Umbilical cord blood transplantation from unrelated donors in patients with Philadelphia chromosome-positive acute lymphoblastic leukemia

José Luis Piñana,[1] Jaime Sanz,[2] Alessandra Picardi,[3] Christelle Ferrá,[4] Rodrigo Martino,[5] Pere Barba,[6] Marta Gonzalez-Vicent,[7] María Jesús Pascual,[8] Carmen Martín,[9] Amparo Verdeguer,[10] Cristina Diaz de Heredia,[11] Pau Montesinos,[2] José-María Ribera,[4] Miguel Sanz,[2,12] William Arcese,[3] and Guillermo Sanz[2] on behalf of the GETH and GITMO groups

[1]Department of Hematology, Hospital Clínico Universitario, Valencia, Spain; [2]Department of Hematology, Hospital Universitario La Fe, Valencia, Spain; [3]UOC Trapianto di Cellule Staminali, Università di Roma Tor Vergata, Roma, Italy; [4]Department of Clinical Hematology, ICO-Hospital Universitari Germans Trias I Pujol, Jose Carreras Research Institute, Badalona, Spain; [5]Department of Hematology, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain; [6]Department of Hematology, Hospital Universitari Vall D'Hebron, Barcelona, Spain; [7]Department of Hematology, Hospital Niño Jesús, Madrid, Spain; [8]Department of Hematology, Hospital Carlos Haya, Málaga, Spain; [9]Department of Hematology, Hospital Reina Sofía, Córdoba, Spain; [10]Pediatric Oncology Unit, Hospital Universitario La Fe, Valencia, Spain; [11]Department of Pediatrics, Hospital Universitari Vall D'Hebron, Barcelona, Spain; [12]Medicine Department, Universitat de Valencia, Valencia, Spain

ABSTRACT

There are very few disease-specific studies focusing on outcomes of umbilical cord blood transplantation for Philadelphia chromosome-positive acute lymphoblastic leukemia. We report the outcome of 45 patients with Philadelphia chromosome-positive acute lymphoblastic leukemia who underwent myeloablative single unit cord blood transplantation from unrelated donors within the GETH/GITMO cooperative group. Conditioning regimens were based on combinations of thiotepa, busulfan, cyclophospamide or fludarabine, and antithymocyte globulin. At the time of transplantation, 35 patients (78%) were in first complete remission, four (8%) in second complete remission and six (14%) in third or subsequent response. The cumulative incidence of myeloid engraftment was 96% at a median time of 20 days and significantly better for patients receiving higher doses of CD34$^+$ cells. The incidence of acute grade II-IV graft-*versus*-host disease was 31%, while that of overall chronic graft-*versus*-host disease was 53%. Treatment-related mortality was 17% at day +100 and 31% at 5 years. The 5-year relapse, event-free survival and overall survival rates were 31%, 36% and 44%, respectively. Although the event-free and overall survival rates in patients without *BCR/ABL* transcripts detectable at time of transplant were better than those in whom *BCR/ABL* transcripts were detected (46% *versus* 24% and 60% *versus* 30%, respectively) these differences were not statistically significant in the univariate analysis (P=0.07). These results demonstrate that umbilical cord blood transplantation from unrelated donors can be a curative treatment for a substantial number of patients with Philadelphia chromosome-positive acute lymphoblastic leukemia.

Introduction

Although tyrosine kinase inhibitors (TKI) have consistently increased the complete remission rates[1-4] in patients with Philadelphia chromosome–positive acute lymphoblastic leukemia (Ph$^+$ ALL), allogeneic stem cell transplantation is still the mainstream post-remission therapy in this setting.[5,6] For patients with Ph$^+$ ALL lacking a suitable human leukocyte antigen (HLA)-compatible donor, umbilical cord blood transplantation (UCBT) may be a feasible, alternative source of stem cells.

