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Single Cord Blood Combined with HLA-Mismatched Third Party Donor Cells: Comparable Results to Matched Unrelated Donor Transplantation in High-Risk Patients with Hematologic Disorders

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A B S T R A C T

Matched unrelated donor (MUD) transplantation is the first alternative in the absence of a matched sibling donor. For patients without a suitable adult donor, we have adopted the dual stem cell transplantation protocol consisting of cord blood (CB) in combination with $CD34^+$ cells from a third party HLA-mismatched donor. We analyzed the outcomes of patients undergoing both procedures in a single center. Starting in 2004, a total of 20 patients with high-risk disease underwent 22 dual transplants and 25 patients underwent myeloablative MUD transplantation. The 30-day cumulative incidence of neutrophil engraftment was similar in both groups (91% and 95%), with a median time to engraftment of 14 and 16 days, respectively. Grade II-IV acute graft-versus-host disease was more frequent in the MUD group (40% versus 5%). Except for a tendency toward a higher incidence of viral hemorrhagic cystitis in the dual transplantation group, posttransplantation infectious events were comparable in the 2 groups. The 3-year cumulative incidence rates of relapse (41% versus 44%) and nonrelapse mortality (30% versus 25%) were similar in the MUD and dual transplantation cohorts. Estimated 3-year overall survival and disease-free survival were 47% and 41%, respectively, with no survival advantage for either group. In our experience, dual transplantation offers survival rates comparable to those from myeloablative MUD transplantation with similar nonrelapse mortality rates.

INTRODUCTION

In the absence of an HLA-matched sibling donor, an unrelated adult donor (MUD) matched at 8 of 8 alleles for HLA-A, -B, -C, and -DRB1 is considered the first alternative for allogeneic stem cell transplantation (SCT). However, only 50% of patients have a suitable donor in a median of 4 months in Europe [1]. Unrelated umbilical cord blood (CB) is being increasingly used as an alternative stem cell source for adult patients eligible for allogeneic SCT but lacking an HLA-matched adult donor [2-4]. Late engraftment, with the subsequent increased risk of serious early neutropenia-related infections, prolonged length of hospital stay, and high nonrelapse mortality (NRM), are considered some of the main limitations of CB transplantation [5,6]. To overcome these drawbacks, which are related mainly to the limited number of cells available in a single unit, several strategies have been explored, including CB in vitro culture expansion, double CB transplantation, and direct intrabone transplantation of CB cells [7-10]. In this context, single CB transplantation with the coinfusion of mobilized and selected $CD34^+$ cells from a third party HLA-mismatched donor (TPD), known as dual SCT, has been shown to reduce the period of posttransplantation neutropenia and related early morbidity and mortality associated with single CB transplantation [11-15].

The objective of this study was to analyze toxicity and survival rates of adults who underwent dual SCT in our center and to compare these rates with those in a cohort of patients who underwent myeloablative MUD SCT in our center in the same time period.

PATIENTS AND METHODS

Eligibility

The study was approved by the hospital's Institutional Review Board, and all donors and recipients gave written informed consent. The study population included consecutive adult patients with high-risk hematologic disease who underwent dual or myeloablative MUD SCT from March 2004 and had a minimum posttransplantation follow-up of 6 months.

Graft Selection and Processing

For CB units, a match of at least 4 of 6 HLA loci, considering low resolution for HLA-A and HLA-B and high resolution for HLA-DR, was required. CB units were selected based on total nucleated cell (TNC) dose and $CD34^+$ and HLA matching as determined before freezing, focusing on cell dose at the expense of matching. CB units with higher cryopreserved cell doses after volume reduction, with a minimum of 2×10^7 TNCs/kg and 1×10^5 $CD34^+$ cells/kg, were preferred. ABO compatibility was a secondary selection criterion.

