Biol Blood Marrow Transplant 26 (2020) 358–366

Biology of Blood and Marrow Transplantation

journal homepage: www.bbmt.org

Haploidentical

Prospective Randomized Study Comparing Myeloablative Unrelated Umbilical Cord Blood Transplantation versus HLA-Haploidentical Related Stem Cell Transplantation for Adults with Hematologic Malignancies

Jaime Sanz[1,2,3,*], Juan Montoro[1], Carlos Solano[3,4], David Valcárcel[5], Antonia Sampol[6], Christelle Ferrá[7], Rocío Parody[8], Ignacio Lorenzo[1,2], Pau Montesinos[1,2], Guillermo Ortí[5], Juan C. Hernández-Boluda[3,4], Aitana Balaguer-Roselló[1], Manuel Guerreiro[1], Carlos Carretero[1], Guillermo F. Sanz[1,2,3], Miguel A. Sanz[1,2,3], José Luis Piñana[1,2]

[1] *Hematology Department, Hospital Universitari i Politecnic La Fe, Instituto de Investigación Sanitaria La Fe, Valencia, Spain*
[2] *Centro de Investigación Biomédica en Red de Cancer, Instituto Carlos III, Madrid, Spain*
[3] *Department of Medicine, University of Valencia, Valencia, Spain*
[4] *Hematology Department, Hospital Clínico Universitario de Valencia, Valencia, Spain*
[5] *Hematology Department, Hospital Universitari Vall dHebron, Barcelona, Spain*
[6] *Hematology Department, Hospital Universitari Son Espases, Palma de Mallorca, Spain*
[7] *Hematology Department, Institut Català d'Oncologia, Institut de Recerca contra la Leucemia Josep Carreras, Hospital Germans Trias i Pujol, Universidad Autónoma de Barcelona, Badalona, Spain*
[8] *Hematology Department, Instituto Catalán de Oncología–Hospital Duran i Reynals, Barcelona, Spain*

Article history:
Received 17 July 2019
Accepted 12 October 2019

Keywords:
Umbilical cord blood transplantation
Haploidentical transplantation
Alternative donor transplantation
Hematologic malignancy
Allogeneic stem cell transplantation clinical trial

A B S T R A C T

In this prospective randomized study, we compared the outcomes of single-unit umbilical cord blood transplantation (UCBT) and unmanipulated haploidentical stem cell transplantation (haplo-SCT) with post-transplantation cyclophosphamide (PTCy) in adults with hematologic malignancies. All patients received a myeloablative conditioning (MAC) regimen consisting of thiotepa, busulfan, and fludarabine, with antithymocyte globulin (ATG) added for UCBT recipients. Nineteen patients were randomized to UCBT and the other 26 to haplo-HSCT. Four patients (15%) allocated to the haplo-HSCT arm lacked a suitable donor and were crossed over to the UCBT arm. Finally, 23 underwent UCBT and 22 underwent haplo-HSCT. The cumulative incidence of neutrophil recovery was 87% at a median of 19 days (range, 13 to 24 days) in the UCBT arm versus 100% at a median of 17 days (range, 13 to 25 days) in the haplo-SCT arm (P = .04). Platelet recovery was 70% at a median of 40 days (range, 18 to 129 days) in the UCBT arm versus 86% at a median of 24 days (range, 12 to 127 days) in the haplo-HCT arm (P = .02). Rates of acute graft-versus-host disease (GVHD) grade II-IV or grade III-IV, overall chronic GVHD, and extensive chronic GVHD in the UCBT and Haplo-SCT arms were 43% versus 36% (P = .8), 9% versus 9% (P = 1), 66% versus 43% (P = .04), and 41% versus 23% (P = .2), respectively. Two-year nonrelapse mortality and relapse in the 2 arms were 52% versus 23% (P = .06) and 17% versus 23% (P = .5), respectively. Two-year disease-free survival, overall survival, and GVHD/relapse-free survival in the 2 arms were 30% versus 54% (P = .2), 35% versus 59% (P = .1), and 17% versus 40% (P = .04), respectively. Our data show that in the context of an MAC regimen, haplo-SCT with PTCy provides improved outcomes compared with ATG-containing single-unit UCBT.

INTRODUCTION

Over the past several decades, remarkable progress has been made in several areas of allogeneic hematopoietic stem cell transplantation (HSCT) that have extended the indications for this procedure to an increasing number of patients. One of the most important achievements has been the use of alternative stem cell sources and donors other than HLA-identical siblings or unrelated donors, in umbilical cord blood transplantation (UCBT) or haploidentical stem cell transplantation (haplo-SCT). These alternative transplantation strategies allow for universal donor availability and the ability to gain quick access to HSCT programs for those patients in need.

Financial disclosure: See Acknowledgments on page 365.
*Correspondence and reprint requests: Jaime Sanz, Servicio de Hematología, Hospital Universitari i politècnic La Fe, Avinguda Fernando Abril Martorell, 106, 46026 Valencia, Spain.
E-mail address: sanz_jai@gva.es (J. Sanz).

https://doi.org/10.1016/j.bbmt.2019.10.014
1083-8791/

The most recent surveys on transplantation activity recorded in the European Society for Blood and Marrow Transplantation and Center for International Blood and Marrow Transplant Research databases confirm a clear trend in donor choice, with increasing use of haplo-SCT and decreasing use of UCBT [1,2]. Although several factors may explain this trend, the number of studies comparing the 2 procedures are very limited, and the existing studies are mainly retrospective. A pilot study of the Blood and Marrow Transplant Clinical Trials Network (BMT CTN) compared the results of 2 parallel multicenter phase 2 trials for individuals with leukemia or lymphoma and no suitable related donor designed to evaluate the efficacy of UCBT (BMT CTN 0604) and haplo-HSCT (BMT CTN 0603) using reduced-intensity conditioning platforms at 27 transplantation centers in the United States [3]. This study showed lower transplantation- related mortality (TRM) in favor of haplo-SCT but a higher relapse rate, which ultimately resulted in similar efficacy in terms of disease-free survival (DFS) and a statistically nonsignificant 8% improvement in overall survival (OS) [3], setting the stage for a currently ongoing multicenter randomized clinical trial to assess the relative efficacy of these 2 strategies [4]. To complete the few reports available comparing both procedures, 2 subsequent retrospective registry-based studies of European Society for Blood and Marrow Transplantation (EBMT)-Eurocord have recently provided discordant results [5,6]. Although the first report did not find statistical differences in the main outcomes after unmanipulated haplo-HSCT and UCBT in patients with acute leukemia [5,6], the more recent study found that haplo-HSCT is associated with better OS compared with UCBT [5,6].

To assess the relative efficacy of haplo-HSCT and UCBT after myeloablative conditioning (MAC) regimens, we designed a multicenter prospective randomized study to compare the clinical outcomes in adults with hematologic malignancies using homogeneous transplantation platforms.