Some studies have shown that, compared to transplants from matched or partially matched unrelated donors, UCBT provides at least similar survival in patients with ALL.[7-9] These studies included variable proportions of Ph$^+$ ALL patients (10-30%), but specific outcomes for this subtype of ALL were not reported. Information on disease-specific outcomes, in particular on patients with Ph$^+$ ALL undergoing UCBT from unrelated donors is extremely limited. In fact, as far as we know, only one pilot study of eight patients with Ph$^+$ ALL who underwent UCBT has been published so far.[10]

We report here the outcome of a relatively large series of 45 patients with Ph$^+$ ALL who underwent busulfan-based myeloablative single-unit UCBT from unrelated donors. Apart from confirming the safety and efficacy of the procedure in this specific disease, an additional aim of the study was to identify variables influencing short- and long-term outcomes.

Methods

A detailed description of the methods is provided in the *Online Supplementary Methods*.

Patients

From June 1999 to December 2011, we included 45 consecutive Ph+ ALL patients undergoing myeloablative single-unit UCBT from

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Correspondence: jlpinana@gmail.com

an unrelated donor within the *Grupo Español de Trasplante Hematopoyético* (GETH) and the Rome Transplant Network of the *Gruppo Italiano Trapianto Midollo Osseo* (GITMO). Most of the patients were included in two subsequent prospective trials: TSCU-GETH2005 and TSCU-GETH/GITMO2008 (registered with EudraCT with code 2008-000927-24). The institutional review board approved the protocol and written informed consent was obtained from all patients according to the Declaration of Helsinki.

Selection of cord blood units and transplant characteristics

Umbilical cord blood units were required to be HLA-matched with the recipient at ≥ 4/6 loci. Until 2005 the cord blood units were required to contain a total nucleated cell dose ≥1.5x10^7/kg recipient's body weight, while the requirements in 2006 and 2007 were a total nucleated cell dose ≥2x10^7/kg and a CD34$^+$ cell dose ≥1x10^5/kg recipient's body weight. From 2008 up to present the minimum cell dose criteria of the UCB units were a total nucleated cell count >150x10^7 and a CD34$^+$ cell count >70x10^5.

All patients received thiotepa, busulfan, cyclophospamide or fludarabine, and antithymocyte globulin as reported elsewhere.[11] Graft-*versus*-host disease (GvHD) prophylaxis was based on cyclosporine combined with either long-course prednisone or mycophenolate mofetil.

Disease evaluation and tyroskine kinase inhibitors

Pre-transplant disease status was assessed during the 30 days prior to UCBT. Minimal residual disease was assessed by qualitative or quantitative polymerase chain reaction (PCR) analysis of p190 BCR/ABL mRNA. The PCR was performed in each participating center using TaqMan technology in accordance with the guidelines approved in the Europe Against Cancer Program.[12]

A TKI was given after engraftment at the physicians' discretion. The generally accepted practice before starting TKI therapy included an evaluation of hematopoietic engraftment, ability of oral intake, and the potential risks of drug interactions.

Table 1. Patients' characteristics at UCBT.

Characteristics	Ph+ ALL (n= 45)
Recipients' median age, years (range)	31 (3-47)
Age group, n. (%)	
3-20 years	11 (24)
21-30 years	9 (20)
31-40	18 (40)
>40 years	7 (16)
Male recipient, n. (%)	25 (56)
Median weight, kg (range)	65 (10-94)
Prior TKI, n. (%)	
Imatinib©	33 (73)
Dasatinib©	3 (7)
None	9 (20)
Prior autologous HSCT, n. (%)	1 (2)
Disease status at UCBT, n. (%)	
First complete remission	35 (78)
Second complete remission	4 (8)
Third or subsequent response	6 (14)
BCR/ABL PCR before UCBT, n. (%)	
Positive	23 (51)
Negative	22 (49)
Time from diagnosis to UCBT, months (range)	8 (4-97)
Patient CMV seropositive	30 (67)
Median follow-up for survivors, months (range)	58 (12-129)

TKI: tyrosine kinase inhibitor; HSCT hematopoietic stem cell transplantation: UCBT: umbilical cord blood transplantation: PCR: qualitative or quantitative reverse transcriptase–polymerase chain reaction: CMV: cytomegalovirus. ©One patient received both dasatinib and imatinib before UCBT.

