Transplantation and Cellular Therapy 27 (2021) 424.e1–424.e9

Transplantation and Cellular Therapy

journal homepage: www.tctjournal.org

ASTCT American Society for Transplantation and Cellular Therapy

Full Length Article
Pediatric

Outcomes of Unmanipulated Haploidentical Transplantation Using Post-Transplant Cyclophosphamide (PT-Cy) in Pediatric Patients With Acute Lymphoblastic Leukemia

Annalisa Ruggeri[1,2,*], Jacques-Emmanuel Galimard[3], Olesya Paina[4], Franca Fagioli[5], Abdelghani Tbakhi[6], Akif Yesilipek[7], José Maria Fernandez Navarro[8], Maura Faraci[9], Rose-Marie Hamladji[10], Elena Skorobogatova[11], Amal Al-Seraihy[12], Mikael Sundin[13], Concepcion Herrera[14], Jose Rifón[15], Arnaud Dalissier[3], Franco Locatelli[16], Vanderson Rocha[17], Selim Corbacioglu[18]

[1] *Department of Hematology and Bone marrow Transplantation, IRCCS San Raffaele Scientific Institute, Milano, Italy*
[2] *Cellular Therapy and Immunobiology Working Party, Leiden, The Netherlands*
[3] *EBMT Paediatric Diseases Working Party, Paris, France*
[4] *First State Pavlov Medical University of St. Petersburg Raisa Gorbacheva Memorial Research Institute for Paediatric Oncology, Hematology and Transplantation, St. Petersburg, Russia*
[5] *Onco-Ematologia Pediatrica, Centro Trapianti Cellule Staminali, Ospedale Infantile Regina Margherita, Torino, Italy*
[6] *King Hussein Cancer Centre, Amman, Jordan*
[7] *Medicalpark Antalya Hospital, Antalya, Turkey*
[8] *Hospital Universitario La Fe Servicio de Hematologia Bulevar Sur S/N Valencia, Valencia, Spain*
[9] *Institute G. Gaslini, Genova, Italy*
[10] *Centre Pierre et Marie Curie Alger, Algerie*
[11] *The Russian Children's Research Hospital, Department of Bone Marrow Transplantation, Moscow, Russia*
[12] *King Faisal Specialist Hospital & Research Centre, Riyadh, Saudi Arabia*
[13] *Pediatric Hematology, Immunology and HCT Section, Astrid Lindgren Children's Hospital, Karolinska University Hospital and Division of Pedatrics, CLINTEC, Karolinska Institutet; Stockholm, Sweden.*
[14] *Maimonides Biomedical Research Institute of Cordoba (IMIBIC), Department of Hematology Reina Sofia University Hospital, University of Cordoba, Cordoba, Spain*
[15] *Clínica Universitaria de Navarra Area de Terápia Celular Pamplona, Pamplona, Spain*
[16] *Department of Pediatric Hematology and Oncology, IRCCS Ospedale Pediatrico Bambino Gesù, Rome, Italy*
[17] *Service of Hematology, Transfusion and Cell Therapy and Laboratory of Medical Investigation in Pathogenesis and Directed Therapy in Onco-Immuno-Hematology, HCFMUSP, University of São Paulo Medical School, São Paulo, Brazil*
[18] *Pediatric Hematology, Oncology and Stem Cell Transplantation Department, University of Regensburg, Regensburg, Germany*

Article history:
Received 3 July 2020
Accepted 18 January 2021

Key Words:
Childhood
Acute lymphoblastic leukemia
Haploidentical transplantation
Post-transplantation cyclophosphamide

A B S T R A C T

HLA-haploidentical transplantation (haplo-HCT) using post-transplantation-cyclophosphamide (PT-Cy) is a feasible procedure in children with malignancies. However, large studies on Haplo-HCT with PT-Cy for childhood acute lymphoblastic leukemia (ALL) are lacking. We analyzed haplo-HCT outcomes in 180 children with ALL. Median age was 9 years, and median follow-up was 2.7 years. Disease status was CR1 for 24%, CR2 for 45%, CR+3 for 12%, and active disease for 19%. All patients received PT-Cy day +3 and +4. Bone marrow (BM) was the stem cell source in 115 patients (64%). Cumulative incidence of 42-day engraftment was 88.9%. Cumulative incidence of day-100 acute graft-versus-host disease (GVHD) grade II-IV was 28%, and 2-year chronic GVHD was 21.9%. At 2 years, cumulative incidence of nonrelapse mortality (NRM) was 19.6%. Cumulative incidence was 41.9% for relapse and 25% for patients in CR1. Estimated 2-year leukemia free survival was 65%, 44%, and 18.8% for patients transplanted in CR1, CR2, CR3+ and 3% at 1 year for active disease. In multivariable analysis for patients in CR1 and CR2, disease status (CR2 [hazard ratio {HR} = 2.19; P = .04]), age at HCT older than 13 (HR = 2.07; P = .03) and use of peripheral blood stem cell (PBSC) (HR = 1.98; P = .04) were independent factors associated with decreased overall survival. Use of PBSC was also associated with higher NRM (HR = 3.13; P = .04). Haplo-HCT with PT-Cy is an option for children with ALL, namely

Financial disclosure: See Acknowledgments on page 431.e8.
*Correspondence and reprint requests: Annalisa Ruggeri, Department of Hematology and Bone Marrow Transplantation, IRCCS San Raffaele Scientific Institute, via Olgettina 58, Milano, Italy.
E-mail address: annalisaruggeri80@hotmail.com (A. Ruggeri).

https://doi.org/10.1016/j.jtct.2021.01.016

those transplanted in CR1 and CR2. Age and disease status remain the most important factors for outcomes. BM cells as a graft source is associated with improved survival.

In the last 10 years, the number of patients with hematological malignancies such as acute leukemias transplanted using haploidentical donors has dramatically increased worldwide [1]. To avoid the risk of graft-versus-host disease (GVHD) in cases of HLA-mismatched transplantation, the use of CD34+ selected grafts after ex vivo T-cell depletion was adopted, but with an excess of graft failure, delayed immune reconstitution, and increased relapse rate.

