Allogeneic Stem Cell Transplantation for Children With Acute Myeloid Leukemia in Second Complete Remission

Franca Fagioli, MD, Marco Zecca, MD,† Franco Locatelli, MD,† Edoardo Lanino, MD,‡ Cornelio Uderzo, MD,§ Paolo Di Bartolomeo, MD,‖ Massimo Berger, MD,* Claudio Favre, MD,¶ Roberto Rondelli, MD,# Andrea Pession, MD,# and Chiara Messina, MD ** on behalf of the AIEOP-HSCT group*

Summary: Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is an effective therapy for patients with relapsed acute myeloid leukemia. In this retrospective, multicenter study, we analyzed the outcome of 63 children (median age, 7 y; range, 0.2 to 17) who received unmanipulated allo-HSCT in second complete remission. Either a matched family donor or an unrelated donor was used in 29 (46%) and 34 (54%) patients, respectively. The stem cell source was bone marrow in 53 children (84%), peripheral blood in 7 (11%), and cord blood in 3 patients (5%). Preparative regimen included total body irradiation in 25 patients (40%). The 5-year estimates of overall survival and leukemia-free survival were 53% [95% confidence interval (CI) 39-66] and 49% (95% CI 35-63), respectively, whereas the cumulative incidence of relapse and transplant-related mortality (TRM) were 26% (95% CI 16-41) and 25% (95% CI 15-40), respectively. In multivariate analysis, the use of a matched family donor predicted a better probability of LFS [relative risk (RR) 2.29, $P = 0.05$]. Both chronic graft-versus-host disease occurrence and age at diagnosis greater than 11 years were associated with an increased TRM (RR 8.08, $P = 0.04$ and RR 4.38, $P = 0.05$, respectively). These results indicate that allo-HSCT is a procedure able to rescue a significant proportion of children with acute myeloid leukemia in second complete remission, especially if an human leukocyte antigen-compatible relative is employed as donor. Both leukemia recurrence and TRM contributed to treatment failure. Optimization of donor selection and of strategies for both prophylaxis and treatment of graft-versus-host disease may improve the results of unrelated donor allo-HSCT.

Key Words: pediatric patients, allogeneic hematopoietic stem cell transplantation, AML in second CR, leukemia relapse

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Aggressive induction and postremission chemotherapy have remarkably improved the prognosis of children and adolescents with acute myeloid leukemia (AML).[1–5] However, even with modern treatment strategies, 30% to 40% of children who achieve a first complete remission (CR1) experience leukemia recurrence.[4,5] Relapse may occur even in patients given either autologous or allogeneic hematopoietic stem cell transplantation (auto-HSCT and allo-HSCT) as consolidation therapy; the majority of relapses are observed within the first year after therapy discontinuation.[6–8] With the use of salvage chemotherapy, a second CR (CR2) can be obtained in approximately 50% of children with AML who experience disease recurrence.[9–11] However, the duration of CR2 is typically shorter than that of CR1, and the probability of a second relapse may exceed 75%.[12,13]

Both allo-HSCT and auto-HSCT have been considered for consolidating patients with relapsed AML who achieve CR2.[14–19] However, as leukemia relapse remains the major cause of treatment failure after auto-HSCT,[14,15] allo-HSCT may be preferable for these patients. The possible advantages of allo-HSCT refer to the replacement of patient marrow potentially harboring leukemia cells with that of a healthy donor and, especially, to the beneficial effect displayed by donor lymphocytes on the eradication of malignant cells escaping the conditioning regimen and known as graft versus-leukemia effect.[16–21]

Although recently published studies have evaluated the efficacy of HSCT to treat relapsed AML,[14–19] the impact of different variables, such as duration of first remission, karyotype abnormalities or first-line therapy, on the outcome of children undergoing HSCT in CR2 is

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From the *Oncoematologia Pediatrica, Ospedale Infantile Regina Margherita, Torino; †Oncoematologia Pediatrica, IRCCS Policlinico San Matteo, University of Pavia; ‡Dipartimento di Ematologia e Oncologia Pediatrica, IRCSS Giannina Gaslini, Genova; §Clinica Pediatrica, Università di Milano-Bicocca, Ospedale Nuovo San Gerardo, Monza; ‖Centro Trapianti Midollo Osseo, Ospedale di Pescara; ¶Centro Trapianti di Midollo Osseo, Clinica Pediatrica I, Pisa; #Clinica Pediatrica, Ospedale Sant'Orsola Malpighi, Università di Bologna; and **Clinica Pediatrica, Università di Padova, Italy.

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Reprints: Franca Fagioli, MD, Oncoematologia Pediatrica, Ospedale Infantile Regina Margherita, Piazza Polonia 94, 10126 Torino, Italy (e-mail: franca.fagioli@unito.it).

still not completely defined, mainly because of the limited number of patients analyzed and the relatively short follow-up.

We carried out a retrospective, multicenter study aimed at analyzing the impact of leukemia-related, patient-related, and transplant-related factors on leukemia-free survival (LFS), relapse incidence (RI), and transplant-related mortality (TRM) in children with AML who underwent allo-HSCT in CR2.

PATIENTS AND METHODS

Patients

Between January 1989 and December 2004, 63 patients younger than 18 years, with AML in CR2, received allo-HSCT in 1 of 12 Italian pediatric transplant centers (see Appendix). Data concerning patient and disease characteristics, as well as transplantation outcome, were collected by a standardized questionnaire of AIEOP (Associazione Italiana di Ematologia e Oncologia Pediatrica) Registry for each patient enrolled into this study.