METHODS

Study Design

We performed an open-label, prospective, multicenter, randomized trial to compare the efficacy between UCBT and haplo-HSCT after MAC for patients with hematologic malignancies (ClinicalTrials.gov identifier NCT02386332). Patients were included from 6 EBMT centers. Randomization was done in a 1:1 ratio, stratifying by disease type (myeloid malignancies versus acute lymphoblastic leukemia) and disease stage at transplantation (early versus advanced stage). Patients were randomized at the time of indication for "alternative" transplantation. In cases not meeting the minimum criteria required for donor selection of the assigned group, cross-over was allowed to the other arm if eligible. Analysis was planned per treatment received and not on an intention-to-treat basis. The primary endpoint was DFS at 2 years after transplantation. Secondary endpoints included OS, risk of relapse, TRM, neutrophil and platelet engraftment, acute and chronic graft-versus-host disease (GVHD), GVHD and relapse-free survival (GRFS), reactivation of cytomegalovirus (CMV) and Epstein-Barr virus (EBV), immune reconstitution, and transfusion requirements. Enrollment began on April 1, 2015, with a target sample size of 206 patients to be included in 3 years. At the end of the study period, accrual was not met, and the study was closed for recruitment on March 31, 2018, after 45 patients were enrolled.

Eligibility Criteria

Patient characteristics required for study eligibility were age 18 to 55 years; a diagnosis of high-risk acute leukemia in complete remission, myelodysplastic syndrome, or chronic myelogenous leukemia (CML) with an indication for first allogeneic stem cell transplantation; adequate organ function; Eastern Cooperative Oncology Group performance status ≤2; and signed written informed consent.

Regarding graft characteristics, eligibility criteria for UCBT were (1) pre-cryopreserved total nucleated cell (TNC) dose $>15 \times 10^8$; (2) pre-cryopreserved $CD34^+$ cells $>7 \times 10^6$; (3) donor-recipient HLA match ≥ 4/6 at HLA-A, HLA-B (at low resolution using DNA- based typing), and HLA-DRB1 (at high resolution using DNA-based typing); and (4) absence in the receptor of preexisting IgG alloantibodies against the HLA antigens of the donor. Eligibility criteria for haplo-HSCT were (1) a first- or second-degree relative with 1 complete HLA haplotype shared between donor and recipient; (2) fulfillment of the usual criteria for healthy donor eligibility for mobilization with G-CSF and collection of peripheral blood or bone marrow harvest; and (3) absence in the receptor of preexisting IgG alloantibodies against the HLA antigens of the donor.

Treatment

Conditioning Regimen and GVHD Prophylaxis

All randomized patients received the same MAC regimen consisting of thiotepa (10 mg/kg), busulfan (9.6 mg/kg i.v.), and fludarabine (150 mg/m^2) [7-9]. The patients assigned to the UCBT arm also received antithymocyte globulin (ATG; Thymoglobulin 6 mg/kg; Genzyme Transplant, Cambridge, MA) [7].

For GVHD prophylaxis, patients allocated to the UCBT arm received cyclosporine starting on day -1 and continuing to day +180 (to maintain levels of 200 to 400 ng/mL) and prednisone (0.5 mg/kg/day on days +7 through +13, followed by 1 mg/kg/day on days +14 through +28 and discontinuation after the last dose on day +28). Patients allocated to the haplo-HSCT arm received cyclophosphamide ++(50 mg/kg/day i.v.) on days +3 and +4, cyclosporine to maintain a level of 200 to 400 ng/mL on days +5 to day +180, and mycophenolate mofetil 10 mg/kg/8 hours on days +5 to +35.

Supportive Care

G-CSF was initiated on day +7 at a dose of 5 μg/kg/day and continued until the absolute neutrophil count was $>1 \times 10^9$/L for 3 consecutive days. Procedures for chemotherapy-induced emesis, management of infections in neutropenic patients, and transfusion support in patients undergoing allogeneic HSCT were performed according to standard institutional guidelines. During neutropenia, patients were isolated in HEPA-filtered rooms. Mold-covering broad-spectrum antifungal prophylaxis was recommended until day +100 and in all patients receiving >15 mg/ day of prednisone for GVHD treatment.

For CMV management, from the beginning, each institution chose between preemptive antiviral therapy guided by PCR or universal prophylaxis strategies. The preemptive approach involved PCR monitoring twice weekly until day +100 and weekly until day +180 and initiation of oral valganciclovir for patients with confirmed detection of >400 copies/mL. Prophylaxis consisted of high-dose acyclovir 500 mg/m^2/8 hours i.v. until neutrophil recovery, followed by valganciclovir as described previously [10]. Once the strategy was chosen, it was maintained in both arms of the study. Treatment of CMV disease, CMV reinfections, and infections resistant to ganciclovir was provided in accordance with institutional standards. Cotrimoxazole was administered to all patients from day -8 through day -2 and then resumed at the time of engraftment until 1 year after transplantation or 3 months after cessation of immunosuppressive treatment, whichever occurred later. Quantification of EBV DNA by quantitative real-time PCR was done weekly from day +7 until day +180. In the event of a detected progressive increase in viral load, the decision to initiate early treatment with rituximab was made individually and according to the standard institutional guidelines of each participating center.

Definitions

Myeloid recovery was defined as the first day of an absolute neutrophil count of $\geq .5 \times 10^9$/L lasting for ≥3 consecutive days. Platelet recovery was defined as the first day of a platelet count of $\geq 20 \times 10^9$/L without transfusion support for 7 consecutive days. Patients who survived for >28 days after transplantation and who failed to achieve myeloid recovery were considered to have primary graft failure. Acute GVHD (aGVHD) and chronic GVHD (cGVHD) were defined and graded according to standard criteria [11-14]. Disease stage at the time of transplantation was classified as early stage, for patients in first complete remission [CR1] for acute leukemia or myelodysplastic syndromes or first chronic phase for CML, or advanced stage, for patients in second or further CR for acute leukemia or myelodysplastic syndromes or second or further chronic phase for CML. The Disease Risk Index (DRI) was used to stratify patients according to disease type and stage [15], and the validated Hematopoietic Cell Transplantation-Specific Comorbidity Index (HCT-CI) was used to stratify patients according to pretransplantation comorbidities [16] Nonrelapse mortality (NRM) was defined as death from any cause without evidence of relapse. DFS was defined as survival from the time of transplantation without evidence of disease relapse. Sinusoidal obstruction syndrome [17], CMV infection and disease [18], EBV-associated post-transplantation lymphoproliferative disorder (PTLD) [19], invasive fungal infection [20], and hemorrhagic cystitis were diagnosed according to consensus criteria.

Statistical Analysis

Our hypothesis was that NRM would be significantly lower but relapse significantly higher after haplo-HSCT versus UCBT, resulting in similar DFS in the 2 arms. The primary analysis was the comparison of DFS in each treatment arm. The target sample size was 103 patients per arm to detect a ≥20% difference in the probability of DFS at 2 years in either of the 2 arms

considering a type I error of 5% and a statistical power of 80%. Patient and transplantation characteristics were compared using the chi-square test for categorical variables and the Wilcoxon test for continuous variables. The probabilities of myeloid and platelet engraftment, aGVHD and cGVHD, TRM, relapse, sinusoidal obstruction syndrome, EBV-PTLD, CMV infection and disease, invasive fungal infection, and hemorrhagic cystitis were calculated using the method of cumulative incidence (competing risk) [21,22]. Cumulative incidence in competing risks data were considered as follows: for myeloid and platelet engraftment, early death or second HSCT with no evidence of engraftment; for aGVHD, death before day +100; for cGVHD, death without development of cGVHD; for NRM, relapse; for relapse, death with no previous relapse; for other post-transplantation events, death or relapse with no previous event. Unadjusted time-to-event analyses were performed using the Kaplan-Meier method [23] and the log-rank test for comparisons [24]. Survival data were calculated from the day of stem cell infusion. In the analysis of DFS, relapse or death in CR, whichever occurred first, was considered an uncensored event. For the analysis of GRFS, extensive cGVHD, relapse, graft failure, and death were considered uncensored events. Patient follow-up was updated on April 1, 2019. All patients were followed for at least 1 year or until death. Statistical analyses were conducted using R (The CRAN Project) [25].

