized, multicenter study comparing the inception of cyclosporine- A (CsA) on day –7 to conventional CsA (on day –1) to evaluate the influence of this modification on graft-versus-host disease (GVHD), treatment-related mortality (TRM), relapse rate (RR), and event-free survival (EFS) in children with hematologic malignancies given unrelated donor (UD) hematopoietic stem cell transplantation (HSCT). Between 1997 and 2002, 152 children transplanted for acute leukemia (102), myelodysplastic syndromes (23), chronic myelogenous leukemia (20), and non-Hodgkin lymphoma (7) were enrolled in the study and randomized to receive either early CsA (group 1, N = 72) or conventional CsA (group 2, N = 80), after stratification according to HLA compatibility and disease phase. The cumulative incidence of both grade II-IV and grade II-IV acute GVHD (aGVHD), as well as of chronic GVHD (cGVHD), did not differ between the 2 groups. No significant differences were observed also with regard to TRM and RR. The 8-year Kaplan-Meier estimates of EFS were 56% in group 1, and 46% in group 2 (P = NS). In the Cox model, advanced disease phase, male recipient, older donor, and occurrence of grade III-IV aGVHD predicted inferior overall EFS. These data indicate that early inception of CsA does not improve posttransplantation outcome of children with hematologic malignancies given UD-HSCT.

Biol Blood Marrow Transplant 15: 741-748 (2009) © 2009 American Society for Blood and Marrow Transplantation

KEY WORDS: CsA, GVHD, UD HSCT, Children, Acute leukemia

INTRODUCTION

Hematopoietic stem cell transplantation (HSCT) from an HLA-identical sibling has become an established treatment for children with hematologic malignancies, bone marrow failure syndromes, and various inborn errors [1-3]. Because <1 of 4 patients has an HLA-matched sibling, the use of unrelated donors (UD) has significantly increased over the last few years. We recently reported that the outcome of children with acute leukemia given UD-HSCT has significantly improved over time, becoming comparable to that of patients transplanted from a compatible relative [4]. The main factors that have significantly contributed

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Financial disclosure: See Acknowledgments on page 747.

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Received January 15, 2009; accepted March 6, 2009

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1083-8791/09/156-0001$36.00/0
doi:10.1016/j.bbmt.2009.03.004

to this improvement are a better selection of UDs, because of a more precise characterization of HLA loci [5], and refinements in both prophylaxis and treatment of graft-versus-host disease (GVHD). Despite this improvement, however, GVHD and transplantation-related toxicity are still major contributors to both early and late morbidity and mortality in UD-HSCT. Usually, in Italy, GVHD prophylaxis for children with malignancies given UD-HSCT includes serotherapy (namely, antilymphocyte globulin (ALG) or antithymocyte globulin [ATG]), cyclosporine-A (CsA) from day –1, and short-course methotrexate (MTX) [6]. However, a single center study by Lamparelli et al. [7], and a retrospective analysis from our group [8], suggested that early administration of CsA (from day −7 before HSCT) may improve outcome. This effect was interpreted to be because of higher levels of the drug at time of transplantation, resulting both in a reduction of the cytokine storm induced by the conditioning regimen and in a blunted early activation of donor T cells [9]. To test prospectively in a controlled trial whether early inception of CsA can improve the results of UD-HSCT, the transplant subcommittee of the Italian Association of Pediatric Hematology and Oncology (AIEOP) designed a randomized study comparing early inception of CsA (ie, from day −7, during conditioning regimen) with conventional CsA (ie, from day −1). The primary endpoints of the study were the incidence of acute and chronic GVHD (aGVHD, cGVHD), treatment-related mortality (TRM), relapse rate (RR), and event-free survival (EFS). Moreover, the impact of other patient-, donor-, and transplantation-related factors on TRM, RR, and EFS was assessed.

PATIENTS AND METHODS

Between September 1997 and October 2002, 152 consecutive children 18 years or younger (median age: 9 years), undergoing first HSCT from an UD for hematologic malignancies in 9 HSCT centers affiliated to AIEOP, were enrolled in this prospective study. UDs were identified through a network of national and international bone marrow donor registries, as previously described [10].

The major endpoint of the study was to determine whether the early inception of CsA could improve EFS probability, compared to conventional CsA. Randomization by a 1:1 allocation ratio after stratification according to HLA compatibility and disease phase, as described below, was centralized at the Department of Pediatrics, Ospedale Sant'Orsola, Bologna, by 1 of the investigators (R.R.) who was not involved in the clinical management of the patients.

To calculate the sample size, a 2-sided log-rank test was used. To obtain a study significance level of .05 and a study power of .80 and supposing an EFS probability at 5 years of .60 for patients given early CsA and .45 for the second arm of the study, 100 patients per arm with an accrual time of 5 years and an overall study duration of 7 years were planned. To monitor the results of the trial, interim analyses were performed 3 years after the beginning of the study and, subsequently, every year. It was decided that patient enrolment should be closed when the difference in overall survival (OS) between the 2 arms reached a P value of .05. The analysis was based on the intention-to-treat principle. At the fifth year, futility analysis based on the EFS estimates observed in the 2 arms indicated a low probability (<30% under the most extreme hypothesis) that the study detected a significant difference if the planned sample size (100 patients per arm) was reached. Patient accrual was then stopped.