Donors of the HLA-mismatched $CD34^+$ cells were sought from among each patient's first-degree relatives. Given the reported high incidence of graft failure related to the use of maternal donors [11,12], nonmaternal relatives were preferred. If no relatives were available, then an unrelated individual was selected as the donor. Granulocyte colony-stimulating factor, 10 μg/kg/day, was administered for 4 consecutive days to all donors, and cells were collected with a continuous-flow apheresis device. $CD34^+$ cells were selected by a positive immunomagnetic technique (CliniMACS; Miltenyi Biotec, Cologne, Germany) to obtain a final product with $2.5\text{-}3 \times 10^6$ $CD34^+$ cells/kg and $<1 \times 10^4$ $CD3^+$ cells/kg of recipient body weight, as described previously [11,12].

Within the MUD cohort, donors were identified by the Spanish national donor registry (REDMO) to have at least 8/8 high-resolution HLA matching at loci A, B, C, and DRB1. Males or females without a history of previous

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pregnancy and bone marrow (BM) donors without major ABO incompatibility were preferred. For cytomegalovirus (CMV)-negative patients, CMV-negative donors were preferable.

Conditioning Regimen and Graft-versus-Host Disease Prophylaxis

The conditioning regimen for dual SCT included fludarabine 30 mg/m^2 (days −8 to −5), antithymocyte globulin (ATG) 2 mg/kg (days −2 and −1), cyclophosphamide 60 mg/kg (days −4 and −3), and i.v. busulfan 3.2 mg/kg (days −6 and −5) (oral busulfan 4 mg/kg uo to 2006) or 10 Gy of fractionated total body irradiation (TBI). CB cells were infused on day 0, followed by the TPD cells either the same day or on day +1 posttransplantation. As graft-versus-host disease (GVHD) prophylaxis, patients received cyclosporine (CsA) from day −5, first i.v. and then orally, and metilprednisolone 2 mg/kg from day −2 and tapered until suspension on day +10. In the absence of GVHD manifestations, CsA was tapered from day +60.

Conditioning for MUD transplantation included i.v. busulfan 3.2 mg/kg for 4 days (except for 1 patient who received oral busulfan) and either cyclophosphamide 60 mg/kg (days −3 and −2), fludarabine 40 mg/m^2 for 4 days, or 12 Gy of fractionated TBI. ATG 2.5 mg/kg on days −3, −2, and −1 was included in all but 2 MUD transplantations. GVHD prophylaxis was provided by CsA starting on day −7 and a short course of methotrexate (15 mg/m^2 on day +1 and 10 mg/m^2 on days +3, +6, and +11) . In the absence of GVHD manifestations, CsA was tapered starting on day +60.

Supportive Care

Patients were housed in high-efficiency particulate air–filtered rooms. Patients undergoing dual SCT received granulocyte colony-stimulating factor, 5 mg/kg/day s.c., from day +1 until neutrophil recovery. All dual and MUD SCT recipients received oral quinolone as antibacterial prophylaxis. Prophylaxis against *Pneumocystis jiroveci* consisted of cotrimoxazole (320/1600 mg sulfamethoxazole/trimethoprim daily) from day −7 to day −2, then resumed after engraftment and maintained for a minimum of 1 year. Intravenous itraconazole 200 mg/day or micafungin 50 mg/day (since 2010) were administered as antifungal prophylaxis. Acyclovir was used as anti-herpesvirus prophylaxis. Galactomannan testing and CMV polymerase chain reaction analysis were performed twice weekly, and Epstein-Barr virus (EBV) polymerase chain reaction analysis was performed every other week until day +100 or indefinitely for those with active GVHD. All blood products were irradiated and leukocyte-depleted. Nonspecific i.v. immunoglobulin was administered at a dose of 400 mg/kg every 2-3 weeks from day +7 until hospital discharge and then monthly during the first 100 days after transplantation.