Definitions

Complete remission was defined according to standard morphological criteria as outlined by the International Working Group.[13] A negative molecular status was defined as <1x10^{-4} *BCR-ABL* transcript copies, assessed by qualitative or quantitative PCR. Graft failure was defined as the failure to achieve myeloid engraftment in patients alive at day +28 after transplantation. Secondary graft failure was defined as the loss of previously achieved engraftment. Acute and chronic GvHD were diagnosed on the basis of previously published criteria.[14,15] For event-free survival, hematologic relapse, death and graft failure were considered as treatment failure.

Statistical analysis

The primary endpoint of this study was long-term event-free survival after UCBT. Secondary endpoints were engraftment, regimen-related toxicity, overall survival, relapse rate, and non-relapse mortality. Engraftment, non-relapse mortality, GvHD, and relapse were estimated by the cumulative incidence method.[16] Unadjusted time-to-event analyses were performed using the Kaplan–Meier estimate,[17] and, for comparisons, log-rank tests.[18] Statistical analyses were conducted using R version 2.12.2 (the CRAN project) with packages, survival v2.36-10, Design 2.3-0, prodlim v1.2.1 and cmprsk v2.2-2.[19]

Results

Patients and disease characteristics

Overall, 45 consecutive patients with Ph$^+$ ALL underwent myeloablative UCBT. The patients' main characteristics are summarized in Table 1. Their median age was 31 years (range, 3-47) and 40 patients (89%) were older than 15 years. Thirty-five patients (78%) were transplanted in first complete remission and 36 (80%) had received TKI-based induction/consolidation chemotherapy (33 patients with imatinib, 2 patients with dasatinib and 1 patient with both). Twenty-three patients (51%) had detectable *BCR/ABL* transcripts at the time of UCBT. Thirteen (37%) out of 35 patients in first complete remission had detectable *BCR/ABL* transcripts at the time of UCBT compared to four (100%) out of four in second complete remission and six (100%) out of six patients in third or subsequent response (P=0.05). TKI therapy during induction-consolidation therapy was not associated with any molecular status at UCBT. The median time from diagnosis to transplantation was 8 months (range, 4 to 97). The median follow-up for surviving patients was 58 months (range, 12-129).

Transplant and cord blood unit characteristics

The main transplant and cord blood unit characteristics are summarized in Table 2. Briefly, most patients (53%) were transplanted within the TSCU-GETH/GITMO2008 clinical trial. Two patients received a fully matched umbilical cord blood unit, whereas all the remaining 43 received an HLA-mismatched cord blood unit. There was donor-recipient disparity for one or two of the six antigens in 15 patients (33%) and 28 patients (62%), respectively.

Early regimen-related toxicity

The main early regimen-related toxicity was hemorrhagic cystitis, which affected 33% of patients. Three patients (7%) developed acute renal failure and one additional patient had sinusoidal obstruction syndrome of the liver.

Engraftment

All patients were evaluable for engraftment. Two patients had primary graft failure at 28 days after UCBT. Both patients received an allogeneic stem cell transplant from a haploidentical family donor and are alive and disease-free at 48 and 60 months after transplantation. The remaining 43 patients engrafted at a median time of 20 days (range, 9-44). All patients with myeloid engraftment showed full donor chimerism at the time of leukocyte reconstitution. Three patients experienced secondary graft failure on days +29, +53 and +73, confirmed by the loss of full donor chimerism. The cumulative incidence of myeloid engraftment at 44 days was 96% (95% CI, 90-100). Univariate analysis for myeloid engraftment showed that the number of cryopreserved $CD34^+$ was the only factor influencing myeloid engraftment. The cumulative incidence of myeloid engraftment in patients receiving umbilical cord blood units containing above and below the best cut-off of $2.3x10^5/CD34^+$ cells/kg recipient's body weight was 100% and 93% at a median time of 18 days and 20 days, respectively (P=0.005) (Figure 1).

Thirty-three of the 43 patients with myeloid engraftment had platelet engraftment at a median time of 44 days (range, 10-183). Eight patients died or relapsed between 40 and 185 days after transplantation without platelet engraftment and two of the three patients with secondary graft failure never achieved platelet engraftment. The cumulative incidence of platelet engraftment at 183 days was 73% (95% CI, 60-86). No factor was found to be associated with platelet engraftment.

