CLINICAL RESEARCH

Allogeneic Hematopoietic Stem Cell Transplantation for Philadelphia-Positive Acute Lymphoblastic Leukemia in Children and Adolescents: A Retrospective Multicenter Study of the Italian Association of Pediatric Hematology and Oncology (AIEOP)

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Philadelphia-positive acute lymphoblastic leukemia (Ph+ ALL) still represents a major challenge. We report the experience of the Italian Association of Pediatric Hematology and Oncology (AIEOP) with allogeneic hematopoietic stem cell transplantation (HSCT) in children with Ph+ ALL from 1990 to 2008. Sixty-nine patients received HSCT from either a related (37, 54%) or an unrelated (32, 46%) donor. Twenty-five patients (36%) underwent transplantation before 2000 and 44 (64%) after 2000. Twenty-three patients (33%) received Imatinib mesylate treatment before HSCT and seven (10%) after HSCT. After a median follow-up of 56 months, the overall survival (OS) probability was 51% (95% confidence interval [CI], 38-63), the leukemia-free survival (LFS) was 47% (95% CI, 34-59), transplantation-related mortality (TRM) was 17% (95% CI, 10-30), and relapse incidence (RI) was 36% (95% CI, 26-50). Transplantation in first complete remission, female gender, and lower WBC count at diagnosis were associated with a better LFS in both univariate and multivariate analyses. Patients with p210 transcript had a trend for a worse prognosis compared with those who had the p190 transcript. Our series confirms the role of HSCT in the eradication of Ph+ ALL. Early HSCT is recommended once morphologic remission is obtained.

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INTRODUCTION

In patients with acute lymphoblastic leukemia (ALL), the Philadelphia chromosome (Ph+) or t(9;22) products (ie, p210 and p190), are detected in approximately 5% of children and 30% of adults [1-3]. The disease is heterogeneous in terms of clinical features, such as age at diagnosis, leukocyte count, and initial steroid response. Despite a high response rate to intensive induction chemotherapy, relapse

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frequently occurs, this resulting in the past into a 5-year overall survival (OS) probability often <20% [4,5]. Slow early response to conventional therapy has been reported to predict poor prognosis [6,7].

In the earlier Italian studies conducted in 1970, all patients received a relatively low-intensity chemotherapy and cranial radiotherapy. The first trial having stratified patients on risk-tailored chemotherapy was the study '82; however, no data are available on Ph-positive leukemias in this cohort. It includes 902 patients from 1982 to 1987. The next study was the Study '87, enrolling 632 patients, but also in this study, the Italian Association of Pediatric Hematology and Oncology (AIEOP)-Registry have no data on event-free survival (EFS) and OS for Ph-positive leukemia. The enrolling time for this trial was from 1987 to 1991. In the study 88 were enrolled 396 patients only in few AIEOP centers. This was the first Berlin-Frankfurt-Munster–based trial, and only seven patients were diagnosed as having Ph-positive ALL. The next AIEOP trial was conducted from 1991 to 1995: 1,192 patients were treated, and 20 had Ph-positive ALL. The 5-year EFS reported for these patients was 35%, whereas the 5-year OS was 49.5%. In the next AIEOP trial (AIEOP LLA 95), we treated 1,743 patients from 1995 to 2000. The 5-year EFS was 28.3%, and the OS was 41.8% for patients with Ph-positive ALL. From 2003, the AIEOP centers enrolled Ph-positive patients in the EsPhALL study, in which a randomized arm received imatinib. Most of the patients received stem cell transplantation in first complete remission (CR1). From 2004 to 2009, 178 patients were treated; in 2009, the randomization for good-risk patients was closed to external evidence of superiority of imatinib. The disease-free survival (DFS) rate was 73% for patients who responded well to prednisone versus 62% who responded poorly [8,9].

To date, one of the more widely applied curative therapies for patients with Ph+ ALL is allogeneic hematopoietic stem cell transplantation (HSCT), better results in terms of outcome having been obtained in patients who underwent transplantation early on during the course of the disease [10-14]. In 2000, Aricò et al. [15] reported 326 children and young adults who were treated for Ph+ ALL. HSCT offered advantages in terms of survival when a matched family donor (MFD) was available, and, more importantly, the benefits of HSCT increased over time, thus suggesting that transplantation played a more effective role than continuation of chemotherapy in preventing late relapse. In 2005, the British Consensus study confirmed the benefit of HSCT and showed a 60% 3-year DFS for children undergoing transplantation compared with 36% for children who had not undergone transplantation [16].

