Biol Blood Marrow Transplant 23 (2017) 96–102

Biology of Blood and Marrow Transplantation

journal homepage: www.bbmt.org

ASBMT American Society for Blood and Marrow Transplantation

Clinical Research: Alternative Donors

Unrelated Cord Blood Transplantation for Acute Leukemia Diagnosed in the First Year of Life: Outcomes and Risk Factor Analysis

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Article history:
Received 2 August 2016
Accepted 15 October 2016

Key Words:
Cord blood transplantation
Infant
Acute leukemia
MLL-rearranged leukemia

A B S T R A C T

Infant acute leukemia still has a poor prognosis, and allogeneic hematopoietic stem cell transplantation is indicated in selected patients. Umbilical cord blood (UCB) is an attractive cell source for this population because of the low risk of chronic graft-versus-host disease (GVHD), the strong graft-versus-leukemia effect, and prompt donor availability. This retrospective, registry-based study reported UCB transplantation (UCBT) outcomes in 252 children with acute lymphoblastic leukemia (ALL; n = 157) or acute myelogenous leukemia (AML; n = 95) diagnosed before 1 year of age who received a single-unit UCBT after myeloablative conditioning between 1996 and 2012 in European Society for Blood and Marrow Transplantation centers. Median age at UCBT was 1.1 years, and median follow-up was 42 months. Most patients (57%) received a graft with 1 HLA disparity and were transplanted in first complete remission (CR; 55%). Cumulative incidence function (CIF) of day 100 acute GVHD (grades II to IV) was 40% ± 3% and of 4-year chronic GVHD was 13% ± 2%. CIF of 1-year transplant-related mortality was 23% ± 3% and of 4-year relapse was 27% ± 3%. Leukemia-free-survival (LFS) at 4 years was 50% ± 3%; it was 40% and 66% for those transplanted for ALL and AML, respectively ($P = .001$). LFS was better for patients transplanted in first CR, regardless of diagnosis. In multivariate model, diagnosis of ALL ($P = .001$), advanced disease status at UCBT (<.001), age at diagnosis younger than 3 months ($P = .012$), and date of transplant before 2004 were independently associated with worse LFS. UCBT is a suitable option for patients diagnosed with infant acute leukemia who achieve CR. In this cohort, patients with AML had better survival than those with ALL.

INTRODUCTION

Acute leukemia diagnosed during the first year of life represents about 2.5% to 5% of acute lymphoblastic leukemia (ALL) and 6% to 14% of acute myelogenous leukemia (AML) in childhood [1]. At diagnosis, infant acute leukemia often occurs with hyperleukocytosis, central nervous system

A study on behalf of Eurocord, cord blood committee of cellular therapy and immunobiology working party and pediatric disease working party of European Society for Blood and Marrow Transplantation.

Financial disclosure: See Acknowledgments on page 101.

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http://dx.doi.org/10.1016/j.bbmt.2016.10.014
1083-8791/

involvement (both ALL and AML), frequent *MLL* rearrangement (ALL), and extramedullary disease (AML) [2]. Although the results obtained in the treatment of acute leukemia in children over 1 year of age have improved over the last decades, infant acute leukemia remains an aggressive disease associated with poor outcome [3,4].

In view of the molecular/genetic characteristics of the leukemia clone and of the response to front-line therapy, about one-third of infants with ALL in first complete remission (CR1), one-third to one-half of infants with AML in CR1, and virtually all infants with acute leukemia in CR2 have an indication to receive allogeneic hematopoietic stem cell transplantation (HSCT) usually after a myeloablative conditioning regimen [2,5]. However, the use of myeloablative conditioning in these young and fragile, often previously heavily treated, patients raises concerns, considering their high risk of toxicity, morbidity, and mortality compared with older children [6]. Moreover, the role of HSCT for infant leukemia is still controversial [7].

Umbilical cord blood transplantation (UCBT) represents a suitable stem cell source for pediatric patients with hematologic malignancies, with results of UCBT comparable with those of HSCT using either bone marrow or peripheral blood stem cells in both children and adults [8,9]. Although the role of UCBT in children has been extensively discussed, only scarce data report its use in patients diagnosed in the first year of life (ie, infants) [1]. Thus, we analyzed results of UCBT in patients reported by participating transplant centers to Eurocord and the European Society for Blood and Marrow Transplantation (EBMT), with the objective of providing data on outcome of UCBT in children with infant ALL and AML.

METHODS

Study Design, Inclusion Criteria, and Data Collection

This is a retrospective registry-based study performed by Eurocord, in collaboration with the Pediatric Disease Working Party of EBMT. Patients with acute leukemia diagnosed within the first year of life who received unrelated single-unit UCBT (first transplant) after myeloablative conditioning in EBMT centers from 1996 through 2012 were considered for the study. A total of 252 patients meeting the above criteria were included in the analysis.

Demographic and clinical data were extracted from the Eurocord database and validated. Participating transplant centers were asked to provide missing information and correct discrepancies using a customized questionnaire.

Parents or legal guardians provided informed consent allowing data entry into the Eurocord and EBMT databases for research purposes. The study was conducted in compliance with the Declaration of Helsinki. The Internal Review Board of Eurocord reviewed and approved the study.

Endpoints, Definitions, and Statistical Analysis

The primary endpoint was leukemia-free survival (LFS), defined as being alive and in continuous CR at last follow up.

Secondary endpoints were overall survival (OS), time of neutrophil and platelet recovery, incidence of relapse, transplant-related mortality (TRM), and acute and chronic GVHD. Probabilities were calculated from date of transplantation until the event or censoring. Neutrophil engraftment was defined as achieving absolute neutrophil count $\geq .5 \times 10^9$/L for 3 consecutive days with no evidence of autologous recovery (<5% leucocytes of donor origin in peripheral blood or marrow). Full donor chimerism was defined as presence of ≥95% leucocytes of donor origin in peripheral blood or bone marrow.