Histocompatibility for all donor-recipient pairs was determined by serology for HLA-A and -B antigens and high-resolution allelic technique for DRB1. Pairs matched for HLA-A, -B, and -DRB1 were defined as 6/6 matched loci, whereas pairs in which there was incompatibility for a single HLA locus were defined as 5/6 matched loci. Since 1998, high-resolution molecular typings were performed to characterize HLA class I loci as well, in 130 patients, but the resulting information was not considered for patient stratification.

Patients with acute lymphoblastic leukemia (ALL) in second complete remission (CR), acute myelogenous leukemia (AML) or non-Hodgkin lymphoma (NHL) in first CR, as well as children with low-blast count myelodysplastic syndrome (ie, refractory cytopenia) or chronic myelogenous leukemia (CML) in first chronic phase were assigned to the "low-risk " group, whereas children with more advanced disease were included in the "high-risk " group. Patients were randomized using a computer-generated random list, and stratified according to HLA matching and disease phase. The distribution was homogeneous between the 2 groups (1 and 2) for patient-, donor-, and transplantation-related characteristics, as shown in Table 1.

The source of progenitor cells was bone marrow for all transplants. The parents of all children gave written informed consent and understood the experimental nature the study, which was approved by the institutional review board at each of the participating Institutions.

Pretransplantation preparative regimens were all myeloablative and chosen according to institutional protocols, on the basis of the underlying disease and its phase, as well as on the recipient's age. Most children with ALL or NHL received a preparative regimen containing total body irradiation (TBI), associated with thiotepa and cyclophosphamide (Cy) [4]. The majority of children with AML and with myelodysplastic syndromes (MDS) received a non-TBI containing regimen, including busulphan (Bu), cyclophosphamide

Table 1. Characteristics of Patients According to the Arm of Randomization

	CsA −7	CsA −1	
	N = 72	N = 80	*P* Value
Percentage	(47%)	(53%)	
Diagnosis			
ALL	40 (56%)	39 (49%)	NS#
AML	9 (12%)	14 (17%)	
MDS	12 (17%)	11 (14%)	
CML	6 (8%)	14 (17%)	
Non-Hodgkin lymphoma	5 (7%)	2 (3%)	
Disease phase			
Early	53 (74%)	63 (79%)	
Advanced	19 (26%)	17 (21%)	NS#
Patient sex			
Male	47 (65%)	47 (59%)	
Female	25 (35%)	33 (41%)	NS#
Patient age at dx (years)			
Median	6	8	
Range	(0-15)	(0-17)	.038§
Patient age at HSCT (years)			
Median	8	10	
Range	(1-18)	(1-18)	NS§
Dx-HSCT interval (months)			
Median	27	24	
Range	(3-79)	(3-80)	NS§
HLA matching			
A, B, DRB1 6/6	53 (74%)	63 (79%)	
5/6	19 (26%)	17 (21%)	NS#
Sex matching			
Don F/Rec M	17 (24%)	18 (23%)	
Other combinations	55 (76%)	62 (77%)	NS#
Donor/recipient h-CMV serology			
Neg/neg	18 (25%)	11 (14%)	
Pos donor and/or recipient	54 (75%)	69 (86%)	NS#
Donor age (years)			
Median	35	36	
Range	(20-54)	(23-52)	NS§
Conditioning regimen			
TBI plus chemotherapy	47 (65%)	52 (65%)	
Chemotherapy alone	25 (35%)	28 (35%)	NS#
Nucleated cells infused ($\times 10^8$/kg)			
Median	6.2	5.3	
Range	(1-25)	(0.5-14)	NS§

CsA−7, indicates cyclosporine from day –7; CsA−1 cyclosporine from day –1; ALL, acute lymphoblastic leukemia; AML, acute myelogenous leukemia; MDS, myelodysplastic syndromes; CML, chronic myelogenous leukemia; HSCT, hematopoietic stem cell transplantation; Don, donor; F, female; Rec, recipient; M, male; Neg, negative; Pos, positive; TBI, total body irradiation; h-CMV, human cytomegalovirus.
#indicates two-tailed Fisher's exact test; dx, diagnosis.
§*t*-test

(Cy), and melphalan (Mel) [11], whereas children with CML received a preparative regimen containing TBI and Cy, as previously reported [8].

aGVHD and cGVHD were diagnosed and graded at each transplant Centre according to the Seattle criteria [12,13]. Patients surviving >14 and 100 days posttransplantation were evaluated for aGVHD and cGVHD occurrence, respectively. Treatment of both aGVHD and cGVHD, supportive therapy, as well as prophylaxis and treatment of infections [14,15], were administered according to protocols in use at each participating Institution.