RESULTS

Patient and Donor Characteristics

As shown in Figure 1, 19 and 26 patients were randomized to the UCBT and haplo-HSCT arms, respectively. Four of the patients (15%) allocated to haplo-HSCT did not have a suitable haploidentical donor available (3 due to absence of a first- or second-degree relative and 1 due to the presence of high titers of anti-HLA antibodies with a positive complement-binding test against the donor HLA antigens) and crossed over to a UCBT procedure. In contrast, all patients allocated to the UCBT arm had cord blood units meeting the minimum criteria for suitability. Finally, 23 patients underwent UCBT and 22 patients underwent haplo-HSCT.

Baseline patient characteristics are summarized in Table 1. The apparent differences between the 2 groups in some patient and disease characteristics (diagnosis, disease stage at

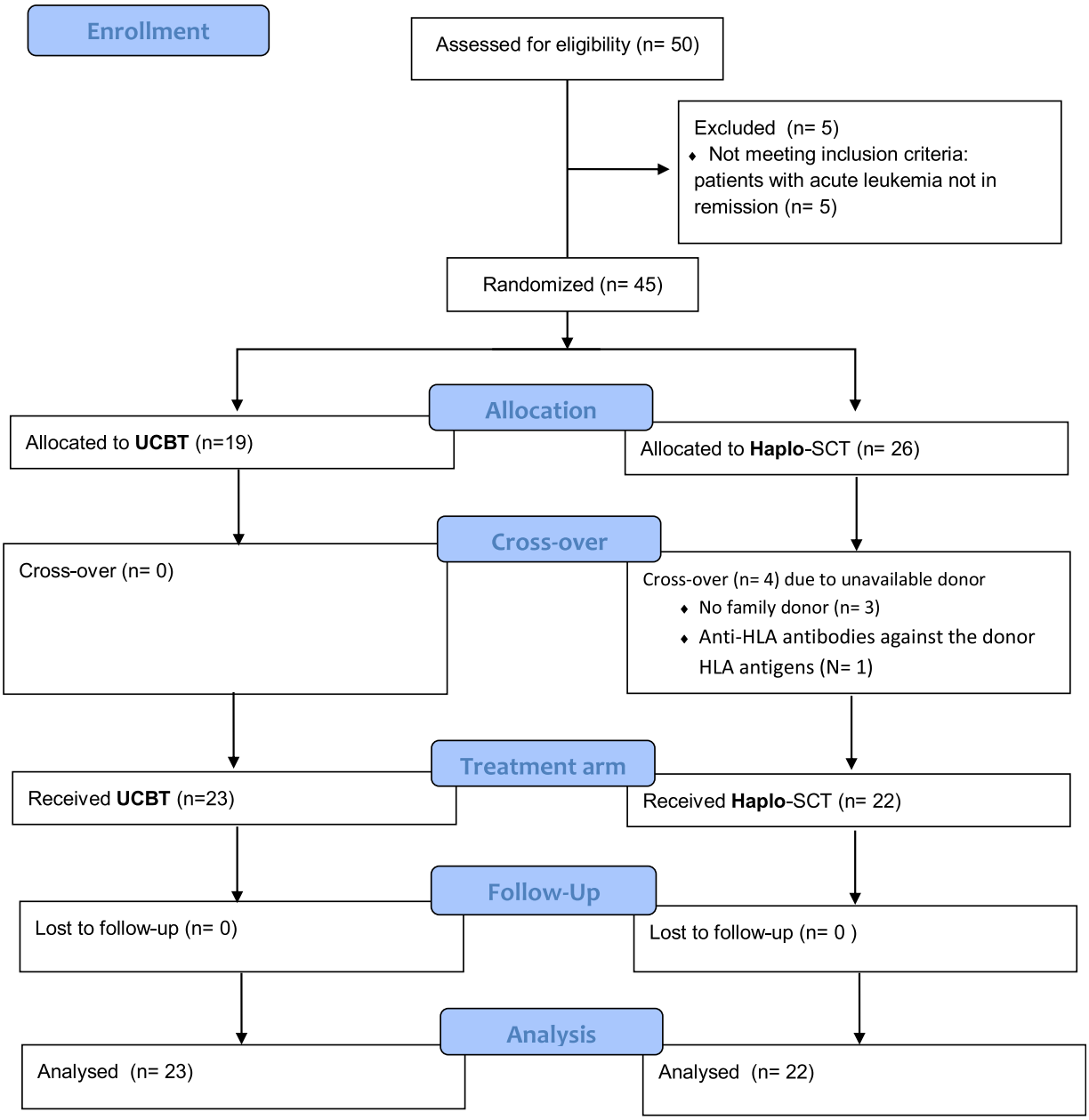

Figure 1. CONSORT flow diagram.

Table 1
Patient Characteristics

Characteristic	UCBT	Haplo-SCT	*P* Value
Number of patients	23	22	
Age, yr			.4
Median	45	41	
Range	24-54	18-55	
Sex, n (%)			.2
Male	11 (48)	15 (68)	
Female	12 (52)	3 (32)	
Weight, kg			.9
Median	68	69	
Range	45-108	46-97	
HCT-CI, n (%)			.7
0-2	13 (57)	11 (50)	
≥3	10 (44)	11 (50)	
Diagnosis, n (%)			.1
Acute myelogenous leukemia	12 (52)	14 (64)	
Acute lymphoblastic leukemia	4 (17)	7 (32)	
Myelodysplastic syndrome	1 (4)	0 (0)	
CML	6 (26)	1 (5)	
Disease stage at transplantation, n (%)			.4
CR1	12 (52)	10 (46)	
Advanced disease	11 (48)	12 (54)	
DRI, n (%)			.3
Low	1 (4)	4 (18)	
Intermediate	10 (44)	10 (46)	
High or very high	12 (52)	8 (36)	
Follow-up of surviving patients, mo			.4
Median	32	32	
Range	21-46	13-46	

Percentages might not sum to 100 because of rounding.

Table 2
Graft- and Transplantation-Related Characteristics

Characteristic	UCBT	Haplo-SCT	*P* Value
HLA compatibility, n (%)*			<.001
6 of 6	0 (0)	0 (0)	
5 of 6	4 (17)	0 (0)	
4 of 6	19 (83)	5 (23)	
3 of 6	0 (0)	17 (77)	
ABO blood group mismatch, n (%)			.3
Major	5 (22)	2 (9)	
Minor	8 (35)	5 (23)	
None	10 (44)	15 (68)	
Donor/recipient sex mismatch, n (%)			.3
Female donor to male recipient	7 (30)	2 (9)	
Other combinations	16 (70)	20 (91)	
Donor/recipient CMV serostatus, n (%)			<.001
+/+	0 (0)	14 (64)	
+/-	0 (0)	3 (14)	
-/+	16 (70)	4 (18)	
-/-	7 (30)	1 (5)	
Number of nucleated cells × 10^8/kg, median (range)			
At cryopreservation	.4 (.2-.8)	—	
At infusion	.3 (.2-.5)	—	
Number of $CD34^+$ cells × 10^6/kg, median (range)			
At cryopreservation	.2 (.09-.4)	—	<.001
At infusion	.2 (.1-.3)	6.6 (2.2-12.4)	
Number of $CD3^+$ cells × 10^8/kg, median (range)	—	2.7 (1.6-16.6)	

transplantation, and DRI) were not statistically significant. The median duration of follow-up for surviving patients was 32 months (range, 21 to 46 months) for the UCBT arm and 32 months (range, 13 to 46 months) for the haplo-SCT arm.