Platelet engraftment was defined as achieving platelet count $\geq 20 \times 10^9$/L unsupported by platelet transfusions for 7 days. Acute and chronic GVHD were graded according to previously published criteria [10,11]. Probabilities of LFS and OS were calculated using the Kaplan-Meier estimates. Cumulative incidence functions (CIFs) were used to estimate incidence of relapse and TRM in a competing risks setting as death and relapse compete with each other. To estimate acute and chronic GVHD incidences, relapse and death were considered as competing events.

A comparison with 2-sided $P < .05$ was considered statistically significant. Variables reaching $P < .10$ in univariate analysis were included in Cox proportional hazard regression models using a backward stepwise selection. Analyses were performed with SPSS version 19 (Inc., Chicago, IL) and Splus software package (MathSoft, Inc., Seattle, WA).

Criteria for poor-risk cytogenetics for ALL were the presence of 1 or more of the following abnormalities: t(4;11), t(1;19), hypodiploidy (<44 chromosomes), and/or any 11q23 abnormality. For AML, poor-risk group was defined according to previously published reports [12,13]. Myeloablative conditioning was defined as a regimen containing either total body irradiation with a dose greater than 6 Gy, a dose of oral busulfan higher than 8 mg/kg, a dose of i.v. busulfan > 6.4 mg/kg, or a myeloablative dose of treosulfan (ie, ≥36 g/m^2 over 3 days). Donor–recipient HLA degree of matching was assessed at the antigen level for HLA-A and -B and at the allele level for HLA-DRB1.

RESULTS

Patient, Disease, and Transplant Characteristics

Patient, disease, and transplant characteristics are summarized in Table 1. Briefly, among the 252 patients included in the study, 95 (38%) were transplanted for AML and 157 (62%) for ALL. Median age at diagnosis and at UCBT was 5.6 months (range, 1 day to 12 months) and 1.1 years (range, .3 to 11 years), respectively. Overall, 138 patients (55%) were transplanted in CR1, 76 (30%) in CR2, and the remaining in

Table 1
Patient, Disease, and Transplant Characteristics (n = 252)

Variable	Value
Patient and disease	
Gender (male)	118 (47)
Disease	
ALL	157 (62)
AML	95 (38)
ALL disease status (assessable n = 154)	
CR1	84 (54)
CR2	50 (33)
>2nd CR	9 (6)
Advanced disease	11 (7)
AML disease status (assessable n = 93)	
CR1	54 (58)
CR2	26 (28)
>2nd CR	2 (2)
Advanced disease	11 (12)
Cytogenetic risk (assessable n = 205)	
Good risk	2 (.8)
Intermediate risk	79 (38)
Poor risk	124 (60)
MLL rearrangement (available for n = 157)	118 (75)
Age at initial diagnosis, mo, median (range)	5.6 (.03-12.0)
Age at diagnosis < 3 months of age, whole cohort	64 (25)
ALL patients	44 (28)
AML patients	20 (21)
Time from diagnosis to transplantation	
Patients in CR1, mo, median (range)	6.1 (2.8-20.7)
Patients in CR2, mo, median (range)	16.7 (5.7-111.6)
Patients beyond CR2, mo, median (range)	
Age at transplantation, yr, median (range)	1.1 (.3-11.0)
Weight at transplantation, kg, median (range)	9.5 (4.0-34.0)
Positive CMV serology	109 (48)
Previous autologous transplant	5 (2)
Transplantation characteristics	
Year of transplantation (assessable n = 252)	
<2004	56 (22)
≥2004	196 (78)
HLA compatibility (assessable n = 188)	
Identical	41 (22)
1 HLA disparity	107 (57)
≥2 HLA disparities	40 (21)
Gender mismatch patient–graft (assessable n = 245)	115 (47)
TNC at cryopreservation, $\times 10^7$/kg, median (range)	12.4 (3.0-44.3)
CD34+ cells at infusion, $\times 10^5$/kg, median (range)	3.9 (.2-323.6)

Values are total number of cases with percents in parentheses, unless otherwise noted.
CMV indicates cytomegalovirus; TNC, total nucleated cell dose collected.

Table 2
Conditioning Regimen and GVHD Prophylaxis

Variable	n (%)
Conditioning regimen (assessable n = 245)	
Bu + Cy	67 (27)
Bu + Cy + melphalan	45 (18)
Bu + Cy + VP16	22 (9)
Bu + fluda + thio	27 (11)
Bu ± other	21 (9)
Treo ± other	15 (6)
Bu + Cy + thio	14 (6)
Cy + TBI	11 (5)
Cy + thio + TBI	10 (4)
Other	13 (5)
TBI containing regimen (assessable n = 252)	
No	218 (88)
Yes	31 (12)
GVHD prophylaxis (assessable n = 236)	
CsA + steroids ± other	135 (57)
CsA	40 (17)
CsA + other	49 (21)
Other	11 (5)
None	1 (<1)
ATG/MoAb use (assessable n = 234)	
Yes	206 (88)
No	28 (12)

Bu indicates busulfan; Cy, cyclophosphamide; VP16, etoposide; fluda, fludarabine; thio, thiotepa; treo; treosulfan; TBI, total body irradiation; CsA, cyclosporine A; ATG, antithymocyte globulin; MoAb, monoclonal antibody.

more advanced disease status, including 16 not in CR at time of UCBT.