The combination of CsA (3 mg/kg/day from day −1), short-course MTX (15 mg/m^2 on day +1, 10 mg/m^2 on days +3, +6, and +11) and ATG (Thymoglobuline®, 3.75 mg/kg from day −4 to day −2) was given to the 80 children randomized to standard CsA. Before receiving the same GVHD prophylaxis, the 72 children allocated to early CsA had also received CsA 1 mg/kg/day from day −7 to day −2. CsA was initially administered intravenously as a 2-hour infusion every 12 hours; when patients were able to tolerate oral intake, CsA was shifted to 6 mg/kg/day twice daily by mouth.

Blood levels of CsA were monitored twice weekly until day +29, once a week from day 30 to day 60 and every 2 weeks from day 60 to day 180 or according to clinical requirements. CsA doses were not adjusted, unless trough blood levels exceeded 300 ng/mL; in the absence of GVHD, CsA was tapered by 20% to 25% every month starting on day +60 and then usually discontinued within the end of month 6.

Statistical Analysis

Data were analyzed as of February 15, 2008. All data were stored in a central data base (AIEOP–BMT Registry), and were analyzed at the AIEOP Operation Office [16].

Demographic and clinical characteristics of the 2 groups of patients were compared using the 2-tailed Fisher's exact test for categoric variables (sex, disease, conditioning regimens, HLA matching, sex matching), as well as Wilcoxon's rank sum test and the *t*-test for continuous variables (age, time interval between diagnosis and HSCT, marrow cell dose) [17].

The primary endpoint of this study was to evaluate the influence of early CsA on EFS, whereas the secondary endpoints were to investigate if this modification had an impact on aGVHD and cGVHD incidence, as well as on RR and TRM. Moreover, we evaluated the impact of the following patient-, donor-, and transplant-related factors on outcome: primary diagnosis, disease phase, recipient sex, recipient age at diagnosis and at HSCT, interval diagnosis-HSCT, donor age and sex, donor-recipient pair HLA compatibility (6/6 loci matched versus 1−locus mismatched), human cytomegalovirus (h-CMV) serology, conditioning regimen (TBI versus chemotherapy), number of nucleated cells infused, aGVHD, and cGVHD occurrence. Patients were censored at time of relapse or death or last follow-up, whichever occurred first [18,19].

The probabilities of OS and EFS were estimated by the Kaplan-Meier method and expressed as percentages and standard error (SE) [20], whereas RR and TRM were calculated as cumulative incidence curves, to adjust the analysis for competing risks [21,22]. For aGVHD and cGVHD, death and relapse were the competing events. For relapse, nonrelapse mortality (NRM) was

the competing event, whereas for TRM, relapse represented the competing event.

The significance of differences between EFS curves was estimated by the log-rank test (Mantel-Cox), whereas Gray's test [23] was used to assess differences in univariate analyses of RR and TRM. All variables with a P value <.05 in univariate analysis were included in a multivariate analysis on EFS, performed using the Cox proportional hazard regression model using a backward selection procedure, whereas the proportional subdistribution hazard regression model [24] was used to perform multivariate analyses of RR and TRM.

Statistical analysis was performed using the STATA package [25] and the R 2.5.0 software package [26].

RESULTS

The median follow-up for survivors in the 2 groups of patients given either early or standard CsA is 85 (range: 56-110) and 82 (range: 53-113) months, respectively (P = NS). The median follow-up for deceased patients in the 2 groups is 4 (range: 1-23) and 6 (range: 0-73) months, respectively (P = NS).

Engraftment and GVHD Occurrence

Eight patients died before engraftment. The remaining 144 patients engrafted; no significant difference for neutrophil or platelet recovery was observed between the 2 groups (data not shown). The 100-day cumulative incidence of grade II-IV aGVHD in patients given either early or standard CsA was 57.1% (SE 5.9) and 67.6% (SE 5.4), respectively (P = NS), whereas the 100-day cumulative incidence of grade III-IV aGVHD was 27.2% (SE 5.3) and 31.1% (SE 5.4), respectively (P = NS).

Fifty-five (47%) of the 118 patients at risk developed cGVHD, which was limited in 27 cases (23%) and extensive in the remaining 28 (24%). The cumulative incidence of cGVHD occurrence was 39.3% (SE 6.6) and 55.4% (SE 6.5) for children given early or standard CsA, respectively (P = NS). cGVHD was never of de novo origin and, thus, a previous occurrence of aGVHD was the only factor predicting the occurrence of cGVHD. None of the other characteristics we analyzed had any influence on the incidence and severity of either aGVHD or cGVHD.

TRM

Forty (26%) children died of transplantation-related causes at a median time of 3 months (range: 0-73) after transplantation. Details on the causes of death are reported in Table 2.

The overall 100-day and 1-year cumulative incidence of TRM for children given early CsA was 9.7% (SE 3.4), and 24% (SE 4.7), whereas the 7-year cumulative incidence of TRM was 23.6% (SE 5.0). For patients allocated to standard CsA, the overall 100-day, 1-year, and 7-year cumulative incidence of TRM was 17.5% (SE 4.2), 23.7% (SE 4.7), and 29.4% (SE 5.2), thus indicating that for none of the three estimations there was any difference between the two groups. In the univariate analysis, the following characteristics were significantly associated with lower 100-day TRM: patient age <9 years (P = .024), negative donor/recipient HCMV serology (P = .044), a nucleated cell dose infused >4.8 × 10^8/kg (P = .017), and grade 0-II aGVHD (P < .0001) (see also Table 3 for details). All these variables lost their prognostic significance in the multivariate model (Table 4).