This study includes only patients with de novo AML. Patients with Down syndrome were excluded from the study. The clinical characteristics of the patients are reported in Table 1.

Briefly, the median age at transplantation was 7 years (range, 0.2 to 17), and the median white blood cells count at diagnosis was 15.1×10^9/L (range, 0.2 to 222).

A successful cytogenetic analysis of the malignant cells was available in 48 of 63 cases (76%). Abnormal karyotypes were classified in the favorable-risk group if *t*(8;21), *t*(15;17) or inv[16] was detected. In patients lacking these favorable anomalies, the presence of monosomy 7, 11q23 abnormalities other than *t*(9;11), monosomy 5, del(5q), abnormal 3q, *t*(6;9) or a complex karyotype defined the poor-risk group. Patients with other abnormalities, and those having a normal karyotype or lacking cytogenetic analysis, were classified in the intermediate-risk group. According to this classification, 21 patients (33%) had a good-risk karyotype, 35 patients (56%) had intermediate-risk karyotype, and 7 patients (11%) had poor-risk karyotype.

The stratification of children according to the protocol they received as first-line therapy is detailed in Table 1. Thirty-eight of the 63 patients (60%) who relapsed had received chemotherapy alone, 16 (25%) auto-HSCT performed in CR1, and 9 (14%) patients had been transplanted from an human leukocyte antigen (HLA)-compatible sibling [matched family donor (MFD)] in CR1. The median interval between diagnosis and first relapse was 15 months (range, 2 to 88). All relapses occurred in the bone marrow (BM). After relapse, patients received different reinduction treatments, according to each single institution policy. Parents or patient guardians signed the appropriate consent form for the transplant procedure.

TABLE 1. Patients Characteristics

No. patients	63 (100%)	
Sex: male/female	36/27 (57%/43%)	
Age at diagnosis (y)	7 (0.2-17)	
FAB classification		
M0	1 (2%)	
M1	19 (30%)	
M2	8 (13%)	
M3/M3v	16 (25%)	
M4	5 (8%)	
M5	11 (17%)	
M6	2 (3%)	
M7	1 (2%)	
White blood cells at diagnosis ($\times 10^9$/L)	15.1 (0.2-222)	
Cytogenetic abnormalities		
t(15;17)	16 (25%)	
inv(16)	1 (2%)	
t(8;21)	4 (6%)	
t(9;11)	2 (3%)	
Other abnormalities	5 (8%)	
Normal karyotype	20 (32%)	
Unknown/failed	15 (24%)	
First-line chemotherapy protocol		
AIEOP LAM 2002	3 (5%)	
AIEOP LAM 92	22 (35%)	
AIEOP LAM 87	6 (10%)	
AIEOP LAM 82	4 (6%)	
AIEOP-GIMEMA 0493 AIDA	13 (21%)	
BFM LAM 93	4 (6%)	
BFM LAM 87	4 (6%)	
Others	7 (11%)	
Diagnosis—first CR (d)	33 (20-144)	
Diagnosis—first relapse (mo)	15 (2-88)	
Site of first relapse		
BM	63 (100%)	
First relapse after		
Chemotherapy alone	38 (60%)	
Chemotherapy + HSCT	25 (40%)	
Type of first HSCT		
Autologus	16 (25%)	
MFD	9 (14%)	
Donor		
MFD	29 (46%)	
MUD	34 (54%)	
Conditioning regimen		
TBI	25 (40%)	
Chemotherapy alone	38 (60%)	
Stem cell source		
BM	53 (84%)	
PB	7 (11%)	
Cord blood	3 (5%)	
GvHD prophylaxis	MFD	MUD
Cs-A or MTX	29	0
Cs-A + steroids	0	3
Cs-A + steroids + ALG	0	3
Cs-A + MTX	0	6
Cs-A + MTX + ALG	0	22

Data are expressed as median and range or as percentage, as appropriate.
ALG indicates antilymphocyte globulin; MTX, methotrexate.

Allo-HSCT

Among the 63 patients, 4 (6%) were given the allograft in CR2 before 1990, 25 (40%) in the time period between 1990 and 1999 and the remaining 34 patients (54%) after 2000.

The conditioning regimen included total body irradiation (TBI 12 Gy over 6 fractions in 3 d) in 25 patients (40%), while 38 patients (60%) received chemotherapy alone (Busulfan-based) as preparation to the allograft. Together with TBI or Busulfan the majority of patients received Cyclophosphamide 120 to 200 mg/Kg. A MFD was employed in 29 patients (46%), whereas the remaining 34 patients (54%) were transplanted from a matched unrelated donor (MUD). Nine patients who underwent allo-HSCT in CR1 from MFD received from the same donor the stem cell rescue. In all donor-recipient pairs, histocompatibility was determined by serology for HLA-A and HLA-B antigens and by DNA typing for HLA-DRB1 locus. In all patients transplanted from a MUD, HLA-DRB1 typing was performed by high-resolution allelic technique. After 1998, all class I and class II (HLA-A, HLA-B, HLA-C, DRβ1, DQα1, and DQβ1) genetic alleles were typed by high resolution 4-digit DNA technique.

The stem cell source was BM for 53 patients (84%), peripheral blood after mobilization with granulocyte-colony stimulating factor for 7 patients (11%), and cord blood stem cells for 3 patients (5%).