Details on disease status for patients with AML and ALL are provided in Table 1. The median time from diagnosis to UCBT for patients in CR1 was 6 months (range, 3 to 21). For 205 patients (81%), information on cytogenetic analysis at diagnosis was available; of these 60% had high-risk disease. Information on MLL gene rearrangement at diagnosis was available for 157 patients (102/157 ALL patients and 55/95 AML patients); of these, 75% were positive for MLL gene rearrangement. Most patients received a UCB unit with 1 HLA mismatch (57%), and donor was gender mismatched with the recipient in 47% of cases. The most commonly used conditioning regimen was busulfan plus cyclophosphamide alone (27%) or in combination with melphalan (18%). Thirty-one patients (12% of the whole population, of which 29 had ALL and 2 AML) received a total body irradiation–based conditioning, and the median age at UCBT for patients receiving total body irradiation was 2.3 years (range, .8 to 11) (Table 2).

Cyclosporine A plus corticosteroids was the most frequently used GVHD prophylaxis (57% of patients), and 82% of patients received antithymocyte globulin. Median total nucleated cell dose and $CD34^+$ cell dose at cryopreservation were 12.4×10^7/kg (range, 3.0 to 44.3×10^7/kg) and 3.9×10^5/kg (range, .19 to 23.6×10^5/kg), respectively.

Engraftment and GVHD

Two hundred twenty-three patients achieved neutrophil recovery, within a median time of 21 days (range, 7 to 55). The CIF of neutrophil engraftment at day 60 was 88% ± 2%. Among patients who achieved neutrophil engraftment, 88% had full donor chimerism at day +100.

Of the 29 patients who failed to engraft, only 5 were alive at last follow-up. Of those, 4 had received a second transplant (1 bone marrow, 1 UCB, and 2 nonspecified). The remaining 24 patients died at a median of 37 days after UCBT (range, 6 to 550), including 5 patients who received a second transplant after graft failure (3 autologous rescues, 1 UCBT, and 1 matched unrelated donor HSCT).

In univariate analysis (Table 3), CIF of day 60 neutrophil engraftment was higher for patients transplanted in CR1 (93% ± 2%) compared with those in other disease status (82% ± 4%) at time of transplantation ($P = .002$). In addition, a total nucleated cell dose at cryopreservation higher than the median value was associated with a better CIF of neutrophil engraftment (91% ± 3% versus 87% ± 3%; $P = .003$). In multivariate analysis (Table 4), disease status at UCBT (CR1) (hazard ratio [HR], 1.46; 95% confidence interval [CI], 1.10 to 1.93; $P = .009$) and higher total nucleated cell dose (as a continuous variable) (HR, 1.02; 95% CI, 1.01 to 1.04; $P = .008$) were independently associated with higher incidence of engraftment.

One hundred patients experienced grades II to IV acute GVHD (62 grade II, 23 grade III, and 15 grade IV) with a median time at onset of 16 days (range, 5 to 100) after UCBT. Thirty-two patients experienced chronic GVHD, 14 with extensive involvement. Among the 32 patients who experienced chronic GVHD, 23 had previously had acute GVHD, whereas 9 experienced de novo chronic GVHD. The CIF of day 100 acute GVHD and 4-year chronic GVHD was 40% ± 3% and 13% ± 2%, respectively. None of the factors studied was associated with the incidence of developing acute or chronic GVHD in the multivariate analysis.

Relapse and TRM

Sixty-seven patients experienced disease recurrence. Overall, CIF of relapse at 4 years was 27% ± 3%; it was 20% ± 4% and 31% ± 4% for patients with AML and ALL, respectively ($P = .12$). Median time to relapse after UCBT was 4.4 months (range, .3 to 64.8); it was 5.4 months (range, .89 to 64.8) and 2.5 months (range, .33-17.4) for ALL and AML patients, respectively ($P = .005$). According to disease status, CIF of relapse at 4 years was 19% ± 4% for patients transplanted in CR1 and 37% ± 5% for those in other disease status (30% for patients in ≥CR2 and 64% for patients with advanced disease) ($P = .001$).

In univariate analysis (Table 3), age at diagnosis ≥ 3 months was associated with lower incidence of relapse (24% ± 4% versus 37% ± 6%; $P = .03$). In multivariate analysis (Table 4), CR1 at time of UCBT (HR, .36; 95% CI, .63 to 3.45; $P < .001$) and age at diagnosis ≥ 3 months (HR, .50; 95% CI, .28 to .88; $P = .016$) were confirmed to be independent factors associated with lower incidence of relapse.

The overall CIF of TRM at 1 year was 23% ± 3% for the whole cohort; it was 29% ± 4% and 13% ± 4% for patients with ALL and AML, respectively ($P = .004$). The CIF of TRM was higher for patients transplanted before 2004 (41% versus 18%; $P < .001$). In multivariate analysis (Table 4), diagnosis of AML (versus ALL) was associated with a lower risk of TRM (HR, .47; 95% CI, .23 to .98; $P = .002$).

A total of 115 patients died: 51 of relapsed disease and 64 of TRM. The most common cause of TRM was infection (n = 31: 8 viral, 6 bacterial, 2 fungal, and 15 not specified), followed by GVHD (n = 9). Among patients in whom the primary cause of death was infection (n = 31), 9 had experienced acute GVHD and 1 had de novo chronic GVHD. Other causes of TRM were interstitial pneumonitis (n = 3), hemorrhage (n = 3), acute respiratory distress syndrome (n = 3), multiorgan failure (n = 3), cardiac toxicity (n = 2), veno-occlusive disease (n = 1), rejection (n = 1), and other causes (n = 8).