Pretransplantation and Posttransplantation Evaluation

Response to therapy before and after transplantation was evaluated based on the National Cancer Institute's criteria as revised by the International Working Group for the Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia [16]. Comorbidities were recorded using the hematopoietic cell transplantation–specific comorbidity index [17]. Posttransplantation bone marrow (BM) analysis and disease restaging were repeated routinely on days +30, +100, +180, and +365 and yearly thereafter, and whenever clinically indicated.

Chimerism was determined by molecular analysis in BM and peripheral blood (PB). BM samples were analyzed on days +30, +100, +180, +365, and once yearly thereafter. PB samples were obtained once a week starting on day +14 in patients with mixed chimerism (MC), once a month during the first year posttransplantation and every other month thereafter. Donor and recipient cells were detected by quantitative analysis of informative microsatellite DNA polymorphisms as described previously [18].

Acute GVHD (aGVHD) was scored according to published consensus criteria [19]. Chronic GVHD (cGVHD) was scored according to the National Institutes of Health's Consensus Development Project [20].

Definitions

Myeloid engraftment was defined as an absolute neutrophil count of $\geq 0.5 \times 10^9$/L for 3 consecutive days. Platelet engraftment was defined as a platelet count of $\geq 20 \times 10^9$/L, without transfusion support, for 3 consecutive days. Patients who survived more than 30 days after transplantation and who failed to achieve myeloid engraftment were considered graft failures. Diagnosis of disease recurrence was based on clinical and pathological criteria.

Statistical Analysis

Quantitative variables are expressed as median and either range or interquartile range (IQR). Qualitative variables are expressed as frequency and percentage. The Fisher exact test or the χ^2 test were used to test for the association between qualitative variables. Comparability of the 2 cohorts (dual and MUD) for the main prognostic features was tested with either the *t* test or the Mann-Whitney *U* test, depending on normality and variance assumptions.

NRM, disease relapse or progression, overall survival (OS), and disease-free survival (DFS) were defined as primary endpoints. NRM was defined as death from any cause without previous disease relapse or progression. OS was defined as the time from transplantation to death from any cause, and surviving patients were censored at last follow-up or at time of subsequent transplantation [21,22]. For those patients with graft failure and rescued with a subsequent transplantation, follow-up was measured from the date of first transplantation until death or last follow-up. DFS was defined as the time from transplantation to relapse, disease progression, or death from any cause, whichever occurred first. Estimates of DFS and OS were calculated using the Kaplan-Meier method, including 95% confidence interval (CI), and differences between both cohorts were compared using the 2-tailed log-rank test.

Estimates of engraftment, NRM, and relapse or progression after transplantation were calculated using cumulative incidence rates and compared by univariate Cox regression. Patient follow-up was updated on April 2012.

RESULTS

Patients

Twenty consecutive adult patients with high-risk hematologic disease who did not have an available HLA-identical related or unrelated donor underwent a total of 22 dual SCTs between March 2004 and May 2011. These patients had a median weight of 70.5 kg (IQR, 56-76 kg). During the same time period, 25 consecutive patients with similar pretransplantation characteristics underwent myeloablative allogeneic MUD SCT. Characteristics of both groups are summarized in Table 1.

In the dual SCT group, 12 patients (60%) underwent SCT in first complete remission (CR1) and 6 patients (30%) had active disease. Eighteen dual SCTs were performed with the busulfan-based preparative regimen, and 2 patients with acute lymphoblastic leukemia received TBI-based conditioning. In addition, 2 patients who exhibited primary CB graft failure were rescued with a second dual SCT using thiotepa, fludarabine, and ATG as the conditioning regimen.

Within the MUD group, 11 patients (44%) underwent SCT in CR1 and 10 (40%) had active disease. Five patients, 3 of them with acute lymphoblastic leukemia, received TBI as part of their conditioning regimens. Patient characteristics were not statistically different between the 2 groups (Table 1).