Table 2 summarizes the graft characteristics. For UCBT, 19 grafts (83%) were matched 4 out of 6 with the recipient. The median TNC and $CD34^+$ cell doses at cryopreservation were .4 × 10^8/kg (range, .2 to .8 × $10^{8/kg}$) and .2 × 10^6/kg (range, .09 to .4 × $10^{6/kg}$), respectively. For haplo-SCT grafts, all patients received mobilized peripheral blood with median $CD34^+$ cells and $CD3^+$ cells of 6.6 × 10^6/kg (range, 2.2 to 12.4 × $10^{6/kg}$) and 2.7 × 10^8/kg (range, 1.6 to 16.6 × $10^{8/kg}$), respectively.

Hematopoietic Engraftment

Neutrophil Recovery

In the UCBT cohort, 1 patient died due to multidrug-resistant *Pseudomonas aeruginosa* bloodstream infection on day +15 after UCB infusion without evidence of myeloid engraftment. Two additional patients experienced primary graft failure, of whom 1 died of graft failure-related complications on day 60 and the other achieved hematopoietic recovery after salvage autologous backup infusion. That patient was subsequently salvaged of relapse with an unrelated donor transplant and remained alive and disease-free after 36 months. These 2 patients received UCB units with TNC doses of 2.1 × 10^8/kg and 4.6 × 10^8/kg and $CD34^+$ cell doses of 1.5 × 10^6/kg and 3 × 10^6/kg, respectively. The remaining 20 patients achieved stable neutrophil recovery at a median time of 19 days (range, 13 to 24 days). In the haplo-SCT cohort, all 22 patients achieved stable neutrophil recovery at a median of 17 days (range, 13 to 25 days).

The cumulative incidence of sustained neutrophil recovery at 30 days was 87% (95% confidence interval [CI], 73% to 100%) in the UCBT arm and 100% (95% CI, 93% to 100%) in the haplo-SCT arm (P = .04) (Figure 2). For the UCBT arm, no variables were associated with neutrophil engraftment. For the haplo-SCT arm, the time to neutrophil recovery was correlated with $CD34^+$ cell dose. The median time to neutrophil recovery was 16 days for patients receiving >6.6 × $10^{6/kg\ CD34^+\ cells}$ and 20 days for patients receiving <6.6 × 10^6/kg $CD34^+$ cells (P = .01).

Platelet Engraftment

Of the 20 patients with neutrophil engraftment in the UCBT cohort, 4 patients died between 24 and 152 days after transplantation without platelet recovery. The remaining 16 patients exhibited platelet engraftment at a median time of 40 days (range, 18 to 129 days). Of the 22 patients in the haplo-SCT cohort, 3 patients died between 54 and 307 days after transplantation without platelet recovery. The remaining 19 patients had platelet engraftment at a median time of 24 days (range, 12 to 127 days). The cumulative incidence of sustained platelet engraftment at 150 days was 70% (95% CI, 51% to 88%) in the UCBT cohort and 86% (95% CI, 72% to 100%) in the haplo-SCT cohort (P = .02).

Transfusion Support

The median number of random donor pooled platelets transfused until day +100 was 15 (range, 5 to 68) in the UCBT

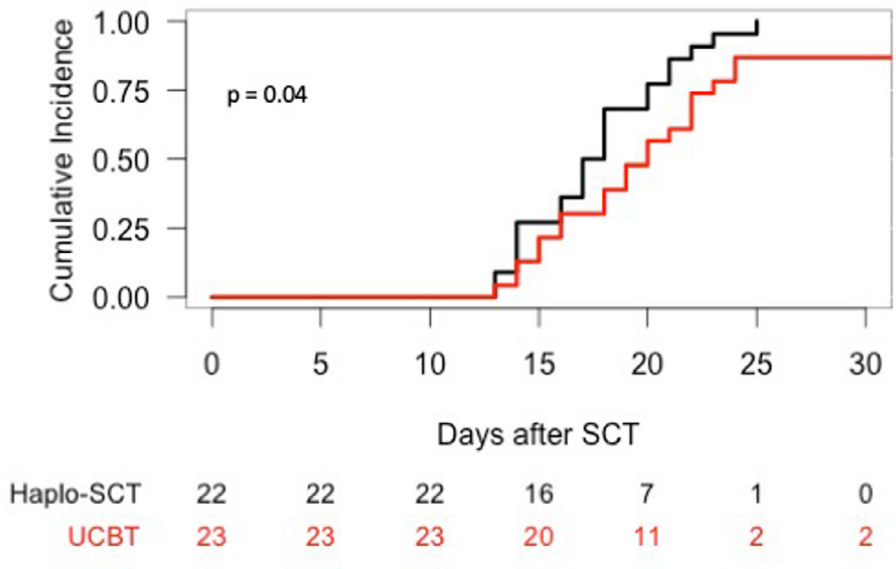

Figure 2. Cumulative incidence of neutrophil recovery according to the type of transplant.

arm and 11 (range, 1 to 130) in the haplo-SCT arm ($P = .1$). The median number of packed red blood cell units transfused until day +100 was 8 (range, 4 to 35) in the UCBT group and 8 (range, 0 to 32) in the haplo-SCT group ($P = 1$).

GVHD

aGVHD

In the UCBT cohort, 15 patients developed aGVHD, including 5 with aGVHD grade I, 8 with grade II, and 2 with grade III. The median time to the development of aGVHD grade II-IV was 40 days (range, 14 to 97 days). In the haplo-SCT cohort, 11 patients developed aGVHD, including grade I in 3 patients, grade II in 6 patients, and grade III in 2 patients. The median time to the development of aGVHD grade II-IV was 21 days (range, 11 to 46 days).

The cumulative incidence of aGVHD at 100 days was 43% (95% CI, 26% to 64%) in the UCBT cohort and 36% (95% CI, 16% to 56%) in the haplo-SCT cohort ($P = .8$) for grade II-IV, whereas for grade III-IV, it was 9% for both arms (p = 1). In the UCBT arm, no variables were associated with aGVHD. In the haplo-SCT arm, $CD34^+$ and $CD3^+$ cell doses of the graft correlated with grade II-IV aGVHD. The cumulative incidence of aGVHD was 83% (95% CI, 54% to 100%) for patients receiving $>8.9 \times 10^{6/kg\ CD34^+\ cells}$ and 19% (95% CI, 0 to 38%) for those receiving $<8.9 \times 10^6$/kg $CD34^+$ cells ($P < .001$). The cumulative incidence of aGVHD was 80% (95% CI, 45% to 100%) for patients receiving $>3.2 \times 10^{8/kg\ CD3^+\ cells}$ and 27% (95% CI, 4% to 49%) for those receiving $<3.2 \times 10^8$/kg $CD3^+$ cells ($P = .04$).

cGVHD

Ten patients in the UCBT cohort developed cGVHD at a median time of 143 days (range, 98 to 266 days). cGVHD was limited in 4 patients and extensive in 10 patients. According to National Institutes of Health severity score, cGVHD was mild in 5 patients, moderate in 1 patient, and severe in 4 patients. In the haplo-SCT cohort, 7 patients developed cGVHD at a median time of 258 days (range, 164 to 550 days). cGVHD was limited in 3 patients and extensive in the remaining 4 patients. According to National Institutes of Health severity score, cGVHD was mild in 4 patients, moderate in 2 patients, and severe in 3 patients.