LFS and OS

Median follow-up duration was 56 months (range, 4 to 203). The probability of 4-year LFS was 49% ± 3%; it was

Table 3
Univariate Analysis of Main Transplant Outcomes

	n	Neutrophil Engraftment		Acute GVHD		TRM		Relapse		OS		LFS	
		Percent	*P*	Percent	*P*	Percent	*P*	Percent	*P*	Percent ± SE	*P*	Percent	*P*
All patients	252	88 ± 3		40 ± 3		23 ± 3		27 ± 4		53 ± 3		49 ± 3	
Diagnosis													
ALL	157	87 ± 3	.180	39 ± 4	.537	29 ± 4	.004	31 ± 4	.123	45 ± 4	<.001	40 ± 4	<.001
AML	95	92 ± 3		41 ± 5		13 ± 3		20 ± 4		68 ± 5		66 ± 5	
Age at diagnosis													
<3 mo	64	88 ± 4	.801	39 ± 6	.945	25 ± 3	.488	37 ± 6	.037	40 ± 6	.012	36 ± 6	.016
≥3 mo	188	89 ± 2		41 ± 4		22 ± 4		24 ± 3		58 ± 4		54 ± 4	
Age at UCBT													
≤1.14 yr	126	90 ± 3	.202	38 ± 4	.828	19 ± 4	.113	29 ± 4	.521	58 ± 5	.163	52 ± 5	.386
>1.14 yr	126	87 ± 3		42 ± 5		26 ± 4		25 ± 4		49 ± 5		47 ± 5	
Patient gender													
Male	118	86 ± 3	.293	39 ± 5	.810	25 ± 4	.603	21 ± 4	.033	58 ± 5	.225	54 ± 5	.173
Female	134	91 ± 3		40 ± 4		21 ± 4		32 ± 4		48 ± 5		45 ± 4	
Year of UCBT													
<2004	56	80 ± 5	.084	38.2 ± 7	.766	41 ± 7	<.001	27 ± 6	.747	41 ± 7	0.0.004	34 ± 4	<.001
≥2004	11796	91 ± 1		41 ± 4		18 ± 3		28 ± 3		57 ± 4		54 ± 4	
Status of disease at UCBT													
CR1	138	93 ± 2	.002	41 ± 4	.436	19 ± 3	.078	19 ± 4	<.001	69 ± 4	<.001	62 ± 4	<.001
Not CR1	109	82 ± 4		38 ± 5		27 ± 4		37 ± 5		35 ± 5		34 ± 5	
Molecular markers													
MLL–	39	NP		NP		NP		NP		56 ± 8	.331	53 ± 5	.782
MLL+	118	NP		NP		NP		NP		60 ± 5		55 ± 8	
Cytogenetics													
Good risk/intermediate	81	NP		NP		NP		NP		57 ± 6	.148	54 ± 6	.263
Poor risk	125	NP		NP		NP		NP		57 ± 5		53 ± 5	
Number of HLA disparities													
0-1 HLA disparities	148	91 ± 2	.627	34 ± 9	.774	20 ± 3	.027	30 ± 4	.110	58 ± 4	.445	50 ± 4	.600
2 HLA disparities	40	88 ± 6		41 ± 3		38 ± 8		18 ± 4		49 ± 8		45 ± 8	
Use of TBI													
No	218	90 ± 2	.107	39 ± 3	.647	21 ± 3	.144	29 ± 3	.041	53 ± 4	.958	49 ± 4	.631
Yes	31	81 ± 8		45 ± 9		32 ± 9		14 ± 6		54 ± 9		54 ± 9	
Use of ATG before day 0													
No	28	87 ± 2	.043	36 ± 9	.936	29 ± 9	.438	29 ± 9	.962	49 ± 9	.595	42 ± 10	.523
Yes	206	100		40 ± 3		21 ± 3		27 ± 3		55 ± 4		52 ± 4	
TNC × 10^7/kg													
≤12.36	115	87 ± 3	.003	40 ± 5	.915	24 ± 4	.318	24 ± 4	.199	54 ± 5	.949	51 ± 5	.790
>12.36	115	91 ± 3		39 ± 5		20 ± 3		31 ± 4		53 ± 5		48 ± 5	

40% ± 5% and 66% ± 5% for patients with ALL and AML, respectively (*P* < .001) (Figure 1).Patients transplanted after 2003 had a better LFS (54% ± 4% versus 34% ± 6%; *P* < .001) than patients transplanted in earlier years.

Table 4
Multivariate Analysis

	HR	95% CI	*P*
LFS			
Diagnosis of AML vs. ALL	.50	.33-.76	.001
Age at diagnosis ≥ 3 mo	.62	.41-.90	.012
CR1 at UCBT	.42	.29-.61	<.001
OS			
Diagnosis of AML vs. ALL	.53	.35-.81	.003
Age at diagnosis ≥ 3 mo	.60	.41-.89	.012
CR1 at UCBT	.37	.25-.55	<.001
Engraftment			
UCBT after 2007	1.32	.99-1.75	.060
CR1 at UCBT	1.39	1.04-1.85	.024
Higher TNC dose*	1.03	1.01-1.04	.005
TRM			
Diagnosis of AML vs. ALL	.46	.22-.95	.036
≥2 HLA disparities	1.87	.99-3.55	.054
Relapse			
Diagnosis of AML vs. ALL	.55	.29-1.03	.060
CR1 at UCBT	.36	.63-3.45	<.001
Age at diagnosis ≥ 3 mo	.50	.28-.88	.016
Acute and chronic GVHD†			

* Higher than the median TNC dose.
† None of the factors included in the multivariate models was associated with the incidence of acute and/or chronic GVHD.

According to disease status, 4-year LFS was 49% ± 6% for patients with ALL in CR1, 24% ± 6% for patients with ALL in CR2, and 0% for patients with more advanced disease (*P* < .001). For patients with AML in CR1, 4-year LFS was 81% ± 5% compared with 58% ± 10% for patients with AML in CR2 and 17% ± 11% for patients with more advanced disease (*P* < .001).