Table 2. Causes of Death in the Study Population

Random	Number of Patients			
	CsA −7		CSA −1	
Cause of death	First 100 Days after HSCT	After 100 Days from HSCT	First 100 Days after HSCT	After 100 Days from HSCT
Acute GVHD	2	1	3	
Chronic GVHD				2
Idiopatic pneumonia	4	2	2	2
Aspergillus pneumonia		1	1	2
h-CMV pneumonia		1	1	1
Fungal infections			1	1
Bacterial sepsis		3	2	1
VOD			1	
MOF			1	
EBV PTLD	1	2	1	
TTP			1	
Total number of patients dead for transplant-related causes	17		23	
Disease progression	4	9	3	13
Total	11	19	17	22

HSCT indicates hematopoietic stem cell transplantion; GVHD, graft-versus-host disease; h-CMV, human cytomegalovirus; VOD, veno-occlusive disease; MOF, multiple organ failure; EBV-PTLD, Epstein-Barr virus-related posttransplant lymphoproliferative disease; TTP, thrombotic thrombocytopenic purpura.

Relapse

Relapse of the underlying disease occurred in 34 (22%) patients at a median time of 6 months (range: 1-69) after the allograft. The cumulative incidence of relapse for children given early or standard CsA was 20.8% (SE 4.8) and 24.2% (SE 4.9), respectively (P = NS) with no difference in the timing of relapse between the 2 groups. The cumulative incidence of relapse for children with ALL allocated in the 2 randomization arms was 17.5% (SE 6.0) and 28.2% (SE 7.2), respectively (P = NS). Disease progression was the direct cause of death in 29 cases. Details on timing of disease-related deaths are reported in Table 2.

Table 3. Univariate Analysis of Variables Potentially Influencing Event-Free Survival, Relapse Rate, and 12-Month Treatment-Related Mortality

Variable		EFS (95% CI)	RR (95% CI)	12-Month TRM (95% CI)
Random	CSA day −7	56% (43-66)	21% (13-33)	24% (16-36)
	CSA day −1	46% (35-57)	24% (16-36)	24% (16-35)
	P value	NS§	NS#	NS#
Disease phase	Early	57% (47-66)	17% (11-25)	23% (17-32)
	Advanced	31% (17-46)	41% (28-61)	25% (14-44)
	P value	0.0004§	0.0006#	NS#
Patient sex	Male	43% (33-53)	29% (21-40)	27% (19-37)
	Female	64% (50-75)	12% (6-24)	19% (11-32)
	P value	0.026§	0.023#	NS#
Patient age at HSCT (years)	<9	53% (40-64)	29% (20-42)	16% (9-28)
	≥9	49% (38-59)	17% (11-27)	30% (21-41)
	P value	NS§	0.044#	0.024#
Dx-HSCT interval (months)	*< 16*	53% (40-63)	23% (15-34)	20% (13-31)
	≥ 16	49% (38-60)	22% (15-34)	27% (19-39)
	P value	NS§	NS#	NS#
HLA matching A, B, DRB1	*6/6*	54% (44-63)	22% (16-31)	19% (14-29)
	5/6	41% (24-56)	23% (13-43)	36% (23-56)
	P value	NS§	NS#	NS#
Sex matching	Don F/Rec M	46% (29-61)	29% (17-48)	26% (15-45)
	Other combinations	52% (43-61)	21% (15-30)	23% (17-32)
	P value	NS§	NS#	NS#
Donor/recipient CMV serology	Neg/neg	54% (34-70)	36% (21-59)	10% (3-30)
	Pos Don and/or Rec	50% (41-59)	20% (14-28)	26% (19-35)
	P value	NS§	NS#	0.044#
Donor age (years)	<35	60% (48-71)	20% (13-32)	16% (10-28)
	≥35	43% (31-53)	24% (16-36)	30% (22-42)
	P value	0.027§	NS#	NS#
Conditioning regimen	TBI plus chemotherapy	51% (41-61)	21% (14-30)	24% (17-35)
	Chemotherapy alone	48% (34-61)	27% (17-42)	23% (14-38)
	P value	NS§	NS#	NS#
Nucleated cells infused ($\times 10^8$/kg)	<4.8	50% (38-61)	15% (9-26)	31% (22-43)
	≥4.8	52% (40-62)	30% (21-42)	17% (10-28)
	P value	NS§	0.012#	0.017#
A, B, 0 group matching	Matched	49% (35-61)	25% (16-40)	25% (16-39)
	Mismatched	54% (37-68)	24% (14-39)	22% (13-36)
	P value	NS§	NS#	NS#
Acute GVHD grade	0-II	60% (50-69)	26% (19-37)	11% (6-19)
	III-IV	32% (19-47)	13% (6-29)	50% (37-68)
	P value	0.0012§	NS#	0.0000#
Chronic GVHD	Absent	64% (51-74)	27% (18-40)	9% (4-20)
	Present	66% (51-77)	19% (11-33)	9% (4-21)
	P value	NS§	NS#	NS#

EFS indicates event-free survival; RR, relapse rate; TRM, treatment-related mortality; CI, confidence interval; CsA −7, cyclosporine from day –7; CsA −1 cyclosporine from day –1; HSCT, hematopoietic stem cell transplantation; Don, donor; F, female; Rec, recipient; M, male; CMV, cytomegalovirus; Neg, negative; Pos, positive; TBI, total body irradiation.
§log-rank test.
#Gray's test.