The 2-year cumulative incidence of any cGVHD and extensive cGVHD in the UCBT and haplo-SCT cohorts was 66% (95% CI, 40% to 91%) and 43% (95% CI, 18% to 68%) ($P = .04$) and 41% (95% CI, 15% to 67%) and 23% (95% CI, 3% to 43%) ($P = .2$), respectively. No variables were associated with the risk of cGVHD.

NRM and Causes of Death

Twelve patients in the UCBT cohort died without previous relapse at a median of 158 days after transplantation (range, 15 to 642 days). Causes of death were infection in 4 patients (1 CMV, 1 *P aeruginosa* bloodstream infection, 1 *Nocardia*, 1 *Mycobacterium tuberculosis*), EBV-PTLD in 2 patients, and GVHD, veno-occlusive disease, primary graft failure, interstitial pneumonitis, cardiac failure, and multiorgan failure of unknown cause in 1 patient each. In the haplo-SCT cohort, 5 patients died without previous relapse at a median time of 93 days after transplantation (range, 54 to 397 days). Causes of death were viral infections in 2 patients (1 adenovirus, 1 syncytial respiratory virus) and veno-occlusive disease, GVHD, and lung carcinoma in 1 patient each. Three additional patients in the UCBT arm and 4 additional patients in the haplo-SCT arm died of relapse-related causes.

The cumulative incidence of NRM at 2 years was 52% (95% CI, 32% to 73%) in the UCBT arm and 23% (95% CI, 5% to 40%) in the haplo-SCT arm ($P = .06$) (Figure 3). No variables were associated with the risk of NRM.

Relapse

Overall, 4 patients in the UCBT cohort relapsed at a median time of 6 months (range, 3 to 10 months) and 6 patients in the haplo-SCT cohort relapsed at a median time of 7 months (range, 2 to 27 months). The 2-year cumulative incidence of relapse was 17% (95% CI, 2% to 33%) in the UCBT arm and 23% (95% CI, 5% to 41%) in the haplo-HCT arm ($P = .5$). No variables were associated with the risk of relapse.

Survival

Seven patients in the UCBT cohort and 11 patients in the haplo-SCT cohort were alive and leukemia-free at the last follow-up. The 2-year DFS was 30% (95% CI, 16% to 57%) for the UCBT arm and 54% (95% CI, 37% to 80%) for the haplo-SCT arm ($P = .2$) (Figure 4A). The OS was 35% (95% CI, 20% to 61%) for

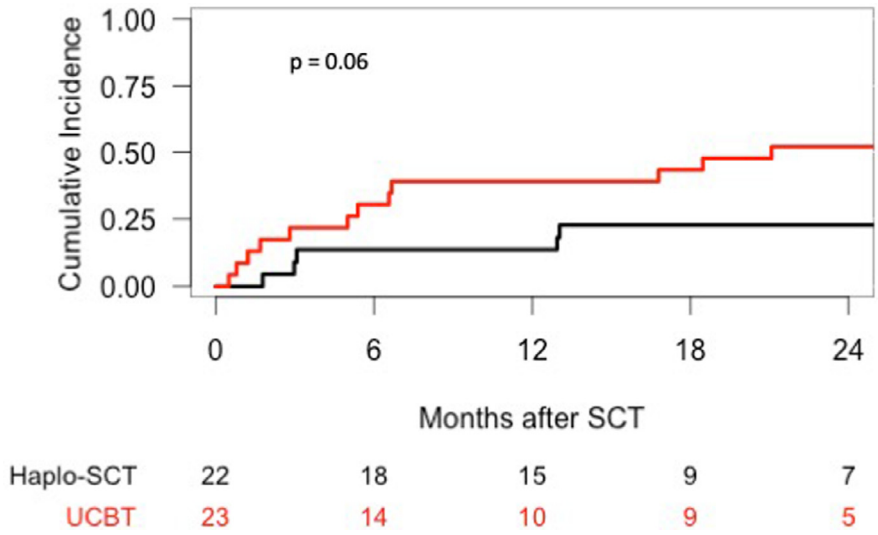

Figure 3. Cumulative incidence of NRM according to the type of transplant.

the UCBT arm and 59% (95% CI, 42% to 84%) for the haplo-SCT arm (P = .1) (Figure 4B). Lower DRI was associated with improved survival. DFS was 49% (95% CI, 32% to 75%) and 30% (95% CI, 15% to 59%) (P = .04), whereas OS was 56% (95% CI, 40% to 82%) and 30% (95% CI, 15% to 59%) (P = .02) for low or intermediate DRI and high or very high DRI, respectively. GRFS at 2 years was 17% (95% CI, 7% to 42%) after UCBT and 40% (95% CI, 24% to 68%) after haplo-SCT (P = .04) (Figure 4C).

Post-Transplantation Events

The cumulative incidence of post-transplantation events according to the type of transplantation is shown in Table 3. In brief, CMV reactivation was similar in the 2 treatment groups. End-organ CMV disease was diagnosed in 5 patients after UCBT (2 pneumonia, 2 gastrointestinal, 1 disseminated disease), and 1 patient had gastrointestinal involvement in the haplo-SCT cohort. Two patients developed monomorphic EBV-PTLD with diffuse large B cell histology on days +120 and +160 after UCBT and were unresponsive to standard immunochemotherapy. Hemorrhagic cystitis was more frequent after haplo-SCT.

Post-Transplantation Immune Reconstitution

The lymphocyte recovery of the different subpopulations, $CD3^+$, $CD4^+$, $CD8^+$, $CD19^+$, and natural killer cells, at 3, 6, 9, 12, 18, and 24 months after UCBT and haplo-SCT is shown in Table 4. In brief, haplo-SCT showed higher $CD4^+$ cell reconstitution until 6 months after transplantation and higher $CD8^+$ cell reconstitution until 2 years post-transplantation. UCBT showed a trend toward better $CD19^+$ cell recovery until 2 years post-transplantation. There was no between-group difference in natural killer cell reconstitution.

DISCUSSION

This prospective randomized study shows that unmanipulated, in the context of an MAC regimen, haplo-SCT with post-transplantation cyclophosphamide (PTCy) provides improved neutrophil and platelet engraftment with a decreased risk of cGVHD and better GRFS compared with ATG containing single-unit UCBT.

The main limitation of this study was the low accrual. The study closed with 45 patients included, out of an expected target sample size of 206 patients over 3 years, demonstrating the difficulty of performing academic randomized trials. In fact, although a randomized trial is ongoing in the US comparing reduced intensity double-unit UCBT versus PTCy unmanipulated haplo-SCT (BMT CTN 1101), it is unlikely that a similar study would be carried out in the myeloablative setting. Nevertheless, despite the limited number of patients, which prevented us from having sufficient statistical power to achieve the primary endpoint, this prospective randomized comparison was carried out in a homogeneously treated population and provides valuable observations that could have a significant impact on clinical practice.