In patients given allo-HSCT from a MFD, graft-versus-host disease (GvHD) prophylaxis consisted of either cyclosporine-A (Cs-A, 1-2 mg/kg starting on day -1) or methotrexate (MTX, 15 mg/m^2 on day $+1$, 10 mg/m^2 on day $+3$, $+6$, and $+11$ and thereafter every week until day 100), as a single drug for all patients. All patients transplanted from a MUD with either BM or peripheral blood progenitors were given Cs-A and short-course MTX as GvHD prophylaxis, whereas cord blood transplantation recipients received Cs-A along with steroids (ie, 6-methyl-prednisolone 1.5 to 2.0 mg/kg/d). Rabbit antithymocyte globulin (3.75 mg/kg/d from day -4 to day -2) was employed in most patients transplanted from an UD (25 children, 75% of the whole number of patients given the allograft from a nonconsanguineous donor).

Supportive therapy, as well as prophylaxis and treatment of infections, was substantially homogenous among centers. Briefly, transplants were performed in rooms with positive pressure filtered flow. Antifungals: oral Nystatin was used until the advent of Fluconazole. Secondary prophylaxis with Amphotericin or Voriconazole was given if the patient was transplanted with a known history of fungal infection. Antiviruses: all patients received Acyclovir, cytomegalovirus (CMV) antigenemia was monitored biweekly and, if CMV positive cells were found a specific antiviral preemptive therapy was started. First-line CMV preemptive therapy consisted of Gancyclovir 10 mg/kg. No routinarely antibacterial therapy was administrated. As *Pneumocystis carinii* prophylaxis: children received oral cotrimoxazole, starting from the day of engraftment, until 3 months after discontinuation of immune suppressive therapy. Broad spectrum antibiotics were given if a patient became febrile. Granulocyte-colony stimulating factor was not routinely used.

Definitions

Patients were considered in CR if they had normal neutrophil and platelet counts, less than 5% blast cells in a BM smear, in the absence of circulating blasts and if there was no extramedullary leukemia cell infiltration.

Neutrophil and platelet engraftment were defined as the first of 3 consecutive days with a neutrophil count greater than 0.5×10^9/L and an unsupported platelet count greater than 50×10^9/L, respectively.

Acute and chronic GvHD were classified according to established criteria.[22] Children with evidence of donor engraftment who survived more than 14 days and more than 90 days from transplantation were evaluated for the occurrence of acute GvHD and chronic GvHD, respectively.

Statistical Analysis

Patient-related, leukemia-related, and transplant-related variables were expressed as medians and ranges, or as percentages as appropriate. The following patient or transplant characteristics were analyzed for their potential impact on outcome: sex; age at diagnosis and at transplantation; white blood cells count at diagnosis; French-American-British (FAB) subtype; cytogenetics abnormalities; interval diagnosis-CR1, interval diagnosis-first relapse; type of first-line treatment (chemotherapy alone vs. chemotherapy plus auto-HSCT vs. chemotherapy plus allo-HSCT); type of donor (MFD vs. MUD); stem cell source; use of TBI as part of the conditioning regimen; year of transplantation (before 2000 vs. after 2000); type of GvHD prophylaxis [monotherapy (Cs-A or MTX alone) vs. combination therapy (Cs-A + MTX or steroids) vs. serotherapy (antithymocyte globulin or monoclonal antibodies associated with Cs-A + MTX or steroids)]; occurrence and severity of acute and chronic GvHD.[23]

For statistical analysis, continuous variables were categorized as follows: each variable was first divided into 4 categories at the 25th, 50th, and 75th percentiles. If the relative event rates (ratio of the observed number of events to the expected number of events in the category, assuming no variation across categories) in 2 or more adjacent categories (and the median time to events) did not differ, those categories were grouped. If no clear pattern was observed for the primary outcomes, the median was taken as the cut point.

The patients were censored at the time of relapse, death, or last follow-up. The probability of survival and LFS were estimated by the Kaplan-Meier method,[24] and expressed as percentages and as a 95% confidence interval (95% CI). Acute and chronic GvHD occurrence, as well as TRM and RI were expressed as cumulative incidence curves, to adjust the analysis for competing risks. The significance of differences between curves was estimated by the log-rank test. All variables with a P value < 0.2 in the univariate analysis were included in a multivariate analysis performed using the Cox proportional hazard regression model.[25–28]

P values < 0.05 were considered statistically significant, *P* values from 0.05 to 0.2 were considered not statistically significant but are shown in the tables in detail, whereas *P* values ≥ 0.2 were reported as NS.

Statistical analysis was performed using the SAS System (SAS Inc, Cary, NC), and the NCSS computer program (Hintze J, 2001, NCSS and PASS, Number Cruncher Statistical System, Kaysville, UT).

RESULTS

The median follow-up was 3 years (range, 0.6 to 14) for surviving patients, and 0.6 years for deceased patients (range, 0.04 to 5.5).

Engraftment and GvHD

The median time to neutrophil engraftment was 15 days (range, 10 to 35), whereas the median time to platelet recovery was 33 days (range, 15 to 135). No differences between MFD and MUD allo-HSCT recipients were found (data not shown). The cumulative incidence of grade II to IV acute GvHD was 49% (95% CI, 38-63), with onset at a median of 17 days from transplantation. No statistically significant differences between MFD or MUD allo-HSCT recipients were found (data not shown). The cumulative incidence of chronic GvHD was 29% (95% CI, 19-43) and involved 17 out of the 59 evaluable patients (ie, surviving in remission for at least 90 d after transplantation), with onset at a median of 4 months after HSCT (range, 3 to 16). Again, no statistically significant differences between MFD or MUD recipients were found (data not shown).

Chronic GvHD was diagnosed as limited in 13 cases and extensive in 4 cases. In 13 of the 17 cases, it followed a previous grade II to IV acute GVHD.

Overall Survival

The 5-year survival probability for the entire cohort was 53% (95% CI 36-66) (Fig. 1).