In multivariate analysis, acute leukemia type (ALL versus AML), age at diagnosis, and disease status at UCBT were independent factors impacting LFS. Patients with diagnosis of AML (HR, .53; 95% CI, .35 to .81; *P* = .003), patients older than 3 months at diagnosis (HR, .61; 95% CI, .41 to .90; *P* = .013) (Figure 2),those who were transplanted in CR1 (HR, .43; 95% CI, .30 to .63; *P* < .001), and those who were transplanted after 2003 (HR, .61; 95% CI, .41 to .90; *P* < .014) had a better probability of LFS.

The probability of OS at 4 years was 53% ± 4% for the whole cohort and 45% ± 4% for patients with ALL and 68% ± 5% for patients with AML (*P* < .001). Among children with ALL, the 4-year OS for patients who receive total body irradiation during the conditioning regimen was 51% ± 10% and 43% ± 5% for those who did not receive it (*P* = .551). The factors significantly associated with OS in both univariate and multivariate analyses were the same as for LFS and are shown in Tables 3 and 4, respectively.

DISCUSSION

Considerable progress has been made in the treatment of pediatric malignant hematologic diseases with improvement

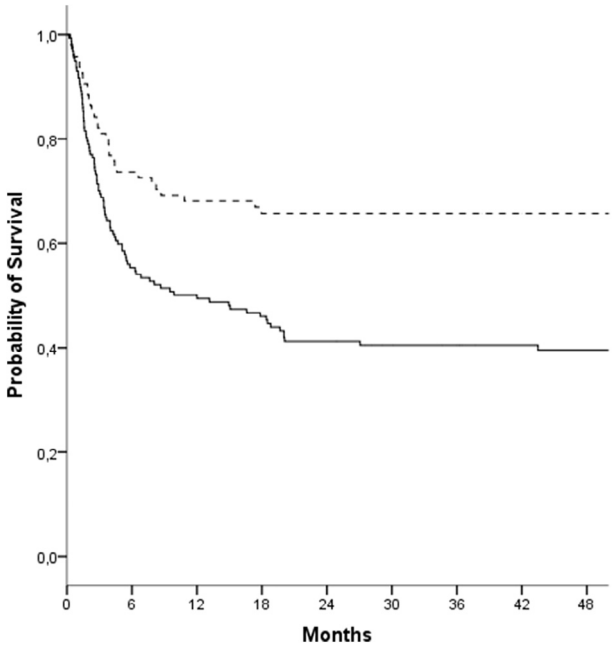

Figure 1. The estimated 4-year probability of LFS in infants with ALL and AML. Solid line represents patients with ALL; dashed line represents patients with AML

in the long-term OS from 10% for children treated between 1968 and 1970 to approximately 90% for children with ALL and around 70% for those with AML treated in the last decade [3,5,14,15]. The reason for such improvement in survival is most likely related to better risk stratification, improvement in supportive care, and more rational delivery of chemotherapy agents during the course of treatment.

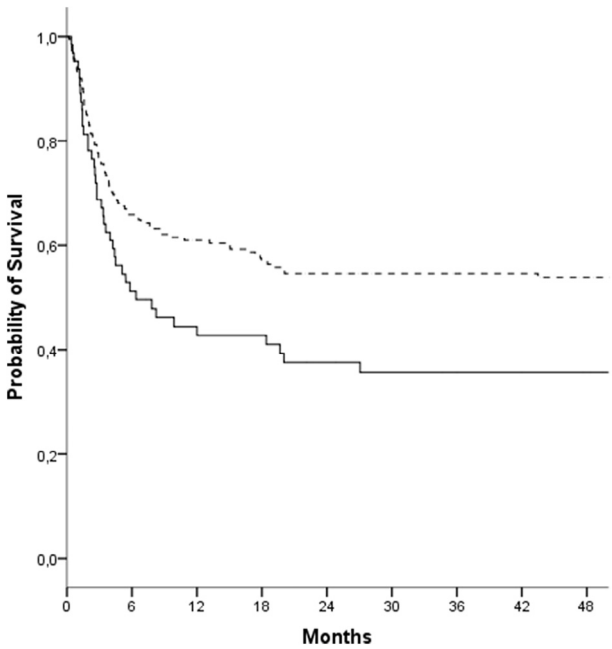

Figure 2. The estimated 4-year probability of LFS in infants according to age at diagnosis. Solid line represents patients < 3 months old at diagnosis of acute leukemia; dashed line represents patients ≥ 3 months at diagnosis of acute leukemia

However, when the onset of acute leukemia occurs within the first year of life, the disease remains associated with poor prognosis. Infant acute leukemia is usually very aggressive because of its biologic and molecular characteristics. In a recent Italian report, 94% of infant AML was classified as high risk [3]. In addition to the intrinsically aggressive characteristics of the disease, physicians have to deal with very fragile organisms. Infants younger than 12 months exhibit immature liver function with some less mature drug metabolism pathways. Therefore, the cumulative incidence of severe toxicities in infants is higher than in older children.

Up to the 1990s, pediatricians have used the same protocol designed for older children to treat infant ALL, but, in view of the poor overall results obtained, specific protocols to treat infants (eg, Interfant-99, MLL03, CCG 1953, POG 9407) have been adopted. Overall, these protocols have been associated with a 5-year OS rate between 40% and 50% [4,16-18]. Depending on the protocol, indication to HSCT differs, and the advantage derived from transplantation performed in CR1 still remains debatable. For the European and Japanese cooperative groups, HSCT significantly increased OS and event-free survival for high-risk patients transplanted in CR1 [16,19]. However, reports by other groups did not show advantages of using HSCT [7].