To minimize potential bias, we used a uniform MAC regimen in the 2 arms, which included thiotepa, busulfan, and fludarabine (TBF regimen). This regimen, first reported by our group in the UCBT setting, had been adopted as the standard for single-unit UCBT by the Spanish Group of Hematopoietic Stem Cell Transplant and demonstrated a high rate of engraftment, a good toxicity profile, and favorable long-term outcomes [8]. Within the Spanish Group of Hematopoietic Stem Cell Transplant platform, we used minimum cell dose requirements based on overall cellularity of the unit and not based on the recipient's body weight, based on our previous experience, in which the recipient's weight was never associated with engraftment and to facilitate unit selection [7,26]. The TBF regimen also showed superiority over other regimens in a study from the EBMT [27]. It has subsequently been used with great success in unmanipulated haplo-SCT with PTCy by the Genova group, and is currently the most commonly used myeloablative regimen in Europe in this setting [9,28,29]. The addition of ATG in UCBT is controversial and may have had an impact on transplantation results. Recent studies have suggested a deleterious effect of high exposure of ATG in UCBT [30,31]. However, we previously showed similar results after single-unit UCBT using the TBF and ATG platform compared with double-unit UCBT after conditioning with a total body irradiation-based regimen at the University of Minnesota [32]. We also reported comparable outcomes with the so-called "haplo-cord" procedure, in which $CD34^+$ cells from a third-party donor are coinfused with the UCB graft [33].

An interesting finding of our study was that 15% of patients allocated to the haplo-HSCT arm did not have a suitable donor available, whereas all patients in the UCBT arm had UCB grafts fulfilling the minimum required criteria. Although most

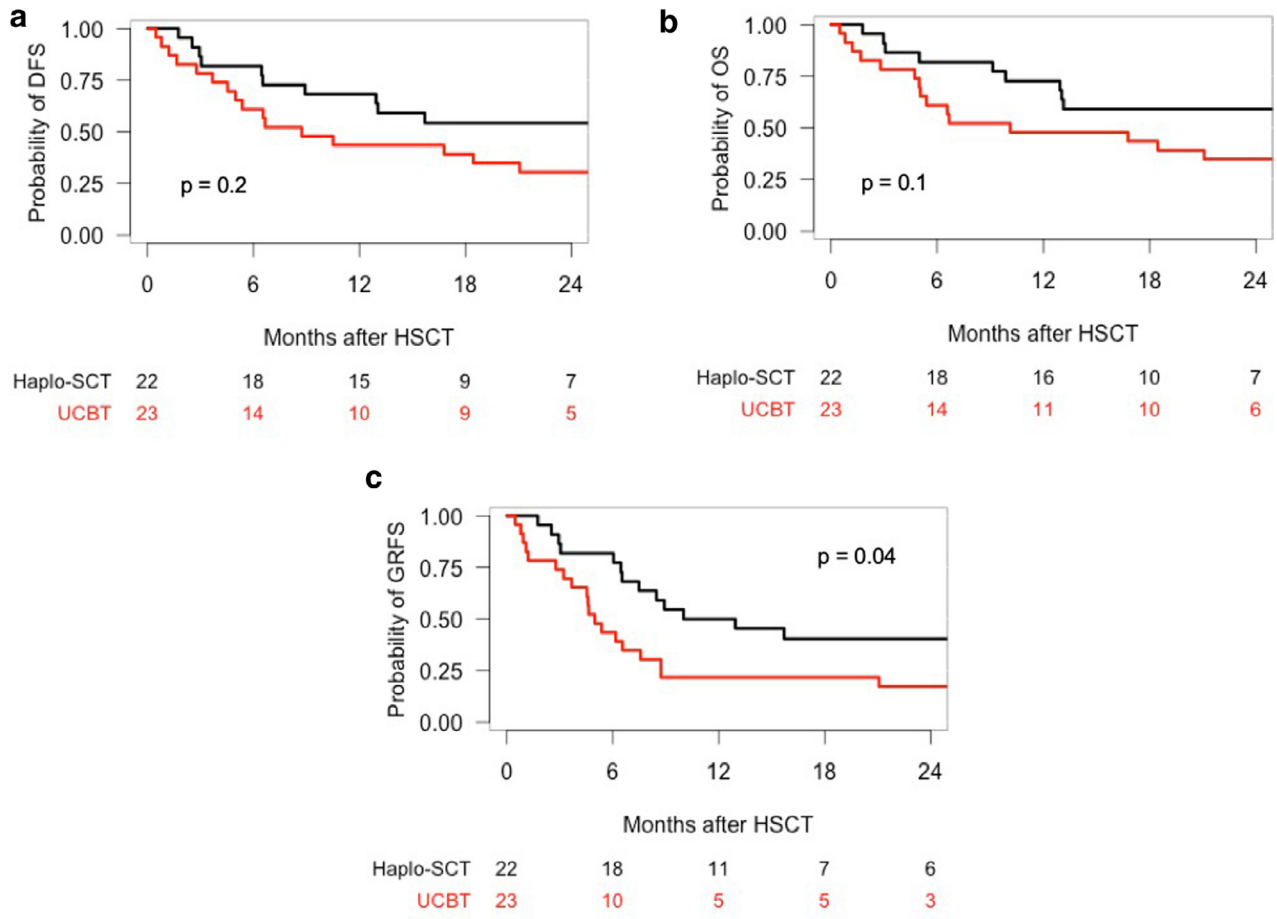

Figure 4. Probability of survival according to the type of transplantation. (A) DFS. (B) OS. (C) GRFS.

Table 3
Post-Transplantation Events According to Type of Transplant

Event	Cumulative Incidence of Post-Transplantation Events, % (95% CI)		
	UCBT	Haplo-SCT	*P* Value
Sinusoidal obstruction syndrome	9 (0-20)	5 (0-13)	.6
CMV infection	43 (23-64)	50 (29-71)	.5
CMV disease	22 (5-39)	5 (0-13)	.1
EBV-PTLD	9 (0-20)	0 (0-0)	.2
Invasive fungal infection	17 (2-33)	9 (0-21)	.4
Hemorrhagic cystitis	26 (8-44)	55 (34-75)	.05

patients will have a haploidentical family donor, that is not always the case, especially for immigrants without an available first- or second-degree relative. Moreover, the presence of anti-HLA antibodies against the donor HLA antigens is a major limitation, because frequently these antibodies are directed against antigens present in various members of the family. This is particularly prevalent in female patients with offspring donors and those receiving transfusion support from family donors. In those scenarios, the use of UCBT may be necessary, and specialized centers should maintain their expertise. Other alternatives, such as desensitization for donor-specific anti-HLA antibodies [34] or the use of mismatched unrelated donor with PTCy [35], have proven safe and effective and could be an appropriate option.

Haplo-SCT was associated with improved neutrophil and platelet recovery, in line with previously published comparisons [5,6]. This finding was expected, given that one of the main limitations of UCBT is the low cellular content of the graft and the delayed hematopoietic recovery. However, it did not translate into higher transfusion requirements, likely due to the increased incidence of hemorrhagic cystitis in the haplo-SCT group. We also observed faster neutrophil engraftment in haplo-SCT recipients with higher numbers of $CD34^+$ cells in the peripheral blood grafts. Although this observation has been widely described in UCBT [36,37], to our knowledge, this is the first time that it has been reported in haplo-SCT with PTCy, for which bone marrow is frequently used. However, this beneficial effect was counterbalanced by an increased risk of aGVHD with higher $CD34^+$ and $CD3^+$ cell doses in the haplo-SCT grafts. These findings may have important clinical implications for donor stem cell collection and graft manipulation, whereas previous studies have reported discordant results regarding the impact of $CD3^+$ cells on GVHD and other post-transplantation events [38-41]. Overall, UCBT and haplo-SCT were associated with a similar risk of aGVHD, as reported previously [5,6]. However, peripheral blood was used as the stem cell source in haplo-SCT, and it has been associated with increased risk of GVHD compared with bone marrow [42,43]. However, haplo-SCT was associated with a lower risk of cGVHD compared with UCBT, demonstrating the effectiveness of PTCy in inducing tolerance and controlling cGVHD [44,45].