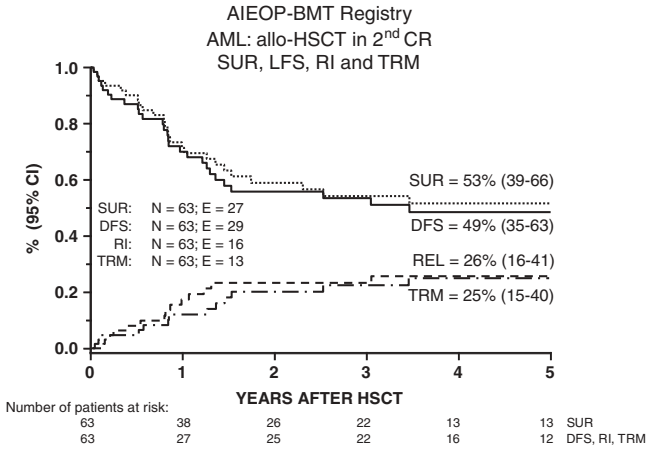

FIGURE 1. Kaplan-Meier analysis of overall survival and LFS, TRM and RI were expressed as cumulative incidence curves, to adjust the analysis for competing risks.

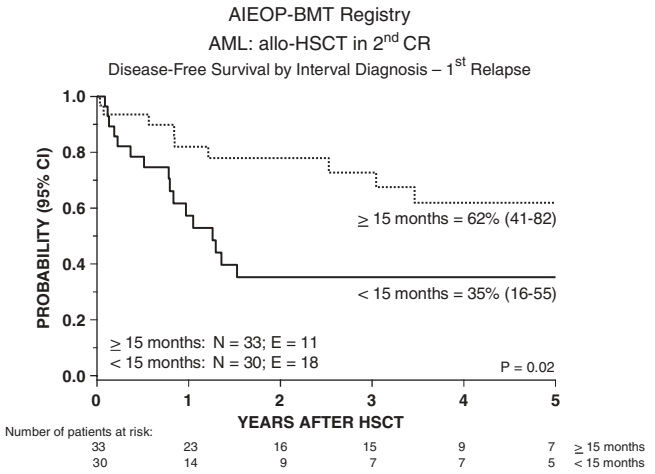

FIGURE 2. Disease-free survival according to duration of CR1.

LFS

The Kaplan-Meier estimate of LFS at 5 years was 49% (95% CI 35-63, Fig. 1). In univariate analysis, the interval between diagnosis and first relapse was the only factor predicting better LFS (35% vs. 62% for patients with duration of CR1 less than or equal/more than 15 mo, $P = 0.02$, see also Fig. 2). Patients given the allograft from a MFD had a better outcome compared with those transplanted from a MUD, although the difference is not statistically significant (62% for MFD allo-HSCT recipient as compared with 37% patients transplanted from a MUD, $P = 0.12$, Fig. 3). A better outcome in terms of LFS was also for patients with AML M3 (66%, 95% CI 38-94) compared with M0-M1-M2 (51%, 95% CI), M4-M5 (46%, 95% CI 19-72), and M6-M7 (0%) ($P = 0.04$) (Table 2). When we compared patients having allo-HSCT from MFD and GvHD prophylaxis with only Cs-A or MTX the LFS was 69% (95% CI 50-88) and 25% (95% CI 0-67) ($P = 0.12$). When all factors having a *P* value < 0.2 were evaluated in a multivariate analysis, only the use of a MFD was significantly associated with better

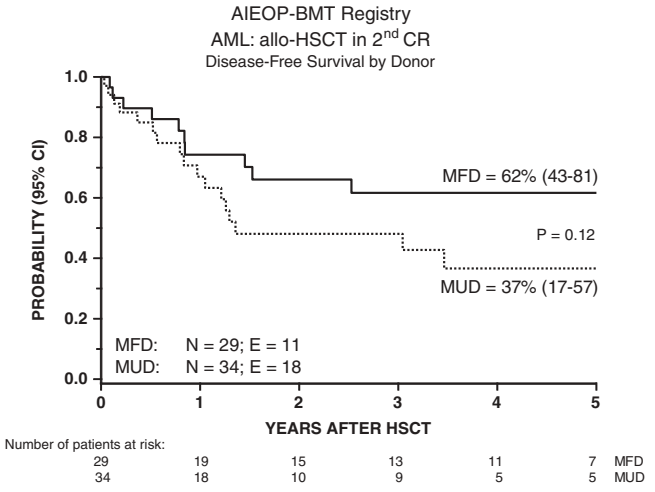

FIGURE 3. Disease-free survival according to donor type.