In univariate analysis, the following variables predicted an increased risk of recurrence: advanced disease phase ($P = .0006$), male sex ($P = .023$), age <9 years ($P = .044$), and a number of nucleated cells infused >4.8 × 10^8/kg ($P = .018$) (Table 3). In multivariate analysis, only advanced disease phase ($P = .0032$), and male sex ($P = .019$) remained significantly correlated with an increased risk of relapse (also see Table 4 for details).

OS and EFS

Overall, 83 (55%) children are alive after HSCT, and 78 of them (51% of the total) are disease free, the 8-year Kaplan–Meier estimate of survival and EFS for the whole cohort of patients being 54.2% (SE 4.1) and 50.9% (SE 4.1), respectively (see also Figure 1); 8-year EFS was 55.6% (SE 5.9) and 46.3% (SE 5.7) for patients given early or standard CsA, respectively (P = NS, see also Figure 2). Noteworthy, there was a trend toward an improvement in the probability of EFS in patients with advanced disease phase given early CsA compared to those receiving standard CsA (42.1%, SE 11.3, versus 17.6%, SE 9.2, respectively, $P = .079$, see also Figure 3).

Univariate analysis of the various patient-, donor-, and transplantation-related factors potentially influencing outcome (Table 3) showed that advanced disease phase ($P = .0004$), recipient male sex ($P = .026$), donor age ≥35 years ($P = .027$), occurrence of grade

Table 4. Multivariate Analysis of Variables Influencing Event-Free Survival, Relapse Rate, and 12-Month Treatment-Related Mortality

	HR	95% CI	*P* Value
EFS			
Disease phase			
Advanced versus Early	3.18	(1.81-5.57)	.0001
Acute GVHD grade			
III-IV versus 0-II	2.69	(1.59-4.54)	.0001
Donor age			
>35 years versus ≤35 years	2.19	(1.31-3.65)	.003
Patient sex			
F veruss M	0.56	(0.33-0.95)	.032
RR			
Disease phase			
Advanced versus early	2.94	(1.43-6.03)	.0032
Patient sex			
F versus M	0.36	(0.16-0.84)	.019
TRM	No variable had any statistical impact		

EFS indicates event-free survival; RR, relapse rate; TRM, treatment-related mortality; RH, relative hazard; CI, confidence interval; F, female; M, male; GVHD, graft-versus-host disease.

III-IV aGVHD ($P = .012$) significantly correlate with inferior EFS. All these factors maintained their prognostic value in the multivariate Cox regression model (Table 4).

DISCUSSION

This prospective, randomized trial demonstrates that early inception of CsA, even if associated with higher blood levels of the drug at time of donor-cell infusion (data not shown), failed to improve patient outcomes. Although CsA, as a calcineurin inhibitor, has the possibility of reducing T cell activation and the cytokine storm associated with the tissue damage induced by the conditioning regimen [9,27], the incidence of both grade II-IV and grade III-IV aGVHD did not differ between the 2 study groups. This lack of effect on the occurrence of aGVHD was associated with a comparable incidence of TRM and of probabilities of EFS in the 2 groups. The discrepancy of our results with those previously reported [7,8], which offered the rationale for our working hypothesis, may be explained by considering different factors. In fact, although in those studies patients were mainly adults affected by a homogeneous disease, namely Ph+ CML, we randomized pediatric patients affected by different hematologic malignancies, mainly acute leukemia. Moreover, all our patients were given ATG, which is known to induce in vivo T cell depletion of the graft, as well as modulation of donor T-lymphocyte function. Previously published reports have shown that the use of ATG can reduce the incidence of grade II-IV GVHD in patients transplanted from an unrelated volunteer [28-30]. Thus, it cannot be excluded that the administration of ATG in our patient population could have obscured the advantage of

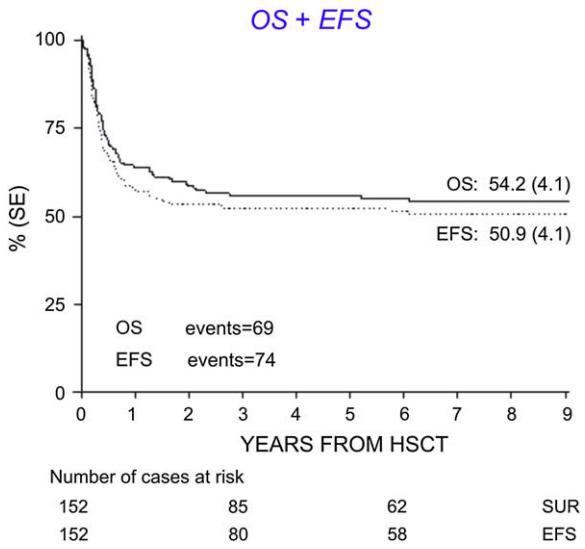

Figure 1. Probability of overall survival (OS), and of event-free survival (EFS) in the whole cohort of patients.