In 2001, imatinib mesylate, a competitive inhibitor of the *bcr-abl* tyrosine kinase resulting from the fusion gene of Ph+ chronic myeloid leukemia (CML), was approved by the US Food and Drug Administration as first-line treatment for patients with CML, and it was also used to treat patients with Ph+ ALL. Later, imatinib mesylate was added to standard chemotherapy and allogeneic HSCT with promising results [17,18]. Although the utility of imatinib for the treatment of Ph+ ALL in children is less well established, a recent Children's Oncology Group (COG) study reported promising results using imatinib together with intensive chemotherapy [19].

The high risk of relapse in patients with positive minimal residual disease (MRD) after HSCT makes the administration of a *bcr-abl*-directed tyrosine kinase inhibitor an attractive therapeutic option to reestablish molecular negativity and prevent relapse. A recent study showed that patients who received imatinib for a 6-month period after related HSCT had a better 1-year EFS compared with an untreated historic control group [19]. However, in a recent study, Kang et al. [20] showed that MRD is not the only predictor of relapse or resistance after first-line therapy. Imatinib may inhibit the *bcr-abl* tyrosine kinase, but other genes may also be involved in relapse, which can occur despite treatment with tyrosine kinase inhibitors.

In order to evaluate the impact of allogeneic HSCT in this very high-risk pediatric patient group, we analyzed the outcomes of 69 consecutive patients under the age of 20 who received myeloablative allogeneic HSCT for Ph+ ALL and were reported to the AIEOP-HSCT Registry between 1990 and 2008.

PATIENTS AND METHODS

Enrolled in the study were 69 patients, younger than 20 years, affected by de novo Ph+ ALL who underwent transplantation between 1990 and 2008. The diagnosis of ALL was established according to conventional criteria. The Philadelphia chromosome t(9;22)(q34;q11.2) or the *bcr-abl* fusion gene was detected by conventional cytogenetic analysis, fluorescent in situ hybridization, or by reverse-transcription polymerase chain reaction.

At diagnosis, median age was 7 years (range, 1-19), whereas median WBC count was 76×10^9/L (range, 30-580). Forty-nine patients were male (71%) and 20 were female (29%). The immunophenotype was B cell precursor ALL for 67 patients (97%) and T cell precursor ALL for two patients (3%). First-line chemotherapy was based on the AIEOP LLA 91 protocol for 13 patients (19%), AIEOP LLA 95 for 16 (23%), and AIEOP LLA 2000 for 27 (39%), whereas 13 patients received other treatment protocols (19%) (Table 1).

Karyotypes were classified according to the International System for Human Cytogenetic Nomenclature [21]. All patients in the study had successful cytogenetic analysis at diagnosis.

Table 1. Patient and Transplantation Characteristics of the 69 Children and Adolescents Enrolled in the Study

Number of patients	69	(100%)
Gender:		
M	49	(71%)
F	20	(29%)
Diagnosis:		
Age at diagnosis (years, median, and range):	7	(1-19)
WBC at diagnosis ($\times 10^9$/L):	76	(30-580)
Immunophenotype		
B lineage	67	(97%)
T lineage	2	(3%)
Molecular analysis		
p190	44	(64%)
p210	13	(19%)
p190/p210	5	(7%)
Unknown	7	(10%)
First-line chemotherapy		
AIEOP ALL 91	13	(19%)
AIEOP ALL 95	16	(23%)
AIEOP ALL 2000	27	(39%)
OTHER PROTOCOLS	13	(19%)
Age at transplantation (years, median, and range)	9	(1-20)
Disease status at HSCT		
First remission	43	(62%)
Second remission	17	(25%)
More advanced disease	9	(13%)
Cytogenetic remission		
Yes	60	(87%)
No	6	(9%)
Unknown	3	(4%)
Donor		
Matched family donor	37	(54%)
Matched unrelated donor	32	(46%)
Stem cell source		
Bone marrow	62	(90%)
Peripheral blood	2	(3%)
Cord blood	5	(7%)
Conditioning regimen		
TBI + chemotherapy	62	(90%)
Chemotherapy alone	7	(10%)
GVHD prophylaxis	MFD	MUD
CyA	33 (89%)	0 (0%)
Cs-A + MTX	4 (11%)	5 (16%)
Cs-A + MTX + ATG	0 (0%)	23 (72%)
Cs-A + MTX + steroids	0 (0%)	1 (3%)
Cs-A + steroids	0 (0%)	2 (6%)
Cs-A + steroids + ATG	0 (0%)	1 (3%)

M indicates male; F, female; TBI, total-body irradiation; MFD, matched family donor; MUD, matched unrelated donor; Cs-A, cyclosporin A; MTX, methotrexate; ATG, antithymocyte globulins.