TABLE 2. Univariate Analysis for LFS

	No. Patients	Events	Kaplan-Meier Probability (%)	(95% CI)	*P*
Overall LFS	63	29	49	(35-63)	—
LFS by sex					
Male	36	14	53	(34-71)	NS
Female	27	15	45	(24-66)	
LFS by age at diagnosis					
< 3 y	16	7	48	(20-76)	0.10
3-7 y	15	5	56	(24-88)	
7-11 y	17	6	62	(36-89)	
≥ 11 y*	15	11	31	(7-55)	
LFS by age at HSCT					
< 5 y	13	6	49	(20-79)	NS
5-9 y	17	6	55	(27-83)	
9-14 y	17	8	43	(15-72)	
≥ 14 y	16	9	49	(24-74)	
LFS by white blood cells at diagnosis					
$< 5 \times 10^9$/L	17	7	44	(14-75)	NS
5-20 $\times 10^9$/L	16	9	36	(8-65)	
20-80 $\times 10^9$/L	15	7	43	(9-77)	
$\geq 80 \times 10^9$/L	15	6	46	(13-78)	
LFS by FAB classification					
M0, M1, M2	28	13	51	(31-71)	0.04
M3	16	4	66	(38-94)	
M4, M5	16	8	46	(19-72)	
M6, M7	3	3	0		
LFS by interval diagnosis first CR (d)					
< 25 d	13	9	30	(2-58)	NS.
25-33 d	18	7	64	(39-90)	
33-50 d	17	6	56	(26-86)	
≥ 50 d	15	7	33	(3-62)	
LFS by interval diagnosis—first relapse (mo)					
< 10 mo	14	8	41	(12-70)	0.11
10-15 mo	16	11	32	(6-57)	
15-30 mo	18	7	46	(12-80)	
≥ 30 mo	15	3	73	(47-99)	
< 15 mo	30	19	35	(16-55)	0.02
≥ 15 mo	33	10	62	(41-82)	
LFS by first-line treatment					
Chemotherapy	38	14	55	(37-73)	NS
Chemotherapy + ABMT	16	10	37	(10-62)	
Allogeneic HSCT from MFD	9	5	52	(17-86)	
LFS by donor type					
MFD	29	11	62	(43-81)	0.12
MUD	34	18	37	(17-57)	
LFS by stem cell source					
BM	53	25	47	(33-62)	NS
PB	3	1	0	—	
Cord blood	7	3	71	(38-100)	
LFS by use of TBI					
No	38	16	56	(38-74)	NS
Yes	25	13	42	(21-63)	
LFS by year of transplantation					
< 2000	29	18	45	(27-63)	NS
≥ 2000	34	11	55	(34-76)	
LFS by GvHD prophylaxis†					
Monotherapy	29	11	62	(43-81)	NS
Combination therapy	9	5	23	(0-61)	
Serotherapy	25	13	41	(18-64)	
Donor = MFD					
Monotherapy	29	11	62	(43-81)	—
Combination therapy	0	0	—	—	
Serotherapy	0	0	—	—	
Donor = MUD					
Monotherapy	0	0	—	—	
Combination therapy	9	5	23	(0-61)	NS
Serotherapy	25	13	41	(18-64)	
LFS by acute GvHD					
Absent	21	11	48	(24-73)	NS
Grade I	11	4	59	(27-90)	

TABLE 2. (*continued*)

	No. Patients	Events	Kaplan-Meier Probability (%)	(95% CI)	*P*
Grade II	24	10	53	(31-74)	
Grade III	4	3	25	(0-63)	
Grade IV	3	1	67	(13-100)	
LFS by chronic GvHD‡					
Absent	42	15	62	(45-79)	NS
Limited	13	7	42	(14-70)	
Extensive	4	3	25	(0-67)	

*Age at diagnosis $\geq$ 11 y vs. age at diagnosis $<$ 11 y: $P = 0.04$.
†GvHD prophylaxis: monotherapy = cyclosporine-A or MTX alone; combination therapy = cyclosporine-A + MTX or steroids; cerotherapy = inclusion of antilymphocyte globulin or monoclonal antibodies in the GvHD prophylaxis schedule.
‡Chronic GvHD analysis was performed only for the 59 children surviving in remission for at least 90 d after HSCT.

LFS [relative risk (RR) 2.29, 95% CI 1.01-5.74, $P = 0.05$] (Table 3).

Relapse

Sixteen of the 63 patients experienced disease recurrence. The overall cumulative RI was 26% (95% CI 16-41) (Fig 1). Univariate analysis showed that both FAB subtype (M0-M1-M2, vs. M3, vs. M4-M5 vs. M6-M7 $P = 0.02$) and interval between diagnosis and first relapse ($<$ 15 mo vs. $\geq$ 15 mo, $P = 0.009$) identify patients at higher risk of disease recurrence. Neither acute nor chronic GvHD influenced the RI. When we compared the RI in patients with allo-HSCT with MFD and GvHD prophylaxis with only Cs-A or MTX the RI was 17% (95% CI 7-42) and 0% (P = NS). In multivariate analysis, only a longer interval between diagnosis and first relapse was associated with a trend toward a lower risk of relapse after the allograft performed in CR2 (RR 0.3, 95% CI 0.07-1.22, $P = 0.09$) (Table 3).

TRM

Thirteen patients died while still in remission for transplant-related causes. The overall cumulative incidence of TRM was 25% (95% CI, 15-40) (Fig. 1). Only the development of chronic GvHD was significantly associated with a higher risk for TRM in univariate

TABLE 3. Results of the Multivariate Analysis for LFS, Relapse, and TRM

	RR	(95% CI)	*P*
LFS			
Age at diagnosis			
3-7 y vs. $<$ 3 y	0.41	(0.11-1.56)	0.19
7-11 y vs. $<$ 3 y	0.36	(0.10-1.32)	0.12
$\geq$ 11 y vs. $<$ 3 y	1.56	(0.52-4.68)	NS
FAB subtype			
M3 vs. M0, M1, M2, M4, M5, M6, or M7	1.27	(0.50-3.24)	NS
Interval diagnosis—first relapse (mo)			
$\geq$ 15 mo vs. $<$ 15 mo	0.80	(0.29-2.21)	NS
Donor			
MUD vs. MFD	2.29	(1.01-5.74)	0.05
Relapse			
FAB subtype			
M3 vs. M0, M1, M2, M4, M5, M6, M7	1.95	(0.59-6.40)	NS
Interval diagnosis—first relapse (mo)			
$\geq$ 15 mo vs. $<$ 15 mo	0.30	(0.07-1.22)	0.09
Donor			
MUD vs. MFD	1.77	(0.53-5.88)	NS
TRM			
Age at diagnosis			
$\geq$ 11 y vs. $<$ 11 y	4.38	(1.01-18.98)	0.05
Type of first-line treatment			
Chemotherapy + ABMT vs. chemotherapy alone	2.26	(0.40-12.89)	NS
Chemotherapy + MFD HSCT vs. chemotherapy alone	4.51	(0.92-22.10)	0.06
Chronic GvHD*			
Limited vs. absent	4.73	(1.02-22.06)	0.05
Extensive vs. absent	8.08	(1.06-61.54)	0.04