Recently, the results of HSCT in patients in CR2 enrolled in the Interfant-99 protocol showed that infants who relapsed within 1 year after diagnosis had 3-year OS rates of 17.5% compared with 25.5% and 44.4% of those who relapsed between 12 and 24 months and beyond 24 months after diagnosis, respectively [19].

For AML, the indication for HSCT depends on both leukemia subtypes and disease status. Criteria for choice might vary according to protocol for patients in CR1, whereas HSCT is consistently indicated for patients in CR2 [3,20-27]. However, most of the literature available discussing the indication for HSCT in pediatric AML is not focused on patients diagnosed as infants.

When HSCT is indicated, physicians have to identify a donor and select the stem cell source. UCB contains hematopoietic stem cells capable of reconstituting the hematopoietic system as well as innate immunity cells and naive T lymphocytes capable to display a strong graft-versus-leukemia effect with low incidence of GVHD [9]. Moreover, the low risk of chronic GVHD after UCBT makes the use of this stem cell source in children very attractive, because it allows GVHD-related disabilities and long-term complications to be avoided [28].

The use of UCBT in children with acute leukemia has been reported at length. Nevertheless, as discussed above, the disease and outcomes are different for patients who have leukemia diagnosed in the first year of life; therefore, our results may add to the current knowledge about the role of UCBT for this specific population. In a retrospective study using data from the Center for International Blood and Marrow Transplant Research and the New York Blood and Placental Program, Eapen et al. [1] reported comparable survival after related and unrelated HSCT, with different donor sources, including UCB, for infants with acute leukemia. Our study differs from their study because it reports UCBT risk factors and outcomes for children of different ages (not only infants) but who were diagnosed in the first 12 months of live.

One may argue that the long period covered in this study may limit our results, as the HSCT field is constantly evolving and treatment for leukemia has changed over time. Of note, in our study OS and LFS were significantly lower for patients transplanted before 2004. Also, TRM was higher for

patients transplanted in the same period. Age younger than 3 months at diagnosis was associated with higher risk of post-transplantation recurrence translating into a poorer LFS. These findings can be interpreted in view of the fact that it is well known that infants below the age of 3 months have a more aggressive leukemia (more MLL rearrangement, more frequent central nervous system involvement, poor response to chemotherapy) in comparison to older infants [4,19].

In our study more than half of the patients underwent UCBT in CR1 and about a third in CR2. As expected, more than 80% of ALL patients exhibited somatic MLL rearrangement.

Importantly, in our series with a median follow-up of 56 months, the incidence of chronic GVHD was low. This finding is of particular interest in infants, because extensive chronic GVHD may be associated with severe morbidity later in life. Contrarily, the incidence of acute GVHD was high (40%; 62 grade II and 38 grades III to IV), but only 9 patients died due to GVHD. This elevated incidence is consistent with a report by Sanders et al. [29] on allogeneic HSCT in which 24 of 39 patients at risk developed grades II to IV acute GVHD.

TRM was lower for patients with AML than for those with ALL. This finding may be explained by the shorter treatment length before transplantation for AML patients as compared with those with ALL. Our results for infants with AML are in accordance with results published by others [3,24].

Better strategies for obtaining disease control before transplantation and to reduce transplant-related toxicity and death are desirable to improve results in this peculiar population of children. The use of immunotherapy [30] (either bispecific antibodies or chimeric antigen receptor T cells in B cell precursor ALL or anti-CD33 monoclonal antibody in AML) in association with conventional chemotherapy may represent a possibility for improvement [30-32]. Also, longer follow-up is needed to assess the long-term side effects after HSCT in these young patients. The Italian cooperative group reported 14% of growth deficiency, 3% of cardiac dysfunction, 9% of hypothyroidism, and 6% of impaired cognitive function in patients treated for infant AML with a protocol including HSCT as postremission treatment in a large proportion of patients [3].

Taken together, our data demonstrate that UCBT is a suitable and effective option for patients with infant acute leukemia in CR. Contrary to what is usually observed in older children, in our study patients with AML had better prognosis than those with ALL. Poor-prognosis patients should be transplanted as soon as they achieve CR1, as suggested by the OS rate greater than 80% reported for AML patients transplanted in CR1 in our study. Patients experiencing a first relapse should be transplanted as soon as a CR2 is achieved and negative or low levels of minimal residual disease before the allograft is pursued to reduce the risk of post-transplantation recurrence.