NRM was unacceptably high after UCBT, higher than the previously published experience with the TBF-ATG platform [7,32,33]. The reasons for this elevated mortality remain unknown and are

Table 4
Post-Transplantation Immune Reconstitution According to Type of Transplant

Parameter	Lymphocytes/mL, median (range)		
	UCBT	Haplo-SCT	*P* Value
3 mo after SCT	N = 14	N = 14	
$CD3^+$	43 (1-370)	402 (54-1501)	<.001
$CD4^+$	34 (0-196)	141 (20-487)	.003
$CD8^+$	11 (0-189)	236 (25-1041)	<.001
$CD19^+$	30 (0-481)	3 (0-266)	.2
NK	157 (30-445)	214 (68-1013)	.3
6 mo after SCT	N = 12	N = 16	
$CD3^+$	60 (2-1199)	834 (45-2484)	<.001
$CD4^+$	47 (1-508)	226 (36-712)	<.001
$CD8^+$	11 (0-552)	425 (11-2135)	<.001
$CD19^+$	122 (0-1211)	22 (0-420)	.3
NK	287 (10-1600)	207 (50-921)	.3
9 mo after SCT	N = 6	N = 13	
$CD3^+$	189 (4-1285)	1061 (116-1980)	.03
$CD4^+$	125 (2-534)	254 (45-576)	.1
$CD8^+$	54 (2-599)	758 (19-1420)	.005
$CD19^+$	398 (11-1016)	64 (0-257)	.07
NK	398 (123-1339)	235 (18-643)	.1
12 mo after SCT	N = 10	N = 12	
$CD3^+$	432 (0-1144)	1429 (43-3363)	.006
$CD4^+$	290 (0-603)	315 (39-763)	.3
$CD8^+$	132 (0-457)	940 (4-2916)	<.001
$CD19^+$	542 (54-1796)	178 (0-866)	.1
NK	350 (147 (1651)	237 (25-860)	.05
18 mo after SCT	N = 7	N = 7	
$CD3^+$	755 (1-1139)	1088 (496-2077)	.1
$CD4^+$	474 (1-570)	325 (231-604)	.4
$CD8^+$	198 (0-561)	669 (247-1744)	.008
$CD19^+$	828 (58-1926)	257 (88-508)	.05
NK	433 (183-1925)	235 (128-362)	.1
24 mo after SCT	N = 3	N = 5	
$CD3^+$	1048 (988-1150)	883 (278-2789)	.7
$CD4^+$	680 (659-706)	274 (204-677)	.05
$CD8^+$	333 (264-442)	670 (283-2071)	.1
$CD19^+$	879 (77-1075)	210 (33-353)	.03
NK	387 (266-655)	248 (90-297)	.08

NK indicates natural killer.

likely multifactorial. Recipients were a high-risk population with approximately 50% of the patients in an advanced disease stage with high or very high DRI and an HCT-CI $\geq$3. Again, the main cause of death was infection, in the context of prolonged impaired immune T cell reconstitution. Together with 2 EBV-PTLD-related deaths, the use of ATG may have contributed to this poor outcome, and results might be improved by omitting ATG from the conditioning regimen. In fact, the haplo-SCT cohort, using the same conditioning but substituting ATG with PTCy, showed improved T cell recovery that translated to decreased NRM. Whether PTCy can be used to prevent GVHD in UCBT is an interesting area for further exploration.

In conclusion, in this randomized comparison using TBF myeloablative conditioning, unmanipulated haplo-SCT with PTCy offered better outcomes than ATG-containing single-unit UCBT in adults with hematologic malignancies. The high NRM observed in this latter platform suggests that further comparisons between haplo-SCT with PTCy and UCBT platforms without ATG are warranted. Our findings could have a significant impact on the practice of transplantation medicine.

ACKNOWLEDGMENTS

Financial disclosure: This study was supported by an unrestricted grant from Adienne.

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

Authorship statement: J.S., M.A.S., J.M., J.L.P., and G.S. designed the study and interpreted the data; J.S. and J.M. performed the statistical analyses; C.S., D.V., A.S., C.F., R.P., I.L., P.M., G.O., J.C.H.B., A.B., M.G., C.C., and G.S. contributed with patient inclusion and data, reviewed the manuscript, and contributed to the final draft; and J.S., J.M., M.A.S., and J.L.P. wrote the manuscript.

REFERENCES

1. Passweg JR, Baldomero H, Bader P, et al. Use of haploidentical stem cell transplantation continues to increase: the 2015 European Society for Blood and Marrow Transplant activity survey report. *Bone Marrow Transplant*. 2017;52:811–817.
2. Niederwieser D, Baldomero H, Szer J, et al. Hematopoietic stem cell transplantation activity worldwide in 2012 and a SWOT analysis of the Worldwide Network for Blood and Marrow Transplantation Group including the global survey. *Bone Marrow Transplant*. 2016;51:778–785.
3. Brunstein CG, Fuchs EJ, Carter SL, et al. Alternative donor transplantation after reduced intensity conditioning: results of parallel phase 2 trials using partially HLA-mismatched related bone marrow or unrelated double umbilical cord blood grafts. *Blood*. 2011;118:282–288.
4. https://clinicaltrials.gov, NCT01597778.
5. Giannotti F, Labopin M, Shouval R, et al. Haploidentical transplantation is associated with better overall survival when compared to single cord blood transplantation: an EBMT-Eurocord study of acute leukemia patients conditioned with thiotepa, busulfan, and fludarabine. *J Hematol Oncol*. 2018;11:110.
6. Ruggeri A, Labopin M, Sanz G, et al. Comparison of outcomes after unrelated cord blood and unmanipulated haploidentical stem cell transplantation in adults with acute leukemia. *Leukemia*. 2015;29:1891–1900.
7. Sanz J, Picardi A, Hernández Boluda JC, et al. Impact of graft-versus-host disease prophylaxis on outcomes after myeloablative single-unit umbilical cord blood transplantation. *Biol Blood Marrow Transplant*. 2013;19:1387–1392.
8. Sanz J, Boluda JC, Martín C, et al. Single-unit umbilical cord blood transplantation from unrelated donors in patients with hematological malignancy using busulfan, thiotepa, fludarabine and ATG as myeloablative conditioning regimen. *Bone Marrow Transplant*. 2012;47:1287–1293.
9. Raiola AM, Dominietto A, Ghiso A, et al. Unmanipulated haploidentical bone marrow transplantation and posttransplantation cyclophosphamide for hematologic malignancies after myeloablative conditioning. *Biol Blood Marrow Transplant*. 2013;19:117–122.
10. Montesinos P, Sanz J, Cantero S, et al. Incidence, risk factors, and outcome of cytomegalovirus infection and disease in patients receiving prophylaxis with oral valganciclovir or intravenous ganciclovir after umbilical cord blood transplantation. *Biol Blood Marrow Transplant*. 2009;15:730–740.
11. Glucksberg H, Storb R, Fefer A, et al. Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched sibling donors. *Transplantation*. 1974;18:295–304.
12. Przepiorka D, Weisdorf D, Martin P, et al. 1994 Consensus Conference on Acute GVHD Grading. *Bone Marrow Transplant*. 1995;15:825–828.
13. Shulman HM, Sullivan KM, Weiden PL, et al. Chronic graft-versus-host syndrome in man. A long-term clinicopathologic study of 20 Seattle patients. *Am J Med*. 1980;69:204–217.
14. 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. 389-401.e1.
15. Armand P, Kim HT, Logan BR, et al. Validation and refinement of the Disease Risk Index for allogeneic stem cell transplantation. *Blood*. 2014;123:3664–3671.
16. Sorror ML, Maris MB, Storb R, et al. Hematopoietic cell transplantation (HCT)-specific comorbidity index: a new tool for risk assessment before allogeneic HCT. *Blood*. 2005;106:2912–2919.
17. Mohty M, Malard F, Abecassis M, et al. Revised diagnosis and severity criteria for sinusoidal obstruction syndrome/veno-occlusive disease in adult patients: a new classification from the European Society for Blood and Marrow Transplantation. *Bone Marrow Transplant*. 2016;51:906–912.
18. Ljungman P, Boeckh M, Hirsch HH, et al. Definitions of cytomegalovirus infection and disease in transplant patients for use in clinical trials. *Clin Infect Dis*. 2017;64:87–91.
19. Styczynski J, van der Velden W, Fox CP, et al. Management of Epstein-Barr virus infections and post-transplant lymphoproliferative disorders in patients after allogeneic hematopoietic stem cell transplantation: Sixth European Conference on Infections in Leukemia (ECIL-6) guidelines. *Haematologica*. 2016;101:803–811.