Multivariate analysis was performed using the Cox proportional hazard regression model. All variables with a P value $<$ 0.2 in univariate analysis were included in the model.
*Chronic GvHD analysis was performed only for the 59 children surviving in remission for at least 90 d.
ABMT indicates autologous BM transplantation.

analysis (9% vs. 41% vs. 50% for patients who did not develop chronic GvHD and for those who experienced limited or extensive chronic GvHD, respectively, $P = 0.03$). Notably, TRM was 13% for patients who had received chemotherapy alone as first-line treatment, 36% for children who had received previous auto-HSCT, and 48% for those previously given allo-HSCT ($P = 0.09$). No significant associations with chronic GvHD was found between patients having received an allo-HSCT in CR1 compared with patients having only chemotherapy or auto-HSCT. When we compared the cumulative TRM incidence for patients underwent MFD transplantation and Cs-A or MTX as only GvHD prophylaxis, the former had 14% (95% CI 5-39) compared with 75% (95% CI 43-100) ($P = 0.01$). Moreover, when factors having P values < 0.2 were analyzed by the Cox proportional hazard regression model, the strongest predictor of TRM was the degree of chronic GvHD (RR 8.08, 95% CI 1.06-64.54, $P = 0.04$), while first-line therapy lost its predictive value for TRM. Patient age at transplantation (≥ 11 y) became a variable associated with a higher incidence of TRM in multivariate analysis (RR 4.38, 95% CI 1.01-18.98, $P = 0.05$) (Table 3). The reason of death varied from respiratory distress, bacterial and fungal infections, and hemorrhagic complications.

DISCUSSION

Allo-HSCT has been demonstrated to be the most effective therapy for patients with relapsed AML.[9–13] In this retrospective, multicenter study our first aim was to describe the outcome of patients with AML in CR2 given allogeneic transplantation and, secondly, to identify factors influencing the probability of LFS, TRM, and RI.

We found that allo-HSCT is able to promote the maintenance of a state of CR2 in around 50% of patients, this confirms the data previously reported by the Seattle group.[18] TRM and disease recurrence contributed equally to treatment failure, the majority of the events being observed in the first 18 months after the allograft.

Patients who were offered allo-HSCT in CR2 represent a selected subgroup of children experiencing a first relapse. Indeed, these patients, besides reaching a new remission, maintained CR2 for a time long enough to be transplanted. This consideration is supported by the observation that patients with good-risk cytogenetic characteristics represented one third of the overall population. In particular, 25% of our patients had acute promyelocytic leukemia with $t(15;17)$. The outcome of this subgroup was particularly encouraging, as only 4 of the 16 patients with AML FAB M3 died either for transplant-related causes (2 children) or for disease recurrence (2 children). These data are in agreement with the results recently reported by Testi and colleagues,[29] who documented a high probability of being rescued by an allograft for children with acute promyelocytic leukemia experiencing disease recurrence after treatment with chemotherapy and all-trans retinoic acid. By contrast, our results are significantly better than those published some years ago by Mandelli et al,[30] who reported a probability of LFS of only 22% in 33, mainly adult patients with acute promyelocytic leukemia given allo-HSCT in CR2.

The only factor found to influence the probability of LFS in multivariate analysis was the type of donor employed, children transplanted from a MFD doing significantly better than those transplanted from an unrelated volunteer. Several factors may have contributed to this difference. Mainly because of HLA polymorphism and the limits of conventional techniques for HLA-typing, historically, increased difficulties for engraftment and augmented incidence of both acute and chronic GvHD, as well as of infectious complications, have been reported in recipients of an UD allograft, this leading to results, in terms of LFS, inferior to those reported using a compatible sibling as donor.[31–33] The lack of confidence on the real HLA identity between donor and recipient has induced to increase the intensity of GvHD prophylaxis, for example, through the use of serotherapy. This, in turn, may promote an increased risk of relapse, owing to attenuation of the graft versus-leukemia effect. Indeed, although the difference is not statistically significant, the difference in the outcome of UD allo-HSCT, the majority of whom received serotherapy before transplantation, and MFD recipients was mainly due to a higher RI in the former.

The type of consolidation therapy, namely chemotherapy alone, auto-HSCT, or allo-HSCT, received during first-line treatment had little or no influence on patient outcome despite lower TRM for patients who had received chemotherapy only as a front-line therapy. This advantage did not translate into a better LFS, as patients given allo-HSCT from an HLA-identical sibling as consolidation therapy of CR1 benefited from a low risk of RI, possibly facilitated by the fact of having received low intensity GvHD prophylaxis, namely MTX alone or low-dose Cs-A for few weeks after the allograft.[34]

We found that a time interval between diagnosis and first relapse equal or longer than 15 months in univariate analysis predicted both a reduced RI and a better LFS. This variable was associated with a favorable trend toward reduced RI also in multivariate analysis.