Graft-versus-host disease

Twenty-eight patients developed acute GvHD at a median time of 23 days (range, 8-128) after transplantation. The acute GvHD was grade I in 13 patients, grade II in six patients, grade III in five patients, and grade IV in four patients. The skin was involved in 26 patients (grade I in 12, grade II in 10, grade III in 3 and grade IV in 1), the gut in 11 patients (grade I in 4, grade II in 2, grade III in 3 and grade IV in 2), and the liver in 7 patients (grade I in 3, grade II in 1 and grade III in 3). The cumulative incidence of grade II-IV and III-IV acute GvHD at 100 days was 31% (95% CI, 18-45) and 20% (95% CI, 8-32), respectively.

Chronic GvHD occurred in 17 of 32 evaluable patients at a median time of 130 days (range, 94-659). Chronic GvHD was limited in nine patients and extensive in eight. The 5-year cumulative incidence of overall and extensive chronic GvHD was 53% (95% CI, 36-70) and 28% (95% CI, 10-47). No factor was found to be associated with the development of acute or chronic GvHD.

Non-relapse mortality, relapse and causes of death

Regarding non-relapse mortality, univariate analysis did not show any statistical significant differences between children (age <20 years) and adults. The fludarabine-containing conditioning regimen was associated with significantly lower non-relapse mortality, while being treated with the TSCU-GETH2005 and TSCU-GETH/GITMO2008 protocols, as well as not having minimal residual disease prior to the UCBT, showed a trend for lower non-relapse mortality in univariate analysis (*Online Supplementary Table S1*). Fourteen patients died without prior relapse at a median time of 86 days (range, 40-316). The cumulative incidences of non-relapse mortality at 100 days, 180 days, and 5 years were 17% (95% CI, 7-29), 20% (95% CI, 8-32) and 31% (95% CI, 18-45), respectively. The causes of death without relapse were infection in five patients (2 bacterial infections, 1

Table 2. Graft and transplantation characteristics.

Characteristics	Ph+ ALL (n= 45)
Year of transplantation, n. (%)	
1999-2002	8 (18)
2003-2006	13 (29)
2007-2011	24 (53)
HLA compatibility, n. (%)	
6/6, 5/6, 4/6	2 (5), 15 (33), 28 (62)
ABO blood group mismatch, n. (%)	
Major/minor	16 (36) / 12 (27)
Female donor, n. (%)	22 (49)
Female donor to male recipient, n. (%)	12 (27)
Conditioning regimen, n. (%)	
Bu+TT+Cy+ATG	13 (29)
Bu+TT+FU+ATG	32 (71)
Horse ATG*, n. (%)	4 (9)
GvHD prophylaxis	
Cyclosporine A + prednisone	26 (58)
Cyclosporine A + MMF	19 (42)
$CD34^+$ $x10^5$/kg, median (range)¥	
Before freezing	1.7 (0.3-6.7)
Infused	1.4 (0.3-11.1)
TNC $x10^7$/kg, median (range)¥	
Before freezing	3 (1.5-10)
Infused	2.5 (1-7.1)

*Bu: busulfan; TT: thiotepa; Cy: cyclophosphamide; ATG: anti-thymoglobuline; FU: fludarabine; MMF: mycophenolate mofetil; TNC, total nucleated cells. *Type of anti-thymoglobulin used in the conditioning. ¥The median numbers of cryopreserved TNC and $CD34^+$ cells per kg of recipient weight 2008 were $3.3x10^7$/kg (range, 1.9-7.5) and $1.8x10^6$/kg (range, 1-6.7) for 17 patients transplanted after 2008.*