an early start of CsA. When our study was started, histocompatibility was determined by serology for HLA-A and -B antigens and by the high-resolution allelic technique for DRB1. In the second part of the study, high-resolution (allelic level) typing for the HLA loci A, B, C, DRB1, and DQB1 was performed to select donors. Although the resulting information was not considered for patient stratification in the randomization, the selection of the unrelated donors, using high-resolution molecular typing for both HLA class I and II loci, may have contributed to reduce the risk of both aGVHD and TRM, as previously reported [4,5,31], this factor also being able to blunt the benefit derived from an earlier administration of CsA. In any case, our results clearly

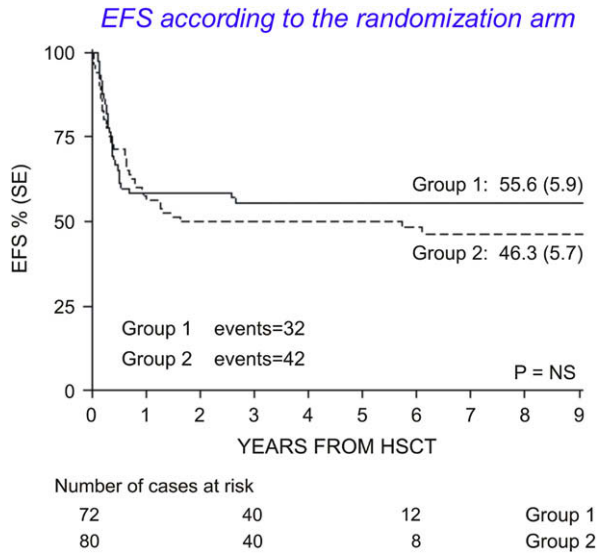

Figure 2. Probability of event-free survival (EFS) according to the group of randomization; group 1 includes patients given CsA from day –7; group 2 includes patients given CsA from day –1; HSCT, hematopoietic stem cell transplantation.

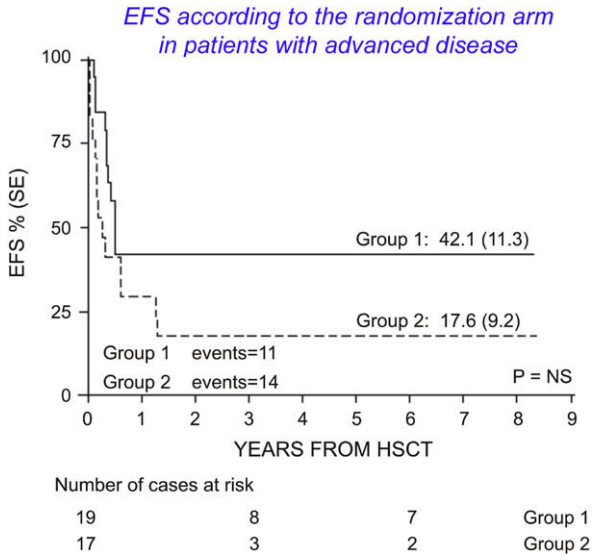

Figure 3. Probability of event-free survival (EFS) according to the group of randomization in patients transplanted in advanced phase; group 1 includes patients given CsA from day −7; group 2 includes patients given CsA from day −1; HSCT, hematopoietic stem cell transplantation.

confirm that data deriving from uncontrolled analyses [7,8] must be validated in prospective randomized trials before being considered sound enough to modify the conventional approaches. When patients with advanced disease phase were analyzed separately, we found a better, although not statistically significant, outcome if they had received CsA from day −7. The reason for this finding remains unclear, although we do not exclude the possibility that, if a larger number of patients with advanced disease had been included in the randomized trial, the value of our working hypothesis might be demonstrated.

Analysis of the overall study group offered us the opportunity to evaluate the impact of many patient-, donor-, and transplantation-related variables on patient outcomes. These results can be of value in designing specific risk-adapted treatment strategies based on recipient characteristics [32].

Our data confirm that the outcome of patients given HSCT from a UD has improved over time, the 51% 8-year EFS we observed in our cohort of children with hematologic malignancies given an UD-HSCT being comparable to the results reported in a cohort of children with ALL transplanted in recent years from an UD [4].

In keeping with Beatty et al. [33], we demonstrate that the final outcome of patients with malignancies transplanted from a 5/6 matched donor does not significantly differ from that of patients given the allograft from a 6/6 matched donor, thus proving that some degree of HLA disparity can be tolerated, even more in young patients who are transplanted from UDs for malignant disease, thus making transplantation an option available to larger numbers of patients. We also confirm that the transplant success is strongly influenced by the disease status at transplantation [6,34,35]. In a previous paper, we showed that the main factor conditioning a poor outcome of patients in need of an allograft is relapse occurring while the search is still ongoing, this being frequently seen for patients with less frequent HLA haplotypes [34]. Thus, although an optimal HLA matching in the donor/recipient pair reduces the risk of TRM [4], our results suggest that when a fully HLA-matched donor is not immediately available, a subset of HLA disparities of little clinical relevance can be accepted, without substantially lowering survival [35]. Clearly, this consideration better applies to patients at higher risk of relapse than to those with a low probability of disease recurrence.