The length of first remission has been demonstrated to be a major prognostic factor for children with relapsed AML.[14,35] Our results suggest that the predictive value of this well-identified prognosis factor on final outcome of patients with relapsed AML may be partially blunted by the effectiveness of allo-HSCT.

The karyotype of malignant cells has also been shown to be one of the most relevant predictor of treatment outcome in childhood AML.[36,37] Interestingly, our children with a poor karyotype had similar 5-year LFS when compared with other patients, while the incidence of leukemia recurrence was higher. However, also the difference in terms of RI was not statistically significant, probably because of the limited number of patients belonging to the poor-risk group.

The only 2 factors unfavorably influencing TRM in multivariate analysis were patient age at diagnosis 11 years and occurrence of chronic GvHD. The observation of a strong correlation between occurrence of chronic GvHD and TRM, with no evidence of a lower risk of relapse for patients experiencing this complication, contrasts with previously published studies, where a protective effect of chronic GvHD against relapse has been reported.[16,36,37] However, these analyses mainly referred to adults, while a study specifically focusing on children did not confirm such a protective effect in patients with AML.[38] There is no obvious explanation for the increased risk of TRM in patients older than 11 years at diagnosis. Despite the low number in each group, another intriguing observation obtained from our analysis regards the MFD group and their GvHD prophylaxis. In comparison with a previous study,[39] we had no proof that the MTX-based prophylaxis correlates to a lower RI (0% vs. 17%, P = NS), but, by contrast, the cumulative TRM was significantly higher (75% vs. 14%, $P = 0.01$).

In conclusion, our study supports the use of allogeneic HSCT for children with AML in CR2, especially if an HLA-compatible relative is available and occurrence of chronic GvHD is successfully prevented. As both leukemia recurrence and TRM contributed to treatment failure, optimization of strategies for both prophylaxis and treatment of GVHD, as well as donor selection, may well improve the results of UD allo-HSCT.

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APPENDIX

The following Centers reported patients to the AIEOP registry:

Oncoematologia Pediatrica, Università di Pavia, Fondazione IRCCS, Policlinico San Matteo, Pavia. Prof F. Locatelli: 20 patients.

Dipartimento di Ematologia e Oncologia, IRCCS G Gaslini, Genova. Dr G. Dini: 7 patients.

Clinica Pediatrica Ospedale S. Gerardo, Università di Milano, Monza. Dr C. Uderzo: 7 patients.

Dipartimento di Pediatria, Università di Padova. Prof C. Messina: 6 patients.

Centro Trapianti Midollo Osseo, Ospedale di Pescara. Dr P. Di Bartolomeo: 5 patients.

Oncoematologia Pediatrica, Ospedale Infantile Regina Margherita. Dr F. Fagioli: 5 patients.

Centro Trapianti di Midollo Osseo, Clinica Pediatrica I, Pisa. Dr C. Favre: 4 patients.

Clinica Pediatrica, Università di Bologna. Prof A. Pession: 3 patients.

Dipartimento di Pediatria, Università di Brescia. Dr F. Porta: 2 patients.

Dipartimento di Biotecnologie Cellulari ed Ematologia, Università di Roma La Sapienza. Prof R. Foà: 2 patients.

Dipartimento di Pediatria, Università di Trieste. Dr M. Andolina 2 patients.

REFERENCES

1. Lowenberg B, Downing JR, Burnett A. Acute myeloid leukemia. *N Engl J Med*. 1999;341:1051–1062.
2. Mayer RJ, Davis RB, Schiffer CA, et al. Intensive postremission chemotherapy in adults with acute myeloid leukaemia. *N Engl J Med*. 1994;331:896–903.
3. Bishop JF, Matthews JP, Young GA, et al. A randomized study of high-dose cytarabine in induction in acute myeloid leukemia. *Blood*. 1996;87:1710–1717.
4. Woods WG, Kobrinsky N, Buckley JD, et al. Timed sequential induction therapy improves postremission outcome in acute myeloid leukemia: a report from the Children's Cancer Group. *Blood*. 1996;87:4979–4989.
5. 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.
6. Locatelli F, Labopin M, Ortega J, et al. Factors influencing outcome and incidence of long-term complications in children who underwent autologous stem cell transplantation for acute myeloid leukemia in first complete remission. *Blood*. 2003;101:16–19.
7. Bonetti F, Zecca M, Pession A, et al. Total body irradiation and melphalan is a safe and effective conditioning regimen for autologous bone marrow transplantation in children with acute myeloid leukemia in first remission. *J Clin Oncol*. 1999;17:3729–3735.
8. Dini G, Boni L, Abla OF, et al. Allogeneic bone marrow transplantation in children with acute myelogenous leukemia in first remission. *Bone Marrow Transplant*. 1994;13:771–776.
9. Keating MJ, Kantarjian H, Smith TL, et al. Response to salvage therapy and survival after relapse in acute myelogenous leukemia. *J Clin Oncol*. 1989;7:1071–1080.
10. Davis CL, Rohatiner AZ, Lim J, et al. The management of recurrent acute myelogenous leukaemia at a single centre over a fifteen-year period. *Hematology*. 1993;83:404–411.
11. Vignetti M, Orsini E, Petti MC, et al. Probability of long-term disease-free survival for acute myeloid leukemia patients after first relapse: a single-centre experience. *Ann Oncol*. 1996;7:933–938.
12. Gale RP, Horowitz MM, Rees JK, et al. Chemotherapy versus transplants for acute myelogenous leukemia in second remission. *Leukemia*. 1996;10:13–19.
13. Fleischhack G, Hasan C, Graf N, et al. IDA-FLAG (idarubicin, fludarabine, cytarabine, G-CSF), an effective remission-induction therapy for poor-prognosis AML of childhood prior to allogeneic or autologous bone marrow transplantation: experiences of a phase II trial. *Br J Haematol*. 1998;102:647–655.
14. Webb DK, Wheatley K, Harrison G, et al. Outcome for children with relapsed acute myeloid leukaemia following initial therapy in the Medical Research Council (MRC) AML 10 trial. *Leukemia*. 1999;13:25–31.
15. Godder K, Eapen M, Laver JH, et al. Autologous hematopoietic stem-cell transplantation for children with acute myeloid leukemia in first or second complete remission: a prognostic factor analysis. *J Clin Oncol*. 2004;22:3798–3804.
16. Aladjidi N, Auvrignon A, Leblanc T, et al. French Society of Pediatric Hematology and Immunology. 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.
17. Michel G, Rocha V, Chevret S, et al. Unrelated cord blood transplantation for childhood acute myeloid leukemia: a Eurocord group analysis. *Blood*. 2003;102:4290–4297.