In another approach, unmanipulated haploidentical grafts with in vivo T-cell-depletion with post-transplantation cyclophosphamide (PT-Cy) [2] helped, to some extent, to overcome the drawbacks of the previous procedure. Unmanipulated HLA-haploidentical donor transplants (haplo-HCT) with PT-Cy added to standard immunosuppressive agents, to overcome HLA mismatches was first adopted in the adult setting with a reduced-intensity conditioning regimen (RIC) and using bone marrow (BM) as a stem cell source and was associated with low incidence of GVHD and graft rejection. Despite its mechanism of action in preventing GVHD not being completely clear, PT-Cy given after graft infusion is able to abrogate the rapidly proliferating alloreactive T-cells in both directions, while preserving hematopoietic stem cells and the slowly dividing memory and regulatory T-cells, because of their high content in aldehyde dehydrogenase [3]. To overcome the excess of relapse reported in adults after RIC haplo-HCT, some authors effectively reported the application of BM and PT-Cy with myeloablative regimens (MAC) or with peripheral blood stem cell (PBSC); however, the last with an increased risk of acute GVHD [4].

HCT is indicated in children with acute lymphoblastic leukemia (ALL) namely in cases of persistence of high minimal residual disease level at the end of consolidation therapy with a high-risk cytogenetic feature or in patients in second complete remission (CR2) according to the timing and the site of disease relapse [5].

To date, few cases of haplo-HCT using PT-Cy in pediatric patients with hematologic malignancies were reported by single center and registry studies, and fewer than 20 patients with ALL were analyzed. Therefore the role of haplo-HCT using PT-Cy for children with ALL is not well established [6,7].

This retrospective and multicenter study on behalf of the Pediatric Disease Working Party (PDWP) of the European Society for Blood and Marrow Transplantation (EBMT) aimed to report the outcomes of pediatric ALL given a non-T-cell–depleted haplo-HCT with PT-Cy. Furthermore, for patients in CR1 and CR2, we performed a risk factors analysis for main HCT outcomes.

METHODS

Patients and Donors

Data were obtained from the PDWP registry of the EBMT. The EBMT is a voluntary working group with participating centers required to report consecutive transplants. Patients were followed up longitudinally. Data were collected on standardized reporting forms and is subject to audits. The Institutional Review Board of the PDWP approved this study. According to EBMT rules, patients or legal guardians provided written informed consent for data collection and use for analysis in accordance with the Declaration of Helsinki. Included are patients aged up to 18 years, with ALL in all disease status at HCT (CR1, CR2, and CR3 or more [CR3+] and active diseases) having received a first allogeneic HCT from 1 January 2011 to 30 June 2019. The graft source was bone marrow or peripheral blood from parents or other relatives mismatched at least at 2 or more HLA loci. Patients received a myeloablative or reduced-intensity conditioning regimen according to the transplant center policy. GVHD prophylaxis included PT-Cy, in combination with calcineurin inhibitors, mycophenolate mofetil or methotrexate according to institutional protocols. Patients with a prior allogeneic transplant (autologous HCT allowed) or ex vivo graft manipulation were excluded.

Outcomes

Leukemia-free survival (LFS) was the primary endpoint. LFS was defined as survival without relapse or progression. Relapse incidence (RI) was defined as leukemia recurrence after transplantation; death without relapse or progression was the competing risk. Overall survival (OS) was defined as death from any cause. Graft-versus-host–free, relapse-free survival (GRFS) was defined as the first event among grade III-IV acute GVHD (aGVHD), severe chronic GVHD (cGVHD), relapse, and death [8]. Nonrelapse mortality (NRM) was defined as death from any cause without relapse; relapse was the competing event. Acute grade II-IV and chronic GVHD were assigned and graded using standard criteria [9,10].

Statistical Analysis

Median values with respective interquartile ranges (IQR), were used to express continuous variables while frequencies in percentages were used for categorical variables. Median follow up was calculated using the reverse of Kaplan-Meier. OS, LFS, and GRFS were calculated using the Kaplan-Meier estimator. The incidence of aGVHD and cGVHD, RI, and NRM were calculated using the cumulative incidence estimator to accommodate competing risks [11]. Competing risks were death for RI, relapse for NRM, relapse or death for aGvHD and cGVHD. As planned, the impact of baseline variables on outcomes was evaluated only on patients transplanted in CR1 and CR2. Univariate analyses were done using the log-rank test for LFS, GRFS, and OS and Gray's test for cumulative incidence. Multivariate analysis was performed using Cox proportional hazards models for overall and leukemia-free survival, relapse, NRM, and GRFS only for patients in first and second remission. The variables included in multivariate models were disease status (CR1 versus CR2), age at haplo-HCT (0-12 years, versus 13 years and older), stem cell source (PBSC versus BM) and myeloablative regimen defined as MAC with total body irradiation (TBI), MAC-chemotherapy based, and RIC. To take into account the center effect, we introduced a random effect (also named frailty model) for each center into the model. The significance level was fixed at.05 and all *P* values are 2-sided. All analyses were done using R software version 4.0.0.

RESULTS

Patient, Disease, and Transplant Characteristics

Patient, disease, and transplant characteristics of the 180 patients included in the study are summarized in Table 1. Median age at transplantation was 9.3 (IQR 6.4-13.7) years. Disease status at transplant was CR1 in 23.8%, CR2 in 45%, CR3 + in 12.2%, and active disease in 19%.

Conditioning regimen was RIC in 22.8% of cases (fludarabine melphalan in 13 patients and based on low-dose TBI in 21 cases). MAC regimens were mainly based on busulfan (51.7%) (thiotepa-busulfan and fludarabine in 38 and busulfan and fludarabine in 27 cases, respectively), whereas TBI >6 Gy was used in 25.6% (41 patients).

The GVHD prophylaxis consisted of high-dose PT-Cy, 50 mg/kg intravenously on days 3 and 4, in combination with other immunosuppressive treatment, as reported in Table 1. Antithymocyte globulin (ATG) was avoided in most of the cases, being reported in 9.4% of patients (n = 17): 9 received thymoglobulin (Sanofi-Aventis, Paris, France) and 5 received Grafalon (Neovii Biotech, Rapperswil, Switzerland), missing information in 3 cases. BM was the most frequently used stem cell source (64%, n = 115), and the median donor age was 37.3 (IQR 28.59-42.79) years.