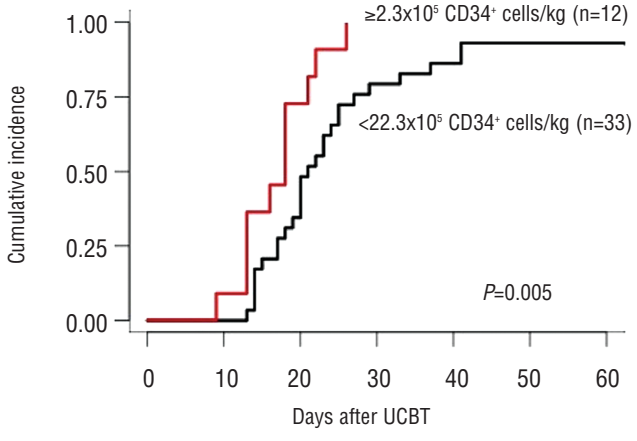

Figure 1. Myeloid engraftment according to the best cut-off of 2.3 $x10^5$ $CD34^+$ cells/kg at the time of freezing the unit.

cytomegalovirus pneumonitis, 1 HHV-6 infection and 1 *Candida krusei* infection), GvHD in three patients, graft failure in three patients, thrombotic microangiopathy in two patients, and multiorgan failure in one patient.

Twelve patients relapsed at a median time of 13 months (range, 2-53), including one patient who had a central nervous system relapse. The 5-year cumulative incidence of relapse was 31% (95% CI, 16-45). Two relapsed patients are still alive and disease-free after salvage therapy at 9 and 31 months. Among the 12 patients who relapsed, three relapsed after discontinuing TKI maintenance therapy, while seven patients who had not received TKI maintenance relapsed and five of them were then treated with TKI therapy. The two patients who relapsed before day 180 also received TKI salvage therapy (patients n. 4 and 7, Table 3). An additional two patients relapsed and died without receiving TKI therapy due to the aggressive behavior of their disease.

Event-free and overall survival

Univariate analyses showed that the fludarabine-containing conditioning regimen was associated with higher event-free and overall survival rates while treatment with the TSCU-GETH2005 and TSCU-GETH/GITMO2008 protocols and no minimal residual disease prior to UCBT showed a trend for higher event-free and overall survival rates (see *Online Supplementary Table S1*). We did not find any differences in event-free or overall survival between children (age <20 years) and adults.

Nineteen patients remained alive and disease-free at their last follow-up (range, 12-129 months) after UCBT. The 5-year event-free and overall survival rates were 36% (95 CI, 20-51) and 44% (95% CI, 28-60), respectively. The 5-year event-free and overall survival rates were 46% (95% CI, 27-80) and 60% (95% CI, 40-88), respectively for patients in molecular remission at the time of transplantation compared to 24% (95% CI, 11-51) and 30% (95% CI, 15-58), respectively for patients with detectable *BCR/ABL* transcripts (P=0.07) (Figure 2).

Post-transplant tyrosine kinase inhibitor therapy

The characteristics of the 19 patients who received TKI therapy after UCBT are summarized in Table 3. TKI therapy was started a median of 6 months (range, 2-62) after transplantation. Eleven patients received imatinib and eight were given dasatinib. Seven patients started TKI therapy because of a relapse (6 hematologic and 1 molecular). One of the six patients with hematologic relapse did not respond, while the remaining five reached a temporary, but prolonged subsequent complete remission (median time of disease control, 25 months; range, 16-38 months) although all finally died from disease progression. The patient who started TKI therapy because of molecular detection of *BCR/ABL* transcripts is still alive and disease-free after 42 months of follow-up. Twelve additional patients started TKI therapy in complete remision as maintenance therapy. Nine of these patients discontinued TKI therapy due to toxicity (6 patients), mainly gastrointestinal, or other reasons. The remaining three patients were still receiving TKI therapy at their last follow-up. To investigate the effect of TKI maintenance for patients in complete remission pre-UCBT, we compared their out-

Table 3. Characteristics of patients treated with tyrosine kinase inhibitors after UCBT (n=19).