PCR analyses showed the presence of the p190 *bcr-abl* fusion protein variant in 44 patients (64%), the p210 form in 13 (19%), whereas five patients had leukemia blasts expressing both the p190 and p210 transcripts (7%). The median age at diagnosis was similar in p190 and p210 patients: 6.1 years (range, 3.1-19) for p190 patients and 5.9 years (range, 3-15.9) for p210 patients ($P > .05$).

The presence of *bcr-abl* RNA copies was measured qualitatively and/or quantitatively in some patients.

Allogeneic HSCT

Median patient age at HSCT was 9 years (range, 1-20); it was 7 (range, 2-20) and 4 years (range, 1-6) for patients who did or did not receive total-body irradiation (TBI), respectively ($P = .005$). At the time of HSCT, 43 patients were in CR1 (62%), 17 in CR2 (25%), and nine were in a more advanced disease phase (13%) (Table 1). All patients received a myeloablative preparative regimen, mainly based on TBI (90%), most frequently combined with thiotepa and cyclophosphamide (33%) (Table 1).

Graft-versus-host disease (GVHD) prophylaxis mainly consisted of cyclosporin A alone in the 37 patients who underwent transplantation from an MFD or of a combination of cyclosporin A, short-term methotrexate ± antithymocyte globulin (72%) in children given the allograft from a matched unrelated donor [MUD] see also Table 1 for details.

Bone marrow was the stem cell source for 62 patients (90%), whereas two patients received peripheral blood stem cells and five patients cord blood. For patients receiving bone marrow cells, the median number of mononuclear cells infused was 3.7×10^8/kg (1.5-6.5).

Supportive therapy, as well as prophylaxis and treatment of infections, were substantially homogeneous among centers. Broad-spectrum antibiotics were given if the patient became febrile. Secondary antifungal prophylaxis was given to patients with a known history of fungal infections. All patients received acyclovir for antiviral prophylaxis. All patients received cotrimoxazole as *Pneumocystis jirovecii* prophylaxis from the day of engraftment until 3 months after the end of immunosuppressive therapy. Granulocyte-colony stimulating factor was not routinely used.

All parents or guardians signed the appropriate informed consent form previously approved by the local ethic committee or the institutional review board.

Imatinib

Twenty-three patients received imatinib mesylate before HSCT (33%). When imatinib mesylate was used after HSCT (seven cases), it was started at a median of 57 days after transplantation (range, 28-69) and continued for a median of 17 months (range, 3-33). The imatinib mesylate dosage was 300 mg/m^2/day.

Definitions

The patients were considered in morphologic CR if they had normal neutrophil and platelet counts, <5% blast cells in a bone marrow smear, and no extramedullary disease. All patients had a lumbar puncture before HSCT to document cerebrospinal fluid remission. Cytogenetic remission was defined as absence of the Philadelphia chromosome in all metaphases obtained from a bone marrow specimen.

Neutrophil and platelet engraftment were defined as the first of 3 consecutive days with a neutrophil count $>0.5 \times 10^9$/L and an unsupported platelet count $>50 \times 10^9$/L, respectively. Acute GVHD (aGVHD) and chronic GVHD (cGVHD) were diagnosed and

graded according to established criteria [22-23]. Children with evidence of donor engraftment who survived more than 14 days and 90 days from transplantation were evaluated for the occurrence of aGVHD and cGVHD, respectively. Relapse was defined on the basis of morphologic evidence of leukemia in bone marrow, or other extramedullary organs. Transplantation-related mortality (TRM) was defined as all causes of nonleukemia death occurring after HSCT. OS was defined as the interval between HSCT and death or the last follow-up, and leukemia-free survival (LFS) was defined as the interval between HSCT and relapse or TRM or the last follow-up, whichever occurred first.