Hematopoietic Recovery and Acute and Chronic Graft-Versus-Host Disease

One hundred sixty-three patients achieved neutrophil recovery, within a median time of 19 days (IQR 16-25 days), being

Table 1
Patient and Disease Characteristics

Variables	All patients (N = 180)	CR1 (N = 43)	CR2 (N = 81)	CR3 ≥3 (N = 22)	Active disease (N = 34)
Age at TX, median [IQR]	9.25 [6.43-13.7]	8.5 [4.2-15.8]	9.4 [6.6-13.1]	11.7 [8-12.7]	8.3 [5-14.9]
Age at transplant					
[0-13]	130 (72.22%)	29 (67.44%)	60 (74.07%)	17 (77.27%)	24 (70.59%)
[13-18]	50 (27.78%)	14 (32.56%)	21 (25.93%)	5 (22.73%)	10 (29.41%)
Time between diagnosis and TX (month), median [IQR]	23.31 [8.59-44.58]	7.1 [5.3-9.1]	28.9 [19.4-44.9]	59 [43.5-83.2]	17 [10.2-33.6]
Year at TX, median (range)	2017 (2011-19)	2017 (2011-19)	2017 (2012-19)	2015 (2012-18)	2015 (2011-19)
Number of TX					
First	173 (96.11%)	43 (100%)	77 (95.06%)	20 (90.91%)	33 (97.06%)
Second (one previous auto)	6 (3.33%)	0 (0%)	4 (4.94%)	1 (4.55%)	1 (2.94%)
Third (Two previous auto)	1 (0.56%)	0 (0%)	0 (0%)	1 (4.55%)	0 (0%)
Disease status at transplantation					
CR1	43 (23.89%)	43 (100%)	0 (0%)	0 (0%)	0 (0%)
CR2	81 (45%)	0 (0%)	81 (100%)	0 (0%)	0 (0%)
CR≥3	22 (12.22%)	0 (0%)	0 (0%)	22 (100%)	0 (0%)
Advanced	34 (18.89%)	0 (0%)	0 (0%)	0 (0%)	34 (100%)
All types					
B	129 (74.57%)	30 (69.77%)	61 (81.33%)	18 (81.82%)	20 (60.61%)
T	39 (22.54%)	13 (30.23%)	13 (17.33%)	1 (4.55%)	12 (36.36%)
Other	5 (2.89%)	0 (0%)	1 (1.33%)	3 (13.64%)	1 (3.03%)
Missing	7	0	6	0	1
Patient gender					
Female	66 (36.67%)	19 (44.19%)	25 (30.86%)	5 (22.73%)	17 (50%)
Male	114 (63.33%)	24 (55.81%)	56 (69.14%)	17 (77.27%)	17 (50%)
Female donor to male recipient					
No	132 (73.33%)	32 (74.42%)	61 (75.31%)	12 (54.55%)	27 (79.41%)
Yes	48 (26.67%)	11 (25.58%)	20 (24.69%)	10 (45.45%)	7 (20.59%)
KPS or Lansky					
<90	45 (25.71%)	6 (14.63%)	16 (20.25%)	4 (19.05%)	19 (55.88%)
≥90	130 (74.29%)	35 (85.37%)	63 (79.75%)	17 (80.95%)	15 (44.12%)
Missing	5	2	2	1	0
CMV Donor to Patient					
Neg to neg	10 (6.54%)	2 (5.56%)	3 (3.9%)	1 (6.25%)	4 (16.67%)
Neg to pos	13 (8.5%)	4 (11.11%)	5 (6.49%)	0 (0%)	4 (16.67%)
Pos to neg	16 (10.46%)	4 (11.11%)	6 (7.79%)	4 (25%)	2 (8.33%)
Pos to pos	114 (74.51%)	26 (72.22%)	63 (81.82%)	11 (68.75%)	14 (58.33%)
Missing	27	7	4	6	10
Stem cell source					
BM	115 (63.89%)	28 (65.12%)	40 (49.38%)	19 (86.36%)	28 (82.35%)
PBSC	65 (36.11%)	15 (34.88%)	41 (50.62%)	3 (13.64%)	6 (17.65%)
In vivo TCD					
No	163 (90.56%)	40 (93.02%)	73 (90.12%)	18 (81.82%)	32 (94.12%)
Yes (ATG)	17 (9.44%)	3 (6.98%)	8 (9.88%)	4 (18.18%)	2 (5.88%)
Conditioning regimen					
MAC/Chemo	93 (51.67%)	21 (48.84%)	38 (46.91%)	11 (50%)	23 (67.65%)
MAC/TBI	46 (25.56%)	15 (34.88%)	23 (28.4%)	5 (22.73%)	3 (8.82%)
RIC	41 (22.78%)	7 (16.28%)	20 (24.69%)	6 (27.27%)	8 (23.53%)
GVHD prophylaxis					
CSA	8 (4.44%)	1 (2.33%)	4 (4.94%)	2 (9.09%)	1 (2.94%)
CSA+MMF	58 (32.22%)	20 (46.51%)	27 (33.3%)	5 (22.73%)	6 (17.65%)
CSA+MMF+TACRO	3 (1.67%)	2 (4.65%)	1 (1.23%)	0 (0%)	0 (0%)
CSA+MTX	6 (3.33%)	3 (6.98%)	2 (2.47%)	0 (0%)	1 (2.94%)
CSA+MTX+MMF	1 (0.56%)	0 (0%)	1 (1.23%)	0 (0%)	0 (0%)
CSA+TACRO	1 (0.56%)	0 (0%)	0 (0%)	1 (4.55%)	0 (0%)
MMF	1 (0.56%)	0 (0%)	0 (0%)	1 (4.55%)	0 (0%)
MMF+SIRO+TACRO	9 (5%)	1 (2.33%)	1 (1.23%)	1 (4.55%)	6 (17.65%)
MMF+TACRO	60 (33.33%)	10 (23.26%)	38 (46.91%)	4 (18.18%)	8 (23.53%)

(*continued*)

Table 1 (*Continued*)

Variables	All patients (N = 180)	CR1 (N = 43)	CR2 (N = 81)	CR3 ≥3 (N = 22)	Active disease (N = 34)
SIRO	1 (0.56%)	0 (0%)	0 (0%)	0 (0%)	1 (2.94%)
SIRO+TACRO	27 (15%)	4 (9.3%)	5 (6.17%)	8 (36.36%)	10 (29.41%)
TACRO	5 (2.78%)	2 (4.65%)	2 (2.47%)	0 (0%)	1 (2.94%)

TX indicates transplantation; KPS, Karnofski performance status; Neg, negative; Pos, positive; TCD, T-cell depletion; CSA, cyclosporine A; MMF, mycophenolate mofetil; TACRO, tacrolimus; MTX, methotrexate; SIRO, sirolimus.

20 days and 16 days in BM and PBSC recipients, respectively. The cumulative incidence of neutrophil engraftment at day 42 and day 60 was 88.9% (83.2-92.7) and 90% (95% confidence interval [CI], 84.5-93.6) respectively. The cumulative incidence of 60 days platelet engraftment was 85.6% (95% CI, 77.7-90.8).

Of the 17 patients who experienced graft failure, the majority were transplanted in active disease status (n = 8), 4 were CR1, and the remaining 5 were in CR2. At last follow-up, 3 patients were alive, 1 after DLI infusion and 1 after a second HCT from the same donor, and 14 patients died (3 after receiving a second HCT from a different donor) at a median of 55 days after haplo-HCT (Supplemental Table S1). No cases of secondary graft failure were reported.