In keeping with a recent study by the National Marrow Donor Program, in our study we did not observe any adverse effect on EFS of donor and recipient h-CMV positive serology [36].The timely diagnosis of h-CMV infection, with preemptive use of drugs, such as ganciclovir, adopted in recent years [13], might have contributed to prevent h-CMV disease and mortality, abrogating any detrimental impact of this viral infection on patient outcome.

We also confirm that donor age plays a significant role in improving EFS, a finding already reported in a study on a large number of patients by the National Marrow Donor Program [36].

We found that both occurrence of grade III-IV aGVHD and recipient male sex predicted an inferior EFS. The detrimental effect played by occurrence of severe GVHD resulted from an increased probability of death due to transplant-related complications, as suggested by the results of univariate analysis on TRM. This unfavorable effect was not compensated by any significant reduction of RR. The reason why male patients had an inferior outcome compared to females is obscure, although, at least in ALL, males have a greater probability of treatment failure even in the context of conventional chemotherapy [37].

In conclusion, our data indicate that an early inception of CsA, although leading to higher blood levels of the drug at time of infusion of donor cells, does not reduce the risk of immune-mediated complications and does not significantly improve patient outcome.

ACKNOWLEDGMENTS

Edoardo Lanino, Franco Locatelli, and Giorgio Dini wrote the paper and equally contributed.

Financial disclosure: This work has been partly supported by grants from Ministero della Salute (RF IGG-2007-650460).