Statistical Analysis

Patient-, disease-, and transplantation-related variables were expressed as median and ranges, or as percentage, as appropriate. The following patient- or transplantation-related variables were analyzed for their potential impact on outcome: gender, WBC at diagnosis, cytogenetics, molecular analysis, disease phase, pre-HSCT imatinib mesylate, post-HSCT imatinib mesylate, donor type, TBI administration, aGVHD and cGVHD occurrence, year of transplantation (before or after 2000), and age. For the statistical analysis, continuous variables, except age, were categorized as follows: each variable was first divided into four categories at the 25th, 50th, and 75th percentiles. If the relative event rates (the ratio of the observed number of events to the expected number of events in the category) in two 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 cutoff point. The patients were censored at the time of relapse, death, or last follow-up. OS and LFS were calculated according to the Kaplan-Meier method. Neutrophil and platelet recovery, aGVHD and cGVHD occurrence, as well as TRM and relapse incident (RI) were expressed as cumulative incidence curves, in order to adjust the analysis for competing risks. Death from any cause and graft rejection were both competing risks to estimate the cumulative incidence of aGVHD and cGVHD. Death in remission was treated as a competing event to calculate the cumulative incidence of relapse. Relapse was considered to be the competing event for calculating TRM. The significance of differences between LFS curves was estimated by the log-rank test (Mantel-Cox), whereas Gray's test was used to assess, in univariate analyses, differences between RI and TRM [24]. All variables having a P value $<.20$ in univariate analyses were included in a multivariate analysis on LFS performed using the Cox proportional regression model [25], whereas the proportional subdistribution hazard regression model was used to perform multivariate analyses of RI and TRM. To examine other possible interactions, variables such as age, type of donor, disease status, cytogenetic remission, and period of transplantation have been included in the multivariate models for OS and LFS even if not statistically significant in univariate analyses. In the multivariate analyses for RI and TRM, aGVHD has been considered as a time-dependent covariate. P values $<.05$ were considered to be statistically significant. Statistical analysis was performed using the SAS System (SAS inc, Cary, NC), Stata software (StataCorp LP, College Station, TX), the NCSS computer program (Hintze, 2001, NCSS PASS, Number Crunched Statistical System, Kaysville, UT), and R 2.5.0 software package.

RESULTS

Engraftment and GVHD

Neutrophil and platelet engraftment occurred after a median time of 18 days (range, 8-37) and 24 days (range, 11-173) after HSCT, respectively. Neutrophil engraftment for MFD and MUD transplantation recipients was obtained after 14 (range, 8-30) and 19 (range, 12-37) days, respectively ($P = .018$). Platelet engraftment for MFD and MUD transplantation recipients was reached after a median time of 23 (range, 11-81) and 24 (range, 16-173) days, respectively (P = NS).

Eighteen patients did not develop aGVHD (26%), 26 patients had grade I aGVHD (38%), 18 patients had grade II aGVHD (26%), six patients presented with grade III aGVHD (9%), and one patient developed grade IV aGVHD (1%). The cumulative incidence of grade II-IV aGVHD was 36% (95% confidence interval [CI], 26-50); it was 30% (95% CI, 18-49), and 44% (95% CI, 30-65) for MFD and MUD transplantation recipients, respectively (P = NS).

Forty-five patients did not develop cGVHD (74%), 10 patients presented with limited cGVHD (10%), and six patients had extensive cGVHD (16%). The cumulative incidence of cGVHD was 27% (95% CI, 18-41); it was 18% (95%, CI 9-36) and 38% (95% CI, 24-62) for MFD and MUD transplantation recipients, respectively ($P = .074$).

OS

The median follow-up time was 56 months (range, 4-218). The 5-year OS probability was 51% (95% CI, 38-63) (Figure 1). In univariate analyses, factors influencing the probability of OS were donor type and patient gender. OS for MFD and MUD recipients was 62% (95% CI, 45-78) and 37% (95% CI, 18-56), respectively ($P = .04$). The OS of females was 79% (95% CI, 60-97) compared with 38% (95% CI, 22-53) for males ($P = .01$).

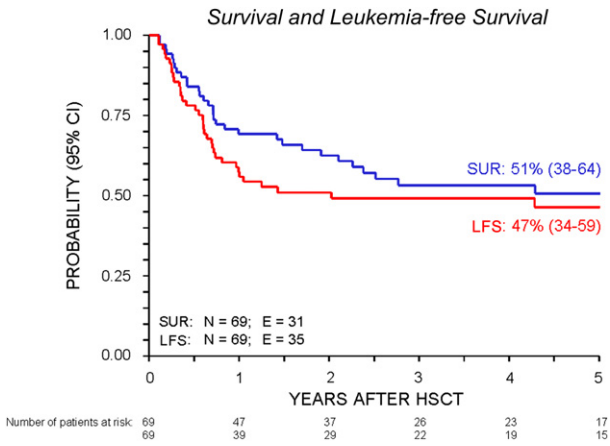

Figure 1. OS and LFS for children given a first allogeneic stem cell transplantation.