Cumulative incidence of day 100 grade II-IV and grade III-IV aGVHD was 28.3% (95% CI, 21.7%-35.2%) and 12.4% (95% CI, 7.9%-17.8%), respectively. The majority of patients experienced grade II aGVHD (n = 30), whereas grade III and grade IV aGVHD were reported in 14 and 9 patients, respectively. Cumulative incidence of 2 years chronic GVHD was 21.9% (95% CI, 16%-29%), and the extensive involvement was 9.5% (95% CI, 5.3%-15%).

When analyzing the group of patients transplanted in CR1 and CR2, for BM and PBSC recipients cumulative incidence of grade II-IV acute GVHD was 28.1% (95%CI 17.7-39.5%) and 37.7% (95%CI 24.8-50.6%), P = .14, and cumulative incidence of grade III-IV aGVHD was 6.2% and 18.9%, P=.04, respectively.

Relapse and Non-Relapse Mortality

Disease recurrence was the most common cause of treatment failure. Cumulative incidence of relapse at 2 years was 25.1%, 37%, and 50.3% for patients in CR1, CR2,and CR3+ and 70.1% for active disease, respectively (Figure 1A).

The overall cumulative incidence of NRM at 2 years was 19.6% (95% CI, 14%-26%) for the whole cohort. In the group of patients transplanted in CR1 and CR2, NRM was 9.8% and 19.1% for patients in CR1 and CR2, respectively (Table 2, Figure 1B). Cumulative incidence of NRM was significantly

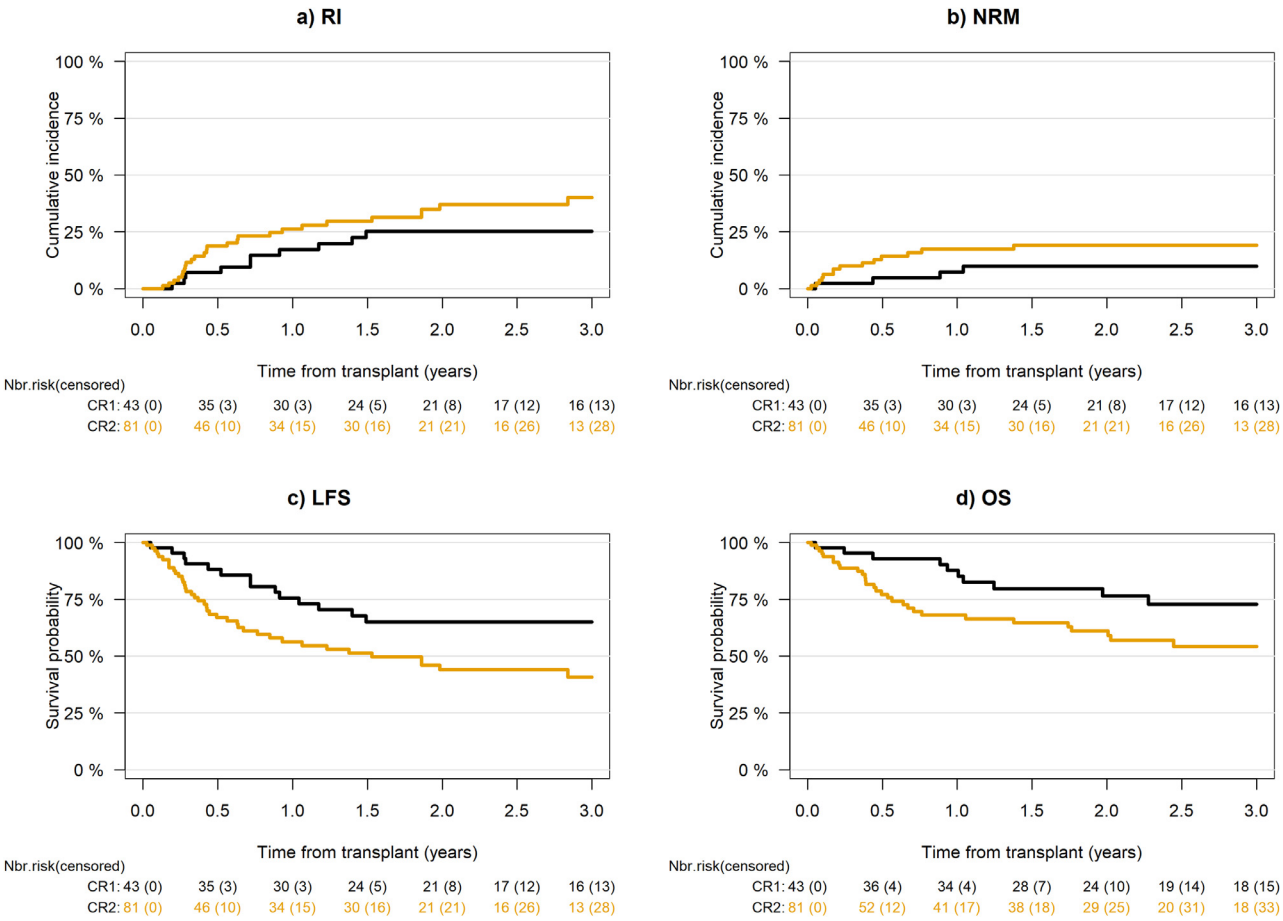

Figure 1. (A) Two-year cumulative RI by CR1 and CR2. (B) Two-year cumulative incidence NRM by CR1 and CR2. (C) Two-year probability of LFS by CR1 and CR2. (D) Two-year probability of OS by CR1 and CR2.