REFERENCES

1. Thomas ED. Karnofsky memorial lecture: marrow transplantation for malignant disease. *J Clin Oncol*. 1983;1:517-531.

2. Champlin RE, Gale RP. The role of bone marrow transplantation in the treatment of hematologic malignancies and solid tumors: a critical review of syngeneic autologous and allogeneic transplant. *Cancer Treat Rep*. 1984;68:145-161.
3. Fischer A, Landais P, Friedrich W, et al. Bone marrow transplantation in Europe for primary immunodeficiencies other than severe combined immunodeficiency: a report from the European Group for BMT and the European Group for Immunodeficiency. *Blood*. 1994;83:1149-1154.
4. Locatelli F, Zecca M, Messina C, et al. Improvement over time in outcome for children with acute lymphoblastic leukaemia in second remission given hematopoietic stem cell transplantation from unrelated donors. *Leukemia*. 2002;16:2228-2237.
5. Petersdorf EW, Gooley TA, Anasetti C, et al. Optimizing outcome after unrelated marrow transplantation by comprehensive matching of HLA class I and II alleles in the donor and recipient. *Blood*. 1998;92:3515-3520.
6. Dini G, Cancedda R, Locatelli F, et al, for the GITMO. Unrelated donor marrow transplantation: an update of the experience of the Italian bone marrow transplant group (GITMO). *Haematologica*. 2001;86:451-456.
7. Lamparelli T, Van Lint MT, Gualandi F, et al. Bone marrow transplantation for chronic myeloid leukemia (CML) from unrelated and sibling donors: single center experience. *Bone Marrow Transplant*. 1997;20:1057-1062.
8. Dini G, Lamparelli T, Rondelli R, et al. Unrelated donor marrow transplantation for chronic myelogenous leukaemia. *Br J Haematol*. 1998;102:544-552.
9. Ferrara JL, Cooke KR, Teshima T. The pathophysiology of acute graft-versus-host disease. *Int J Hematol*. 2003;78:181-187.
10. Dini G, Lanino E, Lamparelli T, et al. Unrelated donor marrow transplantation: initial experience of the Italian bone marrow transplant group (GITMO). *Bone Marrow Transplant*. 1996;17: 55-62.
11. Locatelli F, Nöllke P, Zecca M, et al. Hematopoietic stem cell transplantation (HSCT) in children with juvenile myelomonocytic leukemia (JMML): results of the EWOG-MDS/EBMT trial. *Blood*. 2005;105:410-419.
12. Glucksberg H, Storb R, Fefer A, et al. Clinical manifestations of graft-versus host disease in human recipients of marrow from HLA-matched sibling donors. *Transplantation*. 1974;18:295-304.
13. Storb R, Prentice RL, Sullivan KM, et al. Predictive factors in chronic graft-versus-host disease in patients with aplastic anemia treated by bone marrow transplantation from using HLA-identical sibling donors. *Ann Intern Med*. 1983;98. 461–456.
14. Dallorso S, Rondelli R, Messina C, et al. Clinical benefits of granulocyte colony-stimulating factor therapy after hematopoietic stem cell transplant in children: results of a prospective randomized trial. *Haematologica*. 2002;87:1274-1280.
15. Locatelli F, Percivalle E, Comoli P, et al. Human cytomegalovirus (HCMV) infection in paediatric patients given allogeneic bone marrow transplantation: role of early antiviral treatment for HCMV antigenaemia on patients' outcome. *Br J Haematol*. 1994;88:64-71.
16. Pession A, Rondelli R, Paolucci P, et al. Hematopoietic stem cell transplantation in childhood: report from the bone marrow transplantation group of the Associazione Italiana Ematologia Oncologia Pediatrica (AIEOP). *Haematologica*. 2000;85:638-646.
17. Kalbfleish JD, Prentice RL. Interference based on ranks in the accelerated failure time model. In: Kalbfleish JD, Prentice RL, editors. *The Statistical Analysis of Failure Time Data*. New York: John Wiley; 1980 p. 143-162.
18. Clift R, Goldman JM, Gratwohl A, Horowitz M. Proposals for standardized reporting of results of bone marrow transplantation for leukaemia. *Bone Marrow Transplant*. 1989;4:445-448.
19. Pepe MS, Longton G, Pettinger M, Mori M, Fisher LD, Storb R. Summarizing data on survival, relapse, and chronic graft-versus-host disease after bone marrow transplantation: motivation for and description of new methods. *Br J Haematol*. 1993;83:602-607.
20. Kaplan EL, Meier P. Non parametric estimation from incomplete observations. *J Am Stat Assoc*. 1958;53:457-481.
21. Gooley TA, Leisenring W, Crowley J, Storer BE. Estimation of failure probabilities in the presence of competing risks: new representations of old estimators. *Stat Med*. 1999;18:695-706.
22. Klein JP, Rizzo JD, Zhang MJ, Keiding N. Statistical methods for analysis and presentation of the results of bone marrow transplants. Part I: unadjusted analysis. *Bone Marrow Transplant*. 2001;28:909-915.
23. Gray RJ. A class of K-sample tests for comparing the cumulative incidence of a competing risk. *Ann Stat*. 1988;16:1141-1154.
24. Fine JP, Gray RJ. A proportional hazards model for the subdistribution of a competing risk. *J Am Stat Assoc*. 1999;94:496-509.
25. StataCorp. *STATA Statistical Software: Release 7.0*. College Station, TX: Stata Corporation; 2000.
26. R Foundation for Statistical Computing; R: a language and environment for statistical computing (version 2.5.0). [http://www.R-project.org].
27. Smith CS, Ortega G, Parker L, Shearer WT. Cyclosporine A blocks induction of tumor necrosis factor-alpha in human B lymphocytes. *Biochem Biophys Res Commun*. 1994;204:383-390.
28. Remberger M, Storer B, Ringdén O, Anasetti C. Association between pretransplant thymoglobulin and reduced non-relapse mortality rate after marrow transplantation from unrelated donors. *Bone Marrow Transplant*. 2002;29:391-397.
29. Giebel S, Giorgiani G, Martinetti M, et al. Low incidence of severe acute graft-versus-host disease in children given haematopoietic stem cell transplantation from unrelated donors prospectively matched for HLA class I and II alleles with high-resolution molecular typing. *Bone Marrow Transplantation*. 2003;31:987-994.
30. Bacigalupo A, Lamparelli T, Bruzzi P, et al. Antithymocyte globulin for graft-versus-host disease prophylaxis in transplants from unrelated donors: 2 randomized studies from Gruppo Italiano Trapianti Midollo Osseo (GITMO). *Blood*. 2001;98:2942-2947.
31. Lee SJ, Klein J, Haagenson M, et al. High resolution donor-recipient HLA matching contributes to the success of unrelated donor marrow transplantation. *Blood*. 2007;110:4576-4583.
32. Lanino E, Sacchi N, Peters C, Giardino S, Rocha V, Dini G. Strategies of the donor search for children with second complete remission acute lymphoblastic leukaemia lacking a matched sibling donor. *Bone Marrow Transplant*. 2008;41(Suppl 2):S75-S79.
33. Beatty PG, Anasetti C, Hansen JA, et al. Marrow transplantation from unrelated donors for treatment of hematologic malignancies: effect of mismatching for one HLA locus. *Blood*. 1993;81:249-253.
34. Dini G, Valsecchi MG, Micalizzi C, et al. Impact of marrow unrelated donor search duration on outcome of children with acute lymphoblastic leukemia in second remission. *Bone Marrow Transplant*. 2003;32:325-331.
35. Petersdorf EW, Gooley T, Malkki M, Horowitz M. Clinical significance of donor-recipient HLA matching on survival after myeloablative hematopoietic cell transplantation from unrelated donors. *Tissue Antigens*. 2007;69(Suppl 1):25-30.
36. Kollman C, Howe CW, Anasetti C, et al. Donor characteristics as risk factors in recipients after transplantation of bone marrow from unrelated donors: the effect of donor age. *Blood*. 2001;98: 2043-2051.
37. Conter V, Aricò M, Valsecchi MG, et al. Long-term results of the Italian Association of Pediatric Hematology and Oncology (AIEOP) acute lymphoblastic leukemia studies, 1982-1995. *Leukemia*. 2000;14:2196-2204.