18. Nemecek ER, Gooley TA, Woolfrey AE, et al. Outcome of allogeneic bone marrow transplantation for children with advanced acute myeloid leukaemia. *Bone Marrow Transplant*. 2004;34: 799–806.
19. Eapen M, Horowitz MM, Klein JP, et al. Higher mortality after allogeneic peripheral blood transplantation compared with bone marrow in children and adolescents: the histocompatibility and alternate stem cell source committee of the International Bone Marrow Transplant Registry. *J Clin Oncol*. 2004;22:4872–4880.
20. Horowitz MM, Gale RP, Sondel PM, et al. Graft versus-leukemia reactions after bone marrow transplantation. *Blood*. 1990;75: 555–562.
21. Kolb HJ, Schimidt C, Barrett J, et al. Graft-versus-leukemia reactions in allogeneic chimeras. *Blood*. 2004;103:767–776.
22. Przepiorka D, Weisdorf D, Martin P, et al. 1994 Consensus Conference on Acute GVHD Grading. *Bone Marrow Transplant*. 1995;15:825–828.
23. Byar DP. Identification of prognostic factors. In: Buyse ME, Staquet MJ, Sylvester RJ, eds. *Cancer Clinical Trials: Methods and Practice*. Oxford, UK: Oxford Medical Publications; 1988: 423–443.
24. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. *J Am Stat Assoc*. 1958;53:457–481.
25. Cox DR. Regression models and life tables. *J R Stat Soc B*. 1972; 34:187–202.
26. Klein JP, Rizzo JD, Zhang M-J, et al. Statistical methods for analysis and presentation of the results of bone marrow transplants. Part I: unadjusted analysis. *Bone Marrow Transplant*. 2001;28: 909–915.
27. Gooley TA, Leisenring W, Crowley J, et al. Estimation of failure probabilities in the presence of competing risks: new representations of old estimators. *Stat Med*. 1999;18:695–706.
28. Klein JP, Rizzo JD, Zhang M-J, et al. Statistical methods for the analysis and presentation of the results of bone marrow transplants. Part 2: regression modeling. *Bone Marrow Transplant*. 2001;28: 1001–1011.
29. Testi AM, Biondi A, Lo Coco F, et al. GIMEMA-AIEOP AIDA protocol for the treatment of newly diagnosed acute promyelocytic leukemia (APL) in children. *Blood*. 2005;106:447–453.
30. Mandelli F, Labopin M, Granena A, et al. European survey of bone marrow transplantation in acute promyelocytic leukaemia. Working party on acute leukemia of the European cooperative group for bone marrow transplantation (EBMT). *Bone Marrow Transplant*. 1994;14:293–298.
31. Balduzzi A, Gooley T, Anasetti C, et al. Unrelated donor marrow transplantation in children. *Blood*. 1995;86:3247–3256.
32. Davies SM, Wagner JE, Shu XO, et al. Unrelated donor bone marrow transplantation for children with acute leukemia. *J Clin Oncol*. 1997;15:557–565.
33. 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.
34. Locatelli F, Zecca M, Rondelli R, et al. Graft versus host disease prophylaxis with low-dose cyclosporine-A reduces the risk of relapse in children with acute leukemia given HLA-identical sibling bone marrow transplantation: results of a randomized trial. *Blood*. 2000;95:1572–1579.
35. Stahnke K, Boos J, Bender-Götze C, et al. Duration of first remission predicts remission rates and long-term survival in children with relapsed acute myelogenous leukemia. *Leukemia*. 1998;12: 1534–1538.
36. Martinez-Climent JA, Lane NJ, Rubin CM. Clinical and prognostic significance of chromosomal abnormalities in childhood acute myeloid leukemia de novo. *Leukemia*. 1995;9:95–101.
37. Wheatley K, Burnett AK, Goldstone AH, et al. on behalf of the United Kingdom Medical Research Concil Adult and Children's Leukemia Working Parties. A simple, robust, validated and highly predictive index for the determination of risk-directed therapy in acute myeloid leukemia derived from the MRC AML 10 trial. *Br J Haematol*. 1999;107:69–79.
38. Zecca M, Prete A, Rondelli R, et al. Chronic graft versus-host disease in children: incidence, risk factors, and impact on outcome. *Blood*. 2002;100:1192–1200.
39. Neudorf S, Sanders J, Kobrinsky N, et al. Allogeneic bone marrow transplantation for children with acute myelocytic leukemia in first remission demonstrates a role for graft versus leukemia in the maintenance of disease-free survival. *Blood*. 2004;103:3655–3661.