Table 2
Univariate analysis for patients in CR1 and CR2

Variable	2y OS	2y LFS	2y RI	2y NRM	100d AGVH II-IV	2y GRFS	2y CGVH
Disease status							
CR1	76.5% [59.5-87.1]	65% [48.1-77.7]	25.1% [12.8-39.5]	9.8% [3.1-21.3]	47.5% [31.3-62.1]	53.6% [37.2-67.5]	33.2% [18.8-48.3]
CR2	61.2% [48.4-71.7]	44% [31.6-55.6]	37% [25.2-48.7]	19.1% [10.9-29]	24.7% [15.7-34.8]	30.9% [20.1-42.4]	21.5% [12.1-32.6]
P value	.03	.01	.11	.16	.03	.04	.13
Karnofski performance status							
<90	52.1% [26-73]	47.3% [22.6-68.7]	30.3% [10.1-53.7]	22.4% [6.4-44.2]	26.3% [9.2-47.4]	24.7% [8-46]	27.5% [7.2-52.9]
≥90	69.3% [58.3-77.9]	51.1% [39.9-61.2]	33.7% [23.7-43.9]	15.2% [8.7-23.4]	33% [23.7-42.6]	42.1% [31.4-52.3]	23.2% [14.7-32.9]
P value	.27	.67	.99	.53	.98	.27	.73
Patient age							
[0-13]	71.6% [60-80.4]	54.4% [42.4-64.8]	30.9% [20.8-41.6]	14.7% [8-23.4]	34.1% [24.2-44.2]	41.9% [30.6-52.9]	28.6% [18.6-39.3]
[13-18[	54.5% [35.2-70.3]	44.3% [26.1-61]	37.1% [19.8-54.4]	18.6% [7.3-34]	28.1% [13.8-44.4]	33.3% [17.9-49.5]	18.6% [6.4-35.6]
P value	.05	.25	.46	.56	.65	.29	.35
Gender							
Male	64.2% [51.5-74.4]	47.3% [35-58.7]	36.2% [24.8-47.8]	16.4% [8.9-25.9]	28.8% [18.8-39.4]	36.6% [25.2-48.2]	29.6% [18.8-41.1]
Female	71.3% [53.8-83.1]	59.8% [42.7-73.3]	25.7% [13.1-40.5]	14.5% [5.7-27]	38.6% [24.3-52.8]	44.7% [29-59.2]	19.4% [8.3-33.9]
P value	.43	.56	.51	.91	.31	.96	.24
Female-to-male							
No	70.7% [59.3-79.4]	55.8% [44.2-65.8]	29.4% [19.7-39.7]	14.9% [8.3-23.2]	35.6% [25.8-45.4]	40.5% [29.8-51]	23.9% [14.9-34.2]
Yes	55.9% [35.3-72.3]	40.3% [21.8-58.1]	41.5% [22.2-59.7]	18.3% [6.4-35]	22.2% [8.8-39.4]	37.1% [19.3-55.1]	31.6% [14.5-50.2]
P value	.09	.57	.57	.81	.33	.69	.30
CMV Donor							
Neg	61.7% [30.5-82.2]	63.6% [33-83.1]	21.3% [4.7-45.8]	15.2% [2.1-39.7]	35.7% [12.2-60.4]	44.4% [18.9-67.4]	30.3% [8.2-56.5]
Pos	65.4% [54.1-74.5]	47.6% [36.6-57.8]	35.1% [24.9-45.4]	17.3% [10.3-25.8]	30.9% [21.8-40.3]	35.9% [25.8-46]	24% [15.2-34]
P value	.86	.33	.41	.74	.49	.84	.59
Regimen							
MAC/Chemo	60% [43.8-72.8]	43.6% [29.5-56.9]	38% [24.4-51.6]	18.4% [9.3-29.8]	40% [27-52.7]	25.1% [13.9-38]	22.7% [11.5-36.2]
MAC/TBI	64.1% [45.1-78]	65.3% [46.7-78.8]	17.2% [6.8-31.6]	17.5% [6.9-32.2]	28.6% [14.7-44.1]	54.2% [36.3-69.1]	30.7% [15.7-47.1]
RIC	80.8% [59.8-91.5]	54% [33.4-70.7]	38.3% [19.8-56.6]	7.7% [1.3-22.2]	22.2% [8.8-39.4]	52.3% [31.5-69.5]	25.9% [10.1-45.1]
P value	.59	.11	.12	.38	.17	<.01	.55
Stem cell source							
BM	77.5% [64.8-86]	57.6% [44.3-68.7]	34.7% [23.1-46.6]	7.8% [2.8-16]	28.1% [17.7-39.5]	48.2% [35.2-60.2]	22.2% [12.5-33.7]
PB	51.1% [35.1-65.1]	43.2% [27.8-57.6]	30.3% [16.9-44.9]	26.5% [14.8-39.9]	37.7% [24.8-50.6]	28.3% [16.1-41.8]	31.8% [18-46.4]
P value	**.01**	.13	.44	<.01	.14	.01	.37

Neg indicates negative; Pos, positive.

higher in PBSC recipients 26.5% (95% CI, 14.5%-39.9%) versus BM 7.8% (95% CI, 2.8%-16%), $P < .001$. A total of 86 patients died: 56 of relapsed disease and 30 of NRM. The most common cause of NRM was infections (n = 10), followed by GVHD (n = 8), interstitial pneumonia (n = 3), veno-occlusive disease (n = 2), and other HSCT-related causes (n = 7).

LFS, GRFS, and OS

Median follow-up duration was 2.7 (95% CI, 2.2-3.2) years. In the whole group, the probability of 2-year OS, LFS, and GRFS was 50.8% (95% CI, 42.6%-58.4%), 38.5% (95% CI, 30.8%-46.2%), and 29.2% (95% CI, 22.2%-36.5%).

The probability of 2-year LFS was 38.5% (95% CI, 30.8%-46.2%), being 65% (95% CI, 48.1%-77.7%) for patients in CR1, 44% (95% CI, 31.6%-55.6%) for patients in CR2 (Figure 1C), and 18.8% (95% CI, 4.8%-39.8%) for patients in CR3+; 1-year LFS was 3.2% (95% CI, 0.2%-14%) for patients in active disease. The probability of OS at 2 years was 76.5% for patients in CR1 and 61.2% for those in CR2 (P = .03) (Figure 1D and Supplemental Figure S1).

In patients transplanted in CR1 and CR2, according to the type of conditioning regimen, the 2-year LFS was 65.3% in recipients of MAC TBI-based, 43.6% in MAC-chemotherapy-based, and 54% for RIC, respectively (P = .12) (Table 2). The probability of GRFS at 2 years was 53.6% for patients in CR1 and 30.9% for those in CR2 (P = .04).

Multivariate Analysis for the Patients Transplanted in CR1 and CR2

Multivariate analysis was conducted in patients transplanted either in first or second complete remission (Table 3). Use of PBSC was the only significant factor associated with a significant increased risk of NRM (HR = 3.13, 95% CI, 1.06-9.18; P = .04).

Disease status at haplo-HCT was the only independent factor significantly associated with LFS: CR2 versus CR1 (HR = 2.04, 95% CI, 1.1-3.8; P = .02. Use of TBI-based MAC (HR = 0.51, 95% CI, 0.28-0.92; P = .03) and RIC (HR = 0.46, 95% CI, 0.24-0.89; P = .02) was independently associated with higher GRFS compared to MAC chemotherapy based.