N.	Status at UCBT / molecular status	Month* of TKI onset / type of TKI	Indication of TKI	Month* of interruption and cause#	Month* of relapse	Intervention	Status disease at last follow-up¥	Month* F-up / Survival status
1	CR1/neg	51 / imatinib	H. relapse	-	51	Imatinib	Temporary CR further H. relapse	89 / dead
2	CR/pos	32 / imatinib	H. relapse	-	32	Imatinib	Temporary CR further H. relapse	55 / dead
3	PR/pos	7 / imatinib	H. relapse	-	7	Imatinib	NR	11 / dead
4	CR1/neg	3 / dasatinib	H. relapse	12 / disease progression	3	Nilotinib	Temporary CR further H. relapse	19 / dead
5	CR1/neg	7 / dasatinib	H. relapse	-	6	Dasatinib	Temporary CR further H. relapse	31 / dead
6	CR1/neg	13 / dasatinib	H. relapse	-	13	Dasatininb	Temporary CR further H. relapse	50 / dead
7	CR2/pos	2 / dasatinib	Mol. relapse	.	2	Dasatinib	CR	46 / alive
8	CR1/neg	5 / imatinib	Maintenance therapy	27 / G-I toxicity	42	FLAG-IDA+dasatinib	CR	52 / alive
9	CR1/pos	6 / imatinib	Maintenance therapy	19 / G-I toxicity	20	2nd Allo-HCST	CR	53 / alive
10	CR1/pos	28 / imatinib	Maintenance therapy	48 / physician decision	50	Dasatinib	Progression and NR	53 / dead
11	CR1/neg	33 / dasatinib	Maintenance therapy	H.toxicity→Nilotinib -	-	No	CR	42 / alive
12	CR1/neg	6 / dasatinib	Maintenance therapy	15 / G-I toxicity	-	No	CR	32 / alive
13	CR2/pos	23 / imatinib	Maintenance therapy	62 / physician decision	-	No	CR	82 / alive
14	CR1/neg	2 / imatinib	Maintenance therapy	16 / physician decision	-	No	CR	59 / alive
15	CR1/neg	6 / dasatinib	Maintenance therapy	23 / H. toxicity	-	No	CR	27 / alive
16	CR1/pos	3 / imatinib	Maintenance therapy	-	-	No	CR	54 / alive
17	CR1/neg	6 / dasatinib	Maintenance therapy	12 / H.toxicity	-	No	CR	29 / alive
18	CR1/pos	6 / imatinib	Maintenance therapy	-	-	No	CR	62 / alive
19	CR1/pos	62 / imatinib	Maintenance therapy	-	-	No	CR	70 / alive

*CR: first complete remission; CR2: second complete remission; Neg: negative; pos: positive; PR: partial remission: TKI: tyrosine kinase inhibitors; H. relapse: hematologic relapse; Mol. relapse: molecular relapse; G-I: gastrointestinal: FLAG-IDA: fludarabine, cytarabine and idarubicin; H. toxicity: hematologic toxicity; Allo-HSCT: allogeneic hematopoietic stem cell transplantation; F-up: follow-up. *Calculated from the day of infusion of umbilical cord blood cells. #Dash symbol means that the patient continued under TKI at last follow-up or until death; ¥All patients in CR were negative for minimal residual disease at last follow-up.*

comes to those who did not receive maintenance TKI therapy. A total of 34 patients were included [11 patients were excluded because of early relapse (2 patients) or early mortality (9 patients)]. Seven (32%) out of 22 non-TKI recipients relapsed, compared to three (25%) out of 12 patients who received TKI maintenance. The 5-year event-free survival rates for these two cohorts of patients were 36% (95% CI, 20-66%) and 72% (95% CI, 44-100%), respectively (P=0.05).

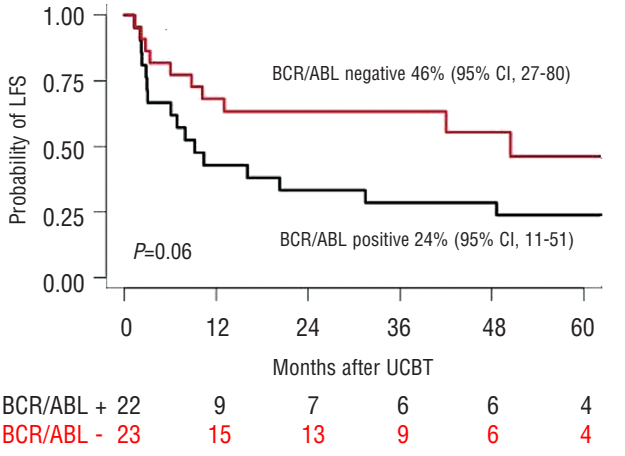

Figure 2. Disease-free survival at 5 years according to the molecular status before UCBT.