ACKNOWLEDGMENTS

The authors thank the Centre Scientifique de Monaco and the following participating centers: **Australia:** Sydney—The Children's Hospital at Westmead; Randwick Sydney Children's Hospital; **Belgium:** Brussels—Cliniques Universitaires St. Luc; Gent—University Hospital Gent; Liege—University of Liege; **Brazil:** Jau Sao Paulo—Hospital Amaral Carvalho; **Canada:** Montréal—Ste-Justine Hospital; **Czech Republic:** Prague—Institute of Hematology and Blood Transfusion; Prague—University Hospital Motol; **Denmark:** Copenhagen—Rigshospitalet; **Finland:** Helsinki University of Helsinki—Hospital for Children & Adolescents; **France:** CHU Bordeaux Groupe Hospitalier Pellegrin-Enfants Onco-Hématologie Pédiatrique; Besançon—Saint Jacques; Clermont-Ferrand—Hotel Dieu CHU/Jean Perrin; Lille-Unité d'Hématologie Pédiatrique Hôpital Jeane de Flandre; Lyon—Debrousse; Marseille—La Timone; Nancy—Brabois; Nantes—Hotel Dieu; Nice—Archet; Paris—Robert Debré; Rennes—Pontchaillou; **Germany:** Dresden—Universitaetsklinikum Dresden; Düsseldorf Universitaetsklinikum; Halle—Martin-Luther-Univ. Halle-Wittenberg; **Hungary:** Budapest—St. László Hospital; Miskolc—Postgraduate Medical School; **Ireland:** Dublin—Our Lady s Hospital for Sick Children; **Israel:** Haifa—Rambam Medical Center; Petach-Tikva—Schneider Children's Medical Center of Israel; Tel-Hashomer—Edmond & Lily Safra Children's Hospital, Chaim Sheba Med Center; **Italy:** Bologna—Azienda Ospedaliero-Universitaria di Bologna; Firenze—Azienda Ospedaliero Universitaria Meyer—Ospedale di Careggi; Genova—Institute G. Gaslini; Monza Ospedale San Gerardo Clinica Pediatrica dell Universita di Milano Bicocca; Padova—Clinica di Oncoematologia Pediatrica; Palermo—Ospedale dei Bambini; Pavia—Fondazione IRCCS Policlinico San Matteo; Pisa—Azienda Ospedaliera Universitaria Pisa; Roma—Ospedale S. Camillo; Roma—Rome Transplant Network; Roma Univ.La Sapienza; Torino—Ospedale Infantile Regina Margherita Onco-Ematologia Pediatrica; Napoli-Hospital Pausilipon'; **Jordan:** Amman—King Hussein Cancer Centre; **Norway:** Rikshospitalet Department of Medicine—Pediatrics; **Poland:** Wroclaw—Wroclaw Medical University; **Portugal:** Lisboa—Inst. Portugues Oncologia; Porto—Inst. Portugues de Oncologia do Porto; **Russia:** Moscow—Russian's Children's Hospital; **Saudi Arabia:** Riyadh—King Faisal Specialist Hospital & Research Centre; **Spain:** Barcelona—Hospital Santa Creu i Sant Pau; Barcelona—Hospital Vall d'Hebron; Córdoba—Hosp. Reina Sofia; Madrid—Hospital Universitario La Paz; Madrid—Niño Jesus Children's Hospital; Málaga—Hospital Carlos Haya; Salamanca—Hospital Clínico; Santander—Hospital U. Marqués de Valdecilla; Sevilla—Hospital Universitario Virgen del Rocío; Valencia Hospital Infantil La Fe; **Sweden:** Stockholm—Karolinska University Hospital Children's Hospital; Uppsala—University Hospital; **Switzerland:** Zürich—University Children's Hospital; **The Netherlands:** Utrecht—University Medical Centre; **Turkey:** Antalya—Akdeniz University Medical School; Izmir—Ege University; **United Kingdom:** Glasgow—Royal Hospital for Sick Children; Birmingham—Birmingham Children's Hospital; Bristol—Royal Hospital for Children; Leeds—Mid Yorkshire Hospitals NHS Trust; Liverpool—Alder Hey Children's NHS Foundation Trust; London—Great Ormond Street Hospital; Newcastle General Hospital Dept. of Paediatric Immunology; Hospital Sheffield Children's Hospital.

Financial disclosure: The authors have nothing to disclose.

Conflict of interest statement: There are no conflicts of interest to report.

Authorship statement: A.R., V.R., E.G., and J.H.D. designed the study. A.R. and F.V. prepared, analyzed data, and wrote the manuscript. F.L. and J.H.D. participated in the writing of the manuscript. G.M., C.D.H., M.A., F.L., M.Z., A.V., K.Y., T.O.B., S.G., J.C., C.P., and P.B. transplanted patients and provided data for the study. All authors edited and approved the manuscript.

REFERENCES

1. Eapen M, Rubinstein P, Zhang MJ, et al. Comparable long-term survival after unrelated and HLA-matched sibling donor hematopoietic stem cell transplantations for acute leukemia in children younger than 18 months. *J Clin Oncol*. 2006;24:145-151.