In multivariate analyses, an early disease phase at transplantation (relative risk [RR] = 0.32; 95% CI, 0.14-0.74; P = .007) and matched family donors (RR = 2.69, 95% CI, 1.13-6.42; P = .026) were confirmed as independent factors associated with a better OS (Table 2).

LFS

The 5-year probability of LFS for all patients analyzed in the study was 47% (95% CI, 34-59) (Figure 1). LFS was better for females compared with males (74% [95% CI, 55-94] versus 34% [95% CI, 19-49], P = .006]) and for children who underwent HSCT in CR1 versus those who underwent transplantation in CR2 or in a more advanced disease phase (56% [95% CI, 40-73], 34% [95% CI 11-57], and 22% [95% CI 0-49], respectively [P = .008], Figure 2). Other variables, such as conditioning regimen with or without TBI, donor type, use of imatinib before or after HSCT, cytogenetic status and aGVHD or cGVHD incidence, had no impact on LFS (Supplementary Table 1).

In multivariate analyses, female gender (RR = 3.59, [95% IC, 1.03-12.59], P = .046), early disease phase at transplantation (RR = 0.27 [95% CI, 0.12-0.61], P = .002), and WBC count at diagnosis below 80 × 10^9/L (RR = 2.38 [95% CI, 1.11-5.12], P = .026) were confirmed as independent factors associated with a better LFS (Table 2).

RI

The median interval from HSCT to relapse was 7 months (range, 2-24). Considering the disease status at HSCT, the median time to relapse was 10 months (range, 3-17), 8 months (range, 2-24), 3 months (range, 3-4), and 173 days (range, 128-238) for patients who underwent transplantation in CR1, CR2, other CR and not in remission, respectively (P = .05).

The overall RI for all patients who underwent transplantation was 36% (95% CI, 26-50). In univariate analyses, RI was higher in males (45% [95% CI, 32-61]) than in females (16% [95% CI, 6-44]; P = .01) and in patients who underwent transplantation in more advanced disease phases (27% [95% CI, 16-45] in CR1 patients, 48%, [95% CI, 29-79] in CR2 patients, and 56%, [95% CI, 31-100] for patients with more advanced disease; P = .05) (Figure 3).

When we analyzed the specific bcr-abl transcripts, namely, p190, p210, or the copresence of both, the RI

Table 2. Results of Multivariate Analysis for Overall Survival (OS), Leukemia-Free Survival (LFS), Relapse Incidence (RI), and Transplantation-Related Mortality (TRM)

	RR	(95% CI)	*P* value
Overall Survival			
Gender			
Male versus female	3.19	(0.88-11.54)	.077
Disease phase			
CR1 versus more advanced disease	0.32	(0.14-0.74)	.007
Donor type			
MUD versus MFD	2.69	(1.13-6.42)	.026
WBC count at diagnosis			
≥80 × 10^9/L versus <80 × 10^9/L	2.00	(0.90-4.45)	.090
Year of transplantation			
≥2000 versus <2000	0.44	(0.16-1.19)	.106
Cytogenetic remission			
yes versus no	3.35	(0.68-16.51)	.138
Age (continuous)	0.99	(0.90-1.09)	.851
LFS			
Gender			
Male versus female	3.59	(1.03-12.59)	.046
Disease phase			
CR1 versus more advanced disease	0.27	(0.12-0.61)	.002
Donor type			
MUD versus MFD	1.96	(0.86-4.45)	.108
WBC count at diagnosis			
≥80 × 10^9/L versus <80 × 10^9/L	2.38	(1.11-5.12)	.026
Year of transplantation			
≥2000 versus <2000	0.41	(0.16-1.07)	.069
Cytogenetic remission			
yes versus no	4.76	(0.98-23.18)	.054
Age (continuous)	1.03	(0.94-1.135)	.469
Relapse incidence			
Gender			
Male versus female	3.68	(1.28-10.56)	.016
Molecular features			
p210 versus p190	0.65	(0.18-2.31)	.506
p190/p210 versus p190	4.72	(1.44-15.52)	.011
Disease phase			
CR1 versus more advanced disease	0.31	(0.13-0.75)	.009
Acute GVHD			
Grade II-IV versus 0-I	0.26	(0.08-0.90)	.033
Year of transplantation			
≥2000 versus <2000	0.79	(0.28-2.20)	.653
Transplantation-related mortality			
Gender			
Male versus female	2.65	(0.45-15.52)	.281
Molecular features			
p210 versus p190	2.84	(0.62-13.11)	.180
p190/p210 versus p190	0	0	<.0001
Disease phase			
CR1 versus more advanced disease	1.19	(0.29-4.78)	.809
Acute GVHD			
Grade II-IV versus 0-I	6.77	(1.40-32.63)	.017
Year of transplantation			
≥2000 versus <2000	0.88	(0.21-3.73)	.859