20. De Pauw B, Walsh TJ, Donnelly JP, et al. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. *Clin Infect Dis*. 2008;46:1813–1821.
21. 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;18:695–706.
22. Gray RJ. A class of K-sample tests for comparing the cumulative incidence of a competing risk. *Ann Stat*. 1988;16:1141–1154.
23. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. *J Am Stat Assoc*. 1958;53:457–481.
24. Mantel N. Evaluation of survival data and two new rank order statistics arising in its consideration. *Cancer Chemother Rep*. 1966;50:163–170.
25. R Core Team. Comprehensive R Archive Network (CRAN). Vienna, Austria: R Foundation for Statistical Computing.
26. Sanz J, Gale RP. One or two umbilical cord blood cell units? Caveat emptor. *Bone Marrow Transplant*. 2017;52:341–343.
27. Ruggeri A, Sanz G, Bittencourt H, et al. Comparison of outcomes after single or double cord blood transplantation in adults with acute leukemia using different types of myeloablative conditioning regimen, a retrospective study on behalf of Eurocord and the Acute Leukemia Working Party of EBMT. *Leukemia*. 2014;28:779–786.
28. Di Bartolomeo P, Santarone S, De Angelis G, et al. Haploidentical, unmanipulated, G-CSF-primed bone marrow transplantation for patients with high-risk hematologic malignancies. *Blood*. 2013;121:849–857.
29. Duléry R, Bastos J, Paviglianiti A, et al. Thiotepa, busulfan and fludarabine conditioning regimen in T-cell replete HLA-haploidentical hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant*. 2019;25:1407–1415.
30. Admiraal R, Lindemans CA, van Kesteren C, et al. Excellent T-cell reconstitution and survival depend on low ATG exposure after pediatric cord blood transplantation. *Blood*. 2016;128:2734–2741.
31. Admiraal R, van Kesteren C, Jol-van der Zijde CM, et al. Association between anti-thymocyte globulin exposure and $CD4^+$ immune reconstitution in paediatric haemopoietic cell transplantation: a multicentre, retrospective pharmacodynamic cohort analysis. *Lancet Haematol*. 2015;2:e194–e203.
32. Sanz J, Wagner JE, Sanz MA, et al. Myeloablative cord blood transplantation in adults with acute leukemia: comparison of two different transplant platforms. *Biol Blood Marrow Transplant*. 2013;19:1725–1730.
33. Sanz J, Kwon M, Bautista G, et al. Single umbilical cord blood with or without $CD34^+$ cells from a third-party donor in adults with leukemia. *Blood Adv*. 2017;1:1047–1055.
34. Ciurea SO, Cao K, Fernadez-Vina M, et al. The European Society for Blood and Marrow Transplantation (EBMT) consensus guidelines for the detection and treatment of donor-specific anti-HLA antibodies (DSA) in haploidentical hematopoietic cell transplantation. *Bone Marrow Transplant*. 2018;53:521–534.
35. Kasamon YL, Ambinder RF, Fuchs EJ, et al. Prospective study of nonmyeloablative, HLA-mismatched unrelated BMT with high-dose posttransplantation cyclophosphamide. *Blood Adv*. 2017;1:288–292.
36. Wagner JE, Barker JN, DeFor TE, et al. Transplantation of unrelated donor umbilical cord blood in 102 patients with malignant and nonmalignant diseases: influence of CD34 cell dose and HLA disparity on treatment-related mortality and survival. *Blood*. 2002;100:1611–1618.
37. Moscardó F, Sanz J, Carbonell F, et al. Effect of $CD8^+$ cell content on umbilical cord blood transplantation in adults with hematological malignancies. *Biol Blood Marrow Transplant*. 2014;20:1744–1750.
38. Cieri N, Greco R, Crucitti L, et al. Post-transplantation cyclophosphamide and sirolimus after haploidentical hematopoietic stem cell transplantation using a treosulfan-based myeloablative conditioning and peripheral blood stem cells. *Biol Blood Marrow Transplant*. 2015;21:1506–1514.
39. Mussetti A, De Philippis C, Carniti C, et al. $CD3^+$ graft cell count influence on chronic GVHD in haploidentical allogeneic transplantation using post-transplant cyclophosphamide. *Bone Marrow Transplant*. 2018;53:1522–1531.
40. McCurdy SR, Zhang MJ, St Martin A, et al. Effect of donor characteristics on haploidentical transplantation with posttransplantation cyclophosphamide. *Blood Adv*. 2018;2:299–307.
41. Solh MM, Dickhaus E, Solomon SR, et al. Fevers post infusion of T-cell replete hla mismatched haploidentical hematopoietic stem cells with post-transplant cyclophosphamide: risk factors and impact on transplant outcomes. *Bone Marrow Transplant*. 2019;54:1756–1763.
42. Ruggeri A, Labopin M, Bacigalupo A, et al. Bone marrow versus mobilized peripheral blood stem cells in haploidentical transplants using posttransplantation cyclophosphamide. *Cancer*. 2018;124:1428–1437.
43. Bashey A, Zhang MJ, McCurdy SR, et al. Mobilized peripheral blood stem cells versus unstimulated bone marrow as a graft source for T-cell-replete haploidentical donor transplantation using post-transplant cyclophosphamide. *J Clin Oncol*. 2017;35:3002–3009.
44. Kanakry CG, O'Donnell PV, Furlong T, et al. Multi-institutional study of post-transplantation cyclophosphamide as single-agent graft-versus-host disease prophylaxis after allogeneic bone marrow transplantation using myeloablative busulfan and fludarabine conditioning. *J Clin Oncol*. 2014;32:3497–3505.
45. 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;14:641–650.