Factors significantly associated with lower OS were CR2 versus CR1 (HR = 2.19, 95% CI, 1.05-4-55; P = .04), age at HCT older than 13 years (HR = 2.07, 95% CI, 1.1-3.91; P = .04), and use of PBSC (HR = 1.98, 95% CI, 1.03-3.81; P = .04) (Figure 2).

DISCUSSION

Our study aimed to report outcomes and their risk factors after Haplo-HCT with PT-Cy in pediatric patients with ALL. To date, there are very few reports published on the use of Haplo-HCT for children with ALL and are limited to a few cases. Published data report the feasibility of this procedure in children with malignant disorders [12,13]. Our series on 180 children with ALL, both receiving MAC and RIC Haplo-HCT showed favorable outcomes of PT-Cy–based GVHD prophylaxis, namely for patients in disease remission. PT-Cy is effective in preventing severe GVHD in children with leukemia receiving unmanipulated HLA-haploidentical transplant, without an excess of NRM.

Disease status remains the most important prognostic factor influencing the risk of disease recurrence and the probability of LFS and OS. Our study was retrospective and registry based, and a high proportion of patients underwent transplantation in active disease status and with relapsed ALL, which usually are associated with poor outcomes [14]. Outcomes of patients transplanted in CR3+ remain poor because of a high

Table 3
Multivariate analysis for patients in CR1 and CR2

Variable	OS		LFS		RI		NRM		GRFS	
	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Disease status										
CR1	1		1		1		1		1	
CR2	2.19 (1.05-4.55)	.04	2.04 (1.1-3.8)	.02	1.91 (0.91-4.01)	.09	2.22 (0.71-6.94)	.17	1.67 (0.97-2.88)	.06
Age at Haplo-HCT										
[0-13]	1		1		1		1		1	
[13-18]	2.07 (1.1-3.91)	.03	1.53 (0.87-2.71)	.14	1.5 (0.75-3.01)	.25	1.58 (0.58-4.28)	.37	1.5 (0.9-2.51)	.12
Stem cell source										
BM	1		1		1		1		1	
PB	1.98 (1.03-3.81)	.04	1.32 (0.75-2.3)	.34	0.86 (0.43-1.75)	.68	3.13 (1.06-9.18)	.04	1.56 (0.95-2.57)	.08
Conditioning regimen										
MAC/Chemo	1		1		1		1		1	
MAC/TBI	0.89 (0.43-1.84)	.76	0.56 (0.28-1.09)	.09	0.4 (0.16-1.01)	.05	0.88 (0.32-2.45)	.81	0.51 (0.28-0.92)	.03
RIC	0.72 (0.31-1.65)	.44	0.64 (0.32-1.27)	.2	0.68 (0.31-1.47)	.33	0.45 (0.09-2.2)	.33	0.46 (0.24-0.89)	.02

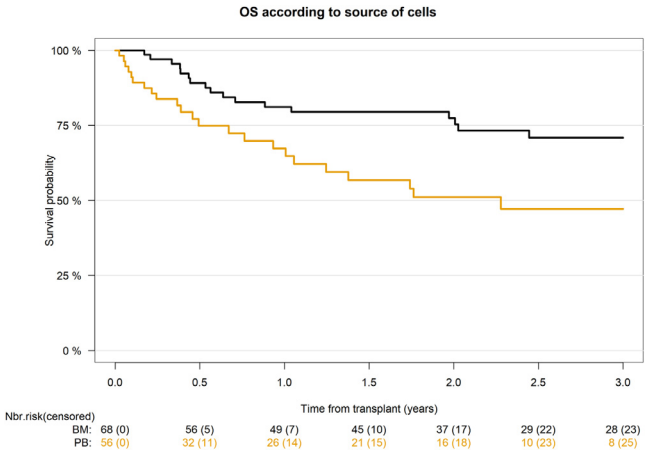

Figure 2. Two-year probability of OS by stem cell source.

incidence of subsequent relapse. Finally, results for patients transplanted in complete remission 1 and 2 were better in terms of disease recurrence and overall results, highlighting the importance of early referral to transplant once the indication is made.

Strategies for obtaining effective disease control before transplantation and to reduce transplant-related toxicity and death are desirable to improve results in pediatric patients with ALL. Importantly, the availability of targeted therapies, such as anti-CD19 or anti CD22 monoclonal antibodies, and the CD19-CAR-T cells may dramatically change the results and the place of HCT in such diseases [15].

On patients in CR1 and CR2, our results confirmed the prognostic impact of age younger than 13 years in overall survival, although benefits have been reported when the adolescent population was treated within the pediatric cooperative group trials [16].

The choice of conditioning regimen should be carefully evaluated taking into consideration the disease risk, as well patient-related factors [17]. In our study, the conditioning regimen was performed according to transplant center policy, and it would be possible that among the 41 patients receiving a reduced-intensity regimen, poor performance status, organ dysfunction, or other clinical conditions may have impacted that decision. We should continue to collect data and to address whether the regimen intensity may influence outcomes and for which population. Additionally, the duration of immunosuppression after HCT could be shorter in RIC recipients to further reduce the risk of disease recurrence and enhance immune recovery. Comparative trials among RIC and MAC in patients eligible to receive both treatments are needed to clearly highlight the place of RIC regimens in childhood ALL.

In the myeloablative setting, recently the results of the "FORUM" prospective randomized trial [18] showed the advantage of the use of TBI-based myeloablative regimens with significant reduction in relapse risk in patients with ALL older than 4 years of age. In our study, when analyzing the patients in CR1 and CR2, the use of a TBI-based MAC regimen was associated with a significantly higher GRFS.

Another important strategy for preventing relapse is the use of post-transplantation target drugs or adoptive immunotherapy [19] that could be performed in a timely manner in the haploidentical HCT setting, because family donors can be rapidly available for subsequent donation when needed [20].

A low incidence of acute GVHD has been reported [7,21] in the setting of unmanipulated haplo-HCT. Of note in our series, 9.6% of patients, namely recipients of PBSC, received the combination of PT-Cy with ATG. One may argue that this strategy has been adopted to decrease the risk of GVHD in the setting of HLA-mismatched HCT, especially in the pediatric population. Furthermore, the action of PT-Cy in preventing GVHD after BM graft has been recently described, not only based on the selective induction of tolerance and intrathymic clonal deletion of allo-reactive T lymphocytes, but also through specific effects on T-cell subsets such as T-regs [22].