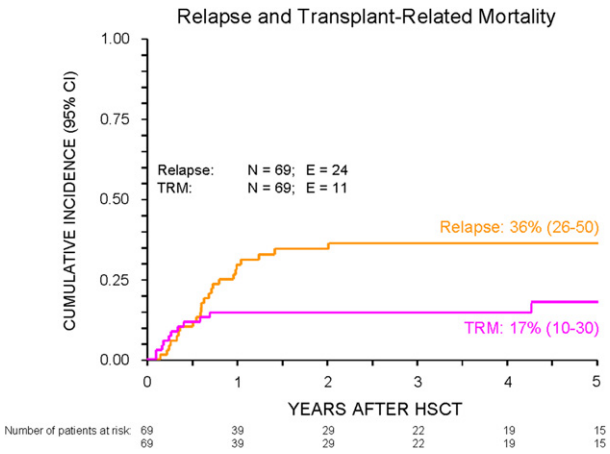

Figure 2. LFS according to remission status.

was 33% (95% CI, 22-51), 31% (95% CI, 14-70), and 80% (95% CI, 52-100), respectively ($P = .057$).

A trend for a lower probability of relapse was found among patients with a WBC count at diagnosis below 80×10^9/L (22%, 95% CI 11-42) compared with patients with a higher WBC count (45%, 95% CI 30-67; $P = .07$) and for patients who developed grade II-IV aGVHD (20%, 95% CI 9-44) compared with those who experienced grade 0-I aGVHD (45%, 95% CI 32-64; $P = .06$).

Other variables, such as cytogenetic abnormalities other than t(9;22), the use of imatinib either before or after HSCT, the donor type, the use of TBI in the preparative regimen, and the occurrence of cGVHD did not predict relapse in the univariate analysis (Supplementary Table 2).

In multivariate analysis, the copresence of both bcr-abl p190 and p210 transcripts (RR = 4.72; $P = .01$) and male gender (RR = 3.68; $P = .016$) were independent risk factors for relapse, whereas early disease phase at transplantation (RR = 0.31; $P = .009$) and the occurrence of grade II-IV aGVHD (RR = 0.26; $P = .033$) were independent favorable prognostic variables for RI (Table 2).

TRM

The overall cumulative incidence of TRM was 17% (95% CI, 10-30). In univariate analyses, the only factor significantly associated with an increased risk of TRM was aGVHD: patients with grade II-IV aGVHD had a TRM incidence of 32% (95% CI, 18-57) versus 9% (95% CI, 3-29) observed in patients with grade 0-I aGVHD, respectively ($P = .0071$). These data were confirmed by comparing patients who experienced grade III-IV aGVHD with those who experienced grade 0-II aGVHD, TRM being 43% (95% CI, 18-100) for the former and 15% (95% CI, 7-29) for the latter ($P = .049$). None of the other variables analyzed were associated with an increased incidence of TRM (Supplementary Table 3). The proportional subdistribution hazard regression model confirmed the impact of grade II-IV aGVHD on TRM (RR = 6.77; $P = .017$).