Discussion

This study shows that single-unit UCBT after a busulfan-based myeloablative conditioning regimen is feasible and should be considered as a real alternative for patients with Ph$^+$ ALL without a suitable HLA-matched donor. Long-term event-free survival can be achieved in a substantial number of patients, particularly in those transplanted in molecular remission. Relapse is a common cause of treatment failure and although most relapses were observed in the first months after UCBT, late events did occur.

Despite the important growth in UCBT activity all over the world, information on disease-specific outcomes is still very limited. In fact, UCBT has been scarcely reported in patients with Ph$^+$ ALL.[10] Limited data are available in registry-based reports of UCBT outcomes in adults with ALL,[6-8,10,20,21] but these reports were mainly focused on comparing UCBT with bone marrow or peripheral blood allogeneic hematopoietic stem cell transplantation (HSCT). Although they included some patients with Ph$^+$ ALL, outcomes were not reported specifically for these patients. We, therefore, discuss our findings in the context of this limited information on UCBT in patients with Ph$^+$ ALL.

This retrospective study included a large series of mostly adult patients with Ph$^+$ ALL, treated with a homogenous strategy of single unit UCBT after busulfan-based myeloablative conditioning in the setting of a cooperative group. Of note, most patients were included in two successive prospective trials (TSCU-GETH2005 and TSCU-GETH/GITMO2008). Although most of the transplants were performed in the TKI era, the use of TKI before and after transplantation was not uniform and was implemented according to each center's criteria.

As in previous reports, single-unit UCBT with a busulfan- and antithymocyte globulin-based myeloablative conditioning regimen enabled high rates of engraftment and fast neutrophil recovery.[22,23] Again the CD34$^+$ cell content at the time of freezing the unit was the major determinant of engraftment.[22,24] Therefore, although there is discussion about the reliability of CD34$^+$ measurements across different laboratories, CD34$^+$ cell counts should be taken into account in graft selection. It is worth noting that while most patients were adults, received highly mismatched units, and the cell dose content of the unit was below the current recommendations,[25] the single unit was sufficient for engraftment. The strategy applied for selection of the most appropriate umbilical cord blood units since 2008 deserves a brief comment, since this selection was based on the total cell doses in the units, irrespective of the recipients' body weight. This strategy led us to obtain optimal median total nucleated and CD34$^+$ cell doses for the recipients' body weight: these doses were higher than the median cell counts in the overall cohort.

An important limitation of UCBT has been concern about a possible high transplant-related mortality of the procedure. However, busulfan-based conditioning was well tolerated with an acceptable toxicity and early non-relapse mortality of 17% at 100 days, and a long-term, 5-year non-relapse mortality of 31%. These results are in line with those in other studies of patients with Ph$^+$ ALL undergoing allogeneic HSCT from related or unrelated donors.[5,26] The choice of conditioning [(busulfan *versus* total body irradiation (TBI)] for allogeneic HSCT in ALL patients is still a matter of debate because of concern about busulfan toxicity. However, we found similar non-relapse mortality to that reported for TBI-based conditioning.[27] In fact, we observed a decrease in non-relapse mortality over time, especially since 2005. Univariate analysis showed that the fludarabine-containing regimen was associated with lower non-relapse mortality, which could be explained by several factors: first, fludarabine replaced cyclophosphamide in 2005, as part of the changes introduced in the TSCU-GETH2005 and TSCU-GETH/GITMO2008 trials. However, other changes were also introduced at the same time, which may have helped to improve the results (switching from oral to intravenous busulfan or oral busulfan but with pharmacokinetically-adjusted dosing, the potential benefit over time of greater experience of the transplant teams, the better cord blood unit selection, improved supportive care). All these changes suggest a refinement in the UCBT procedure over time which led to a lower non-relapse mortality rate. The results of this study should be interpreted with caution, since they are limited to the non-TBI UCBT setting. Future studies analyzing the results of UCBT for Ph$^+$ ALL patients using a TBI-based conditioning regimen would be of interest.