In the group of patients transplanted in CR1 and CR2, the use of PBSC as a stem cell source was associated with significantly lower OS and higher NRM. This is an important finding because the graft cell source is a modifiable factor, meaning that clinicians can make the option to choose bone marrow as the preferred graft source according to patient and disease characteristics. Our results are different from findings reported in the adults [21]; however, we acknowledge that our series is not comparable, reporting only children receiving MAC regimen in the majority of cases. More focused comparisons of graft sources taking into account differences among children and adults are warranted. We were not able to detect statistically significant differences in GVHD according to the stem cell source, despite the higher incidence in PBSC recipients. The lack of difference could be related to the low number of GVHD events found in our series; however, the toxicity and morbidity related to the GVHD ultimately may have an impact in the NRM and OS. Low incidence of chronic GVHD has been reported by a single-center study with the use of G-CSF–primed unmanipulated BM together with harvested PBSC from haploidentical donor with PT-Cy; however, larger number are needed to clearly highlight the possible effect of the combination of the stem cell source [23]. We are aware of the limitations of our study given its retrospective nature. Therefore we should have caution for any conclusion for the analysis of relapse because of the lack of minimal residual disease status before HCT. Also the burden of post-HCT long-term complications should be carefully considered, especially in the pediatric setting to avoid quality-of-life impairment [24].

Also, we were unable to collect data on infectious complications and immune reconstitution to evaluate the efficacy of PT-Cy in the naïve and memory T-cell compartment, which is important also to address the relative benefit of PT-Cy–based approaches over the T-cell α/β–depleted strategy.

Curiously, despite the impact of donor kinship being associated with a decreased risk of relapse, when the mother was the

haplo donor [6], this finding was not systematically demonstrated [25] in the setting of haplo-HCT using PT-Cy.

In summary we have confirmed the dismal prognosis of patients transplanted in active disease and CR3+. For patients transplanted in CR1 and CR2, our results show the feasibility of Haplo-HCT with PT-Cy in children with ALL [6], and importantly we have described outcomes and their risk factors after this strategy in the largest series described so far. The use of BM cells as a graft source and remission status of ALL at transplantation are the most important factors associated with main outcomes. How the PT-Cy strategy might compare with the results of the new techniques in the T-cell depleted haploidentical setting, such as the selective depletion of the α/β T- and B-cells [2], or of unrelated donors HCT, needs to be carefully evaluated, also taking into account the cost-effectiveness of the strategies and the patients' quality of life.

ACKNOWLEDGMENTS

The authors thank the clinical staff and investigators involved in this research (Andrea Pession Azienda Ospedaliero Universitaria, Policlinico S. Orsola-Malpighi Bologna, Bologna, Italy, Jean-Hugues Dalle Hôpital Robert Debre, Pediatric Hematology and Immunology Department, Paris, France, Andrew McDonald Alberts cellular therapy, Netcare Pretoria East Hospital, Pretoria, South Africa, Cristina Diaz de Heredia Hospital Vall d`Hebron, Servicio de Hematologia y Oncologia Pediétrica, Barcelona, Spain, Ali Bazarbachi American University of Beirut Medical Center, Department of Internal Medicine, Beirut, Lebanon, Domenico Russo USD Trapianti di Midollo, Adulti, Universita di Brescia, Brescia, Italy, Tunc Fisgin Altinbas University, Faculty of Medicine, Bahçelievler Medicalpark Hospital, Pediatric Bone Marrow Transplantation Unit, Istanbul, Alexei Maschan Federal Research Center for Pediatric Hematology, Oncology and Immunology, Moscow, Russia, Mahmoud Aljurf King Faisal Specialist Hospital & Research Centre, Oncology (Section of Adult Haematolgy/BMT), Riyadh, Saudi Arabia, Peter J. Shaw The Children`s Hospital at Westmead, Oncology Unit, Sydney, Australia, Tayfun Güngör University Children`s Hospital, The hospital of the eleonore foundation, Division of Stem Cell Transplantation, Steinwiesst, Zurich, Switzerland, H. Emel Ozyurek Samsun Medicalpark Hastanesi, Pediatric Hematology, Atakum, Turkey, Gaelle Guillerm C.H.R.U de Brest, Service Onco-Hematologie, Brest, France, Anca Colita Fundeni Clinical Institute, Pediatric Bone Marrow Transplantation, Bucharest, Romania, Jan Styczynski University Hospital, Collegium Medicum UMK, Pediatric Hematology and Oncology, Bydgoszcz, Poland, Estelle Verburgh University of Cape Town Faculty of Health Sciences, Division of Clinical Haematology, Cape Town, South Africa, Larisa Fechina Regional Children`s Hospital No. 1, Department of Bone Marrow Transplantation, Ekaterinburg, Russia, Nadezda Basara St. Franziskus Hospital, Medizinische Klinik I, Flensburg, Germany, Claudia Sossa FOSCAL-UNAB, Urbanización El Bosque, Floridablanca, Colombia, Emanuele Angelucci Ospedale San Martino, Department of Haematology II, Genova, Italy, Manuel Jurado Chacón Hospital Univ. Virgen de las Nieves, Servicio de Hematología, Granada, Spain, Zafer Gülbas Anadolu Medical Center Hospital, Bone Marrow Transplantation Department, Kocaeli, Turkey, Yves Bertrand Institut d`Hematologie et d`Oncologie Pediatrique, Lyon, France, Antonio Perez Martinez Hospital Universitario La Paz, Hematologia-Oncologia, Madrid, Spain, Cristina Belendez Hospital Univ.Materno Infantil Gregorio Marañon, Onhcohematologia Pediatrica, Madrid, Spain, Gérard Michel Hopital d`Enfants de la Timone, CHU, Département Hématologie Oncologie Pédiatrique, Marseille, France, Marco Zecca Fondazione IRCCS Policlinico San Matteo, Pediatric Hematology-Oncology, Pavia, Italy, Giuseppe Visani AORMN Hospital, Hematology & Transplant Centre, Pesaro, Italy, Eduard Forcade Hôpital Hautleveque, CHU Bordeaux, Pessac, France, Mario Petrini Azienda Ospedaliero Universitaria Pisana, Unità Operativa Ematologia, Pisa, Italy, Mercedes Colorado Araujo Hospital U. Marqués de Valdecilla, Servicio de Hematología-Hemoterapia, Santander, Spain, Jose Antonio Pérez-Simón Hospital Universitario Virgen del Rocío, Servicio de Hematologia y Hemoterapia, Sevilla, Spain, Patrizio Mazza Ospedale Nord, Institute of Haematology, Taranto, Italy) and especially thank the patients who took part.

Financial disclosure: None.

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

Authorship statement: A.R., V.R., and S.C. designed the study. I.M., F.F., A.Y., A.T., J.M.F.N., M.F., F.L., and V.R., were the principal investigators at the centers recruiting the highest number of patients for the study. A.D. was responsible for data management. J.E.G. performed the statistical analysis. A.R. wrote the manuscript. All the authors reviewed and approved the final version of the manuscript.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.jtct.2021.01.016.