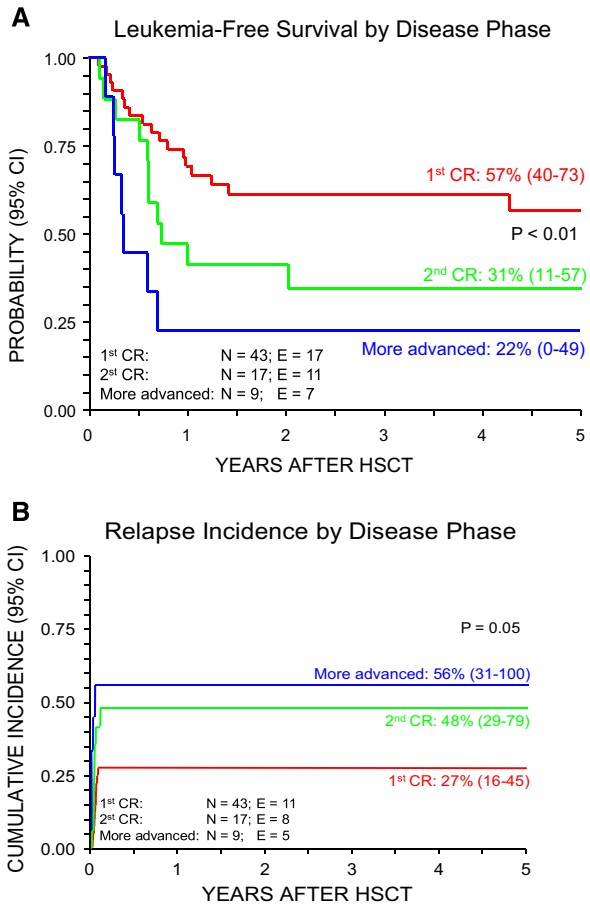

Figure 3. Leukemia-Free Survival by Disease Phase and cumulative incidence of relapse.

Imatinib

Twenty-three patients were treated with imatinib before HSCT and seven after HSCT. No significant differences in terms of LFS, RI, or TRM were observed among patients who did or did not receive imatinib, respectively.

Cytogenetics and Molecular Data

No differences were observed in terms of LFS, RI, or TRM by comparing patients with isolated Philadelphia-chromosome positive with those with additional abnormalities. However, as mentioned previously, there was a higher RI for patients carrying both p190 and p210 transcripts. In detail, four of the five patients with the p190 and p210 transcripts relapsed (80%, 95% CI 52-100), compared with patients only with p190 (33%, CI 95% 22-51) and p210 (31%, 95% CI 14-70; $P = .057$). This finding was also confirmed in the multivariate analysis for RI (RR = 4.72; $P = .011$).

Causes of Death

The main cause of treatment failure was disease progression, which was recorded in 21 patients (68%). Other causes of death were fungal infection (3%), bacteria pneumonia (6%), interstitial pneumonia (3%), cGVHD (3%), multiorgan failure (3%), and finally, cerebral cryptococcosis (3%).

DISCUSSION

Although the majority of patients with Ph+ ALL typically respond to first-line therapy, the duration of remission is short in a vast proportion of patients. In the past, the median EFS after combination chemotherapy was reported to be only approximately 8 months, with a 5-year OS of <20% [4,5]. Because of this dismal outcome, allogeneic HSCT was considered the optimal treatment after induction chemotherapy for patients with a suitable MFD or best-available MUD. Our report summarizes the outcome of 69 pediatric and adolescent patients with Ph+ ALL who underwent allogeneic HSCT in 13 AIEOP-HSCT centers from 1990 to 2008.

Our results demonstrate that many patients can be cured provided that HSCT be performed early during the course of the disease. Indeed, as already reported by other groups [10,11,26], our results underscore the importance of disease remission in determining patients' outcomes, as children who underwent transplantation in CR1 fared significantly better in terms of LFS than those who received HSCT in a more advanced disease phase. The impact of disease status (namely, morphologic CR1) on patient outcome was independent of cytogenetic remission. In this retrospective analysis of 69 children, we did not observe any significant difference in the relapse rate in patients who received imatinib therapy compared with those who did not, even though the negative result might be because of the low number of patients who received imatinib mesylate. Our results are, however, in agreement with data produced by other groups who found no benefit of imatinib mesylate on the outcome when HSCT is performed in an early phase of disease [3].

The factors significantly correlated with a better probability of LFS were being in CR1 at HSCT, female gender, and a WBC count at diagnosis $<80 \times 10^9$/L. Although the role of CR1 status has already been outlined in a number of studies [13,26,27], the impact of gender on outcome has emerged from very few reports [28,29]. Because nearly two-thirds of patients were males, and given the limited number of patients, a nonrandom distribution of "high-risk" features (such as deletion of chromosome 9p) might be the cause of these differences [30]. In particular, other variables typically associated with a better outcome, such as the donor type or the earlier phase of disease, did not show differences in the female versus male cohorts (data not shown).

Other factors typically associated with LFS, in particular, cytogenetic remission at HSCT, the type of donor employed, the use of TBI, or an occurrence of aGVHD or cGVHD, had no significant effect [31-34].