Relapse was a major cause of treatment failure. The relapse rate was 31% after a long, median follow-up of 5 years: this is similar to the rates reported for bone marrow or peripheral blood allogeneic HCST from related or unrelated donors.[28-30] Of note, we observed that around 30% of relapses occurred more than 30 months after UCBT, suggesting a long-lasting period of relapse risk in Ph$^+$ ALL patients after transplantation. The arrival of TKI has offered a new approach to the treatment of relapses in Ph$^+$ ALL patients after transplantation. In our series, five of six

patients with hematologic relapse reached a complete remission after the onset of salvage TKI therapy, which was sustained for several months. These data suggest that TKI are active but, likely, generate a time-exposure resistance or mutational clone selection among the leukemic cells and do, therefore, need to be followed by other strategies such as withdrawal of immunosuppressants, salvage chemotherapies, or second allogeneic HSCT in selected cases. Thus, it appears critical to treat patients with minimal residual disease rather than hematologic relapse in order to improve event-free survival.[31]

On the other hand, the use of TKI after transplantation seems a reasonable approach to prevent relapses, but to date few studies have evaluated this issue and no uniform recommendations have been established. Recent studies evaluating the impact of imatinib after allogeneic HSCT suggested that the TKI decreased the relapse rate and improved survival.[28,30,32] These findings do, however, need to be confirmed in prospective trials and important issues such as the choice of TKI, dosing, optimal timing and duration of therapy need to be defined. In our study, around 26% of the cohort received TKI after transplantation to prevent relapse. However medication was not well tolerated and half of the patients had to suspend therapy, mainly because of gastrointestinal toxicity. We sought to estimate the safety and feasibility of TKI maintenance after UCBT rather than to demonstrate its potential benefit; nevertheless, although the difference was not statistically significant, we observed a better event-free survival with TKI maintenance, and, more importantly, TKI maintenance did not adversely affect the outcomes of UCBT. These results must, however, be interpreted with caution given the low number of patients and the possibility that selection bias could explain the findings.

Finally, a substantial number of patients achieved long-term survival (event-free or not), which again is consistent with existing reports on outcomes after allogeneic HSCT using stem cells from other sources.[28,29,33-35] In fact, previous studies reported disease-free survival and overall survival rates ranging from 35% to 67% and 30% to 60% after related and unrelated donor allogeneic HSCT in Ph$^+$ ALL patients, respectively.[5,28,29] The better results, in terms of event-free and overall survival, in this study for the fludarabine-containing conditioning regimen could be explained by the lower non-relapse mortality found since 2005 but also because patients more often did not have minimal residual disease at UCBT [24 (75%) out of 32 patients receiving fludarabine *versus* 4 (30%) out of 13 patients receiving cyclophosphamide; P=0.008], suggesting that patients had a better remission status at UCBT. The introduction of TKI during induction-consolidation chemotherapy protocols around 2005 is probably the major reason for these improved remissions. An important observation in this study was the impact of molecular minimal residual disease status before UCBT. In fact, patients who had detectable *BCR/ABL* transcripts before UCBT had a trend towards lower event-free and overall survival rates, as has been reported in the minimal residual disease setting.[36] The validity of these results is limited by the use of different PCR methods (quantitative or qualitative) among the different participating centers as well as the lack of standardization of PCR techniques to detect *BCR/ABL* transcripts.

In conclusion, these results show that single-unit UCBT from unrelated donors after myeloablative busulfan-based conditioning may be a curative treatment for a substantial number of patients with Ph$^+$ ALL. Strategies to reduce the relapse rate after transplant with TKI should be investigated. More specific studies on patients with Ph$^+$ ALL undergoing UCBT from unrelated donors are warranted to definitely establish the role of this type of transplantation in the therapeutic algorithm of Ph$^+$ ALL in the TKI era.

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