2. Biondi A, Cimino G, Pieters R, Pui CH. Biological and therapeutic aspects of infant leukemia. *Blood*. 2000;96:24-33.
3. Masetti R, Rondelli R, Fagioli F, et al. Infants with acute myeloid leukemia treated according to the Associazione Italiana di Ematologia e Oncologia Pediatrica 2002/01 protocol have an outcome comparable to that of older children. *Haematologica*. 2014;99:e127-e129.
4. Pieters R, Schrappe M, De Lorenzo P, et al. A treatment protocol for infants younger than 1 year with acute lymphoblastic leukaemia (Interfant-99): an observational study and a multicentre randomised trial. *Lancet*. 2007;370:240-250.
5. Hunger SP, Mullighan CG. Acute lymphoblastic leukemia in children. *N Engl J Med*. 2015;373:1541-1552.
6. Tomizawa D, Koh K, Sato T, et al. Outcome of risk-based therapy for infant acute lymphoblastic leukemia with or without an MLL gene rearrangement, with emphasis on late effects: a final report of two consecutive studies, MLL96 and MLL98, of the Japan Infant Leukemia Study Group. *Leukemia*. 2007;21:2258-2263.
7. Sison EA, Brown P. Does hematopoietic stem cell transplantation benefit infants with acute leukemia? *Hematology Am Soc Hematol Educ Program*. 2013;2013:601-604.
8. Rocha V, Labopin M, Sanz G, et al. Transplants of umbilical-cord blood or bone marrow from unrelated donors in adults with acute leukemia. *N Engl J Med*. 2004;351:2276-2285.
9. Eapen M, Rubinstein P, Zhang MJ, et al. Outcomes of transplantation of unrelated donor umbilical cord blood and bone marrow in children with acute leukaemia: a comparison study. *Lancet*. 2007;369:1947-1954.
10. Przepiorka D, Chan KW, Champlin RE, et al. Prevention of graft-versus-host disease with anti-CD5 ricin A chain immunotoxin after CD3-depleted HLA-nonidentical marrow transplantation in pediatric leukemia patients. *Bone Marrow Transplant*. 1995;16:737-741.
11. Flowers ME, Kansu E, Sullivan KM. Pathophysiology and treatment of graft-versus-host disease. *Hematol Oncol Clin North Am*. 1999;13:1091-1112, viii-ix.
12. Grimwade D, Hills RK, Moorman AV, et al. Refinement of cytogenetic classification in acute myeloid leukemia: determination of prognostic significance of rare recurring chromosomal abnormalities among 5876 younger adult patients treated in the United Kingdom Medical Research Council trials. *Blood*. 2010;116:354-365.
13. Michel G, Cunha R, Ruggeri A, et al. Unrelated cord blood transplantation for childhood acute myelogenous leukemia: the influence of cytogenetic risk group stratification. *Leukemia*. 2016;30:1180-1183.
14. Foresto SA, Youlden DR, Baade PD, Hallahan AR, Aitken JF, Moore AS. The outcomes and treatment burden of childhood acute myeloid leukaemia in Australia, 1997-2008: a report from the Australian Paediatric Cancer Registry. *Pediatr Blood Cancer*. 2015;62:1664-1666.
15. Pession A, Rondelli R, Basso G, et al. Treatment and long-term results in children with acute myeloid leukaemia treated according to the AIEOP AML protocols. *Leukemia*. 2005;19:2043-2053.
16. Koh K, Tomizawa D, Moriya Saito A, et al. Early use of allogeneic hematopoietic stem cell transplantation for infants with MLL gene-rearrangement-positive acute lymphoblastic leukemia. *Leukemia*. 2015;29:290-296.
17. Dreyer ZE, Dinndorf PA, Camitta B, et al. Analysis of the role of hematopoietic stem-cell transplantation in infants with acute lymphoblastic leukemia in first remission and MLL gene rearrangements: a report from the Children's Oncology Group. *J Clin Oncol*. 2011;29:214-222.
18. Kotecha RS, Gottardo NG, Kees UR, Cole CH. The evolution of clinical trials for infant acute lymphoblastic leukemia. *Blood Cancer J*. 2014;4:e200.
19. Driessen EM, de Lorenzo P, Campbell M, et al. Outcome of relapsed infant acute lymphoblastic leukemia treated on the Interfant-99 protocol. *Leukemia*. 2016;30:1184-1187.
20. Webb DK, Wheatley K, Harrison G, Stevens RF, Hann IM. Outcome for children with relapsed acute myeloid leukaemia following initial therapy in the Medical Research Council (MRC) AML 10 trial. MRC Childhood Leukaemia Working Party. *Leukemia*. 1999;13:25-31.
21. Aladjidi N, Auvrignon A, Leblanc T, et al. Outcome in children with relapsed acute myeloid leukemia after initial treatment with the French Leucemie Aique Myeloide Enfant (LAME) 89/91 protocol of the French Society of Pediatric Hematology and Immunology. *J Clin Oncol*. 2003;21:4377-4385.
22. Fagioli F, Zecca M, Locatelli F, et al. Allogeneic stem cell transplantation for children with acute myeloid leukemia in second complete remission. *J Pediatr Hematol Oncol*. 2008;30:575-583.
23. Tsukimoto I, Tawa A, Horibe K, et al. Risk-stratified therapy and the intensive use of cytarabine improves the outcome in childhood acute myeloid leukemia: the AML99 trial from the Japanese Childhood AML Cooperative Study Group. *J Clin Oncol*. 2009;27:4007-4013.
24. Isoyama K, Oda M, Kato K, et al. Long-term outcome of cord blood transplantation from unrelated donors as an initial transplantation procedure for children with AML in Japan. *Bone Marrow Transplant*. 2010;45:69-77.
25. Sander A, Zimmermann M, Dworzak M, et al. Consequent and intensified relapse therapy improved survival in pediatric AML: results of relapse treatment in 379 patients of three consecutive AML-BFM trials. *Leukemia*. 2010;24:1422-1428.
26. Imamura T, Iwamoto S, Kanai R, et al. Outcome in 146 patients with paediatric acute myeloid leukaemia treated according to the AML99 protocol in the period 2003-06 from the Japan Association of Childhood Leukaemia Study. *Br J Haematol*. 2012;159:204-210.
27. Nakayama H, Tabuchi K, Tawa A, et al. Outcome of children with relapsed acute myeloid leukemia following initial therapy under the AML99 protocol. *Int J Hematol*. 2014;100:171-179.
28. Rocha V, Wagner JE Jr, Sobocinski KA, et al. Graft-versus-host disease in children who have received a cord-blood or bone marrow transplant from an HLA-identical sibling. Eurocord and International Bone Marrow Transplant Registry Working Committee on Alternative Donor and Stem Cell Sources. *N Engl J Med*. 2000;342:1846-1854.
29. Sanders JE, Im HJ, Hoffmeister PA, et al. Allogeneic hematopoietic cell transplantation for infants with acute lymphoblastic leukemia. *Blood*. 2005;105:3749-3756.
30. Pollard JA, Loken M, Gerbing RB, et al. CD33 expression and its association with gemtuzumab ozogamicin response: results from the randomized phase III children's oncology group trial AAML0531. *J Clin Oncol*. 2016;34:747-755.
31. Schlegel P, Lang P, Zugmaier G, et al. Pediatric posttransplant relapsed/refractory B-precursor acute lymphoblastic leukemia shows durable remission by therapy with the T-cell engaging bispecific antibody blinatumomab. *Haematologica*. 2014;99:1212-1219.
32. Maude SL, Frey N, Shaw PA, et al. Chimeric antigen receptor T cells for sustained remissions in leukemia. *N Engl J Med*. 2014;371:1507-1517.