The second aspect of our study to highlight is that concerning leukemia recurrence and the graft-versus-leukemia effect. Because a high proportion of relapsed patients did not receive HSCT in CR1 (38%) and also considering the low incidence of TRM (only 17% despite an 18-year enrollment period), if a suitable donor is identified, a low-intensity GVHD prophylaxis might permit a more efficient immunologic effect in what is an otherwise life-threatening disease, characterized by a high incidence of relapse. This speculation is supported by the lower RI observed in patients who developed grade II-IV or III-IV aGVHD. Indeed, as reported previously, patients with grade III-IV GVHD had an RI of 14% compared with 39% for patients having grade 0-II GVHD. Thus, early HSCT with a low-intensity GVHD prophylaxis might improve the outcome. The advantage offered by the occurrence of GVHD in terms of disease recurrence prevention was, however, offset by a higher incidence of TRM, this resulting into a probability of LFS comparable in patients who did or did not experience GVHD. However, more recent data produced by a cooperative group (in imatinib-free patients), have shed some light on the improved benefit of unrelated over related transplantation for Ph+ ALL [26]. That study showed a 5-year LFS rate of 41.4% ± 6.5% and 55.8% ± 5.4% before and after 2000 ($P = .07$) for MUD recipients compared with 38.9% ± 6.6% and 41.1% ± 6.4% for MFD (P = NS) for MFD recipients. In the unrelated donor group, it was observed a lower incidence of relapse (38.2% ± 6.4% and 21.4% ± 4.1% before and after 2000), whereas for the MFD group, the cumulative incidence of relapse was unchanged [26]. This finding could be attributed to a more potent graft-versus-leukemia effect on residual leukemia driven by HLA disparities in the donor/recipient pairs.

Our analysis shows how the outcome of patients is related to molecular features, such as the specific p190 transcript and that LFS is significantly better than in patients who also carry the p210 transcripts. These data have already been established in adult populations in which the p190 fusion was the only independent prognostic factor conferring a significantly better probability of DFS and OS. The heterogeneous clinical outcome of *bcr-abl* ALL patients was first suggested by Secker-Walker and Craig in 1993 [35,36], who discussed the controversy surrounding a case of Ph+ ALL and an ALL blast crisis following Ph+ CML. A favorable prognostic impact of the p190 gene fusion on the clinical outcome of *bcr-abl* ALL patients was

not statistically proven in the GIMEMA ALL 04/89 and 05/93 studies, in which only a trend toward a better OS for the p190+ patients was noted [37-39]. Finally, the prognostic significance of *bcr-abl* transcripts did not seem to be affected by the type of transplantation procedure, as suggested by the results of the multivariate analysis.

Since US Food and Drug Administration approval, imatinib has become the upfront standard treatment for both adults and children with Ph+ ALL, in the attempt to obtain a better control of disease to improve outcomes in patients receiving allogeneic HSCT. However, to date, the role of this therapeutic use of Imatinib in a pediatric setting is still unclear and, for pediatric patients with Ph+ ALL, there have only been limited reports [40]. The COG recently reported excellent 1-year EFS rates in 31 Ph+ ALL patients treated on the COG AALL0031 trial with intensive imatinib and combination chemotherapy and 21 patients who received MFD allogeneic HSCT after upfront chemotherapy and imatinib—both with superior results (EFS 96.7% and 95%, respectively) compared with historic controls [19]. Several reasons might explain why imatinib did not significantly improve transplantation outcomes in our and other Ph+ ALL cohorts [41,42] such as: (1) proto-oncogene *bcr-abl* activity alone is not responsible for the phenotype of Ph+ ALL [43], (2) presence of imatinib-resistant clones [44], (3) acquired resistance to imatinib also occurs, especially involving the *abl* domain [45], (4) or the inability of imatinib to eliminate the leukemia stem cell [46]. Another intriguing explanation for the nonbenefit of imatinib on post-HSCT leukemia relapse has been given in a recent article by Kang et al. [20], in which the result of ALL MRD, monitored by gene profile, is a better predictor of leukemia relapse than the usual specific transcript. This would mean that other tumor escape mechanisms are responsible for leukemia resistance and the subsequent relapse of leukemia clones. By contrast, imatinib might extend the best time to transplantation thus permitting, for patients lacking an MFD, the search for and the recruitment of "the best" unrelated HSCT donor while maintaining the remission status.

In conclusion, our data support performing allogeneic HSCT in pediatric Ph+ ALL as soon as a suitable donor is found, especially if the specific p210 transcript is identified at diagnosis. The role of imatinib following HSCT should be investigated in a larger controlled randomized study.

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SUPPLEMENTARY DATA

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