REFERENCES

1. Passweg JR, Baldomero H, Chabannon C, et al. The EBMT activity survey on hematopoietic-cell transplantation and cellular therapy 2018: CAR-T's come into focus. *Bone Marrow Transplant*. 2020;55(8):1604–1613.
2. Luznik L, O'Donnell PV, Symons HJ, et al. HLA-haploidentical bone marrow transplantation for hematologic malignancies using nonmyeloablative conditioning and high-dose, posttransplantation cyclophosphamide. *Biol Blood Marrow Transplant*. 2008;14(6):641–650.
3. Symons HJ, Zahurak M, Cao Y, et al. Myeloablative haploidentical BMT with posttransplant cyclophosphamide for hematologic malignancies in children and adults. *Blood Adv*. 2020;4(16):3913–3925.
4. Nagler A, Ruggeri A. Haploidentical stem cell transplantation (HaploSCT) for patients with acute leukemia-an update on behalf of the ALWP of the EBMT. *Bone Marrow Transplant*. 2019;54(Suppl 2):713–718.
5. Bader P, Salzmann-Manrique E, Balduzzi A, et al. More precisely defining risk peri-HCT in pediatric ALL: pre- vs post-MRD measures, serial positivity, and risk modeling. *Blood Adv*. 2019;3(21):3393–3405.
6. Berger M, Lanino E, Cesaro S, et al. Feasibility and outcome of haploidentical hematopoietic stem cell transplantation with post-transplant high-dose cyclophosphamide for children and adolescents with hematologic malignancies: an AIEOP-GITMO retrospective multicenter study. *Biol Blood Marrow Transplant*. 2016;22(5):902–909.
7. Klein OR, Buddenbaum J, Tucker N, et al. Nonmyeloablative haploidentical bone marrow transplantation with post-transplantation cyclophosphamide for pediatric and young adult patients with high-risk hematologic malignancies. *Biol Blood Marrow Transplant*. 2017;23(2):325–332.
8. Ruggeri A, Labopin M, Ciceri F, Mohty M, Nagler A. Definition of GvHD-free, relapse-free survival for registry-based studies: an ALWP-EBMT analysis on patients with AML in remission. *Bone Marrow Transplant*. 2016;51(4):610–611.
9. Martino R, Romero P, Subirá M, et al. Comparison of the classic Glucksberg criteria and the IBMTR Severity Index for grading acute graft-versus-host disease following HLA-identical sibling stem cell transplantation. International Bone Marrow Transplant Registry. *Bone Marrow Transplant*. 1999;24(3):283–287.
10. Jagasia MH, Greinix HT, Arora M, et al. National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: I. The 2014 Diagnosis and Staging Working Group Report. *Biol Blood Marrow Transplant*. 2015;21(3):389–401. e1.
11. Gooley TA, Leisenring W, Crowley J, Storer BE. Estimation of failure probabilities in the presence of competing risks: new representations of old estimators. *Stat Med*. 1999;18(6):695–706.
12. Jaiswal SR, Chakrabarti A, Chatterjee S, et al. Haploidentical peripheral blood stem cell transplantation with post-transplantation cyclophosphamide in children with advanced acute leukemia with fludarabine-, busulfan-, and melphalan-based conditioning. *Biol Blood Marrow Transplant*. 2016;22(3):499–504.
13. Saglio F, Berger M, Spadea M, et al. Haploidentical HSCT with post transplantation cyclophosphamide versus unrelated donor HSCT in pediatric patients affected by acute leukemia [e-pub ahead of print September 23,

2020]. *Bone Marrow Transplant*. 2021. https://doi.org/10.1038/s41409-020-01063-2.
14. Balduzzi A, Dalle J-H, Wachowiak J, et al. Transplantation in children and adolescents with acute lymphoblastic leukemia from a matched donor versus an HLA-identical sibling: is the outcome comparable? Results from the International BFM ALL SCT 2007 Study. *Biol Blood Marrow Transplant*. 2019;25(11):2197–2210.
15. McNeer JL, Rau RE, Gupta S, Maude SL, O'Brien MM. Cutting to the front of the line: immunotherapy for childhood acute lymphoblastic leukemia. *Am Soc Clin Oncol Educ Book*. 2020;40:1–12.
16. Boissel N, Baruchel A. Acute lymphoblastic leukemia in adolescent and young adults: treat as adults or as children? *Blood*. 2018;132(4):351–361.
17. Willasch AM, Peters C, Sedláček P, et al. Myeloablative conditioning for allo-HSCT in pediatric ALL: FTBI or chemotherapy?-A multicenter EBMT-PDWP study. *Bone Marrow Transplant*. 2020;55(8):1540–1551.
18. Peters C, Dalle J-H, Locatelli F, et al. Total body irradiation or chemotherapy conditioning in childhood ALL: a multinational, randomized, noninferiority phase III study. *J Clin Oncol*. 2021;39(4):295–307.
19. Pulsipher MA, Langholz B, Wall DA, et al. Risk factors and timing of relapse after allogeneic transplantation in pediatric ALL: for whom and when should interventions be tested? *Bone Marrow Transplant*. 2015;50(9):1173–1179.
20. Dholaria B, Savani BN, Labopin M, et al. Clinical applications of donor lymphocyte infusion from an HLA-haploidentical donor: consensus recommendations from the Acute Leukemia Working Party of the EBMT. *Haematologica*. 2020;105(1):47–58.
21. Ruggeri A, Labopin M, Bacigalupo A, et al. Bone marrow versus mobilized peripheral blood stem cells in haploidentical transplants using posttransplantation cyclophosphamide. *Cancer*. 2018;124(7):1428-1437.
22. Radojcic V, Luznik L. Mechanism of action of posttransplantation cyclophosphamide: more than meets the eye. *J Clin Invest*. 2019;129(6):2189–2191.
23. Uygun V, Karasu G, Daloğlu H, et al. Haploidentical hematopoietic stem cell transplantation with post-transplant high-dose cyclophosphamide in high-risk children: A single-center study. *Pediatr Transplant*. 2019;23(7):e13546.
24. Yen H-J, Eissa HM, Bhatt NS, et al. Patient-reported outcomes in survivors of childhood hematologic malignancies with hematopoietic stem cell transplant. *Blood*. 2020;135(21):1847–1858.
25. Ciurea SO, Al Malki MM, Kongtim P, et al. The European Society for Blood and Marrow Transplantation (EBMT) consensus recommendations for donor selection in haploidentical hematopoietic cell transplantation. *Bone Marrow Transplant*. 2020;55(1):12–24.