Graft Features

In the dual SCT group, the median number of post-processing CB TNCs and CD34$^+$ cells were 2.8×10^7/kg (range, 1.48-4.74 $\times 10^7$/kg) and 1.7×10^5/kg (range, 0.73-3.2 $\times 10^5$/kg), respectively (Table 1). The HLA-mismatched TPD was a sibling (in 62%, 14% of them fully mismatched), another haploidentical relative (father in 13%, son in 9%, and mother in 1 case), or a fully mismatched unrelated individual (in 1 case). The median number of infused TPD CD34$^+$ cells was 2.6×10^6/kg (range, 1.5-3.2 $\times 10^6$/kg), and the median number of CD3$^+$ cells was 0.23×10^4/kg (range, 0.05-1.5 $\times 10^4$/kg).

In the MUD group, the stem cell source was BM in 32% and mobilized PB in 68%. All patients received a 12/12 HLA allele–matched graft. The median number of infused TNC and CD34$^+$ cells was 4.8×10^8/kg (range, 0.8-16$\times 10^8$/kg) and 4×10^6/kg (range, 0.8-19 $\times 10^6$/kg), respectively.

Engraftment and Chimerism

In the dual SCT group, the cumulative incidence of myeloid engraftment at 30 days was 91% (Figure 1), with a median time to engraftment of 14 days (range, 9-28 days).

Table 1
Characteristics of Patients and Transplants

Characteristic	MUD (n = 25)	Dual (n = 20)	*P* Value
Patients			
Age, years, median (IQR)	38 (30-52)	39 (28-49)	.79
Sex, n (%)			
Male	16 (64)	11 (55)	.55
Female	9 (36)	9 (45)	
Disease status at transplantation, n (%)			
Acute myelogenous leukemia/myelodysplastic syndrome	18 (72)	11 (55)	
CR1	7 (28)	8 (40)	.12
Other	11 (44)	3 (15)	
Acute lymphoblastic leukemia	4 (16)	6 (30)	
CR1	3 (12)	3 (15)	.57
Other	1 (4)	3 (15)	
Lymphoproliferative disease	2 (8)	2 (10)	.77
Chronic myelogenous leukemia	1 (4)	1 (5)	.57
Previous transplantation, autologous/allogeneic, n (%)	3 (12)/1 (4)	2 (10)/0 (0)	.68
Time from diagnosis to transplantation months, median (IQR)*	9 (6-14)	8 (6-9)	.11
HCT-CI, n (%)			
0-2	24 (96)	20 (100)	1
≥3	1 (4)	0 (0)	
Transplants			
ABO blood group mismatch, n (%)			
Major	10 (40)	5 (22)	
Minor	2 (8)	3 (13)	
None	13 (52)	14 (63)	
Conditioning, n (%)			
Busulfan-fludarabine	13 (52)	0	
TBI-containing regimen	5 (20)	2 (9)	
Busulfan-fludarabine-cyclophosphamide	0	18 (82)	
ATG-containing regimen	23 (92)	22 (100)	
GVHD prophylaxis, n (%)			
CsA + methotrexate	24 (96)	0	
CsA + steroids	0	20 (90)	
CsA +/− mycophenolate mofetil	0	2 (8)	
CB cells, median (range)†			
TNCs $\times 10^7$/kg	-	2.8 (1.48-4.74)	
$CD34^+ \times 10^5$/kg	-	1.7 (0.73-3.2)	
TPD cells, median (range)‡			
$CD34^+ \times 10^6$/kg	-	2.6 (1.5-3.2)	
$CD3^+ \times 10^4$/kg	-	0.23 (0.05-1.5)	
MUD cells, median (range)‡			
TNCs $\times 10^8$/kg	4.8 (0.8-16)	-	
$CD34^+ \times 10^6$/kg	4 (0.8-19)	-	
HLA match, n (%)			
8/8	25 (100)	-	
6/6	-	0	
5/6	-	4 (18)	
4/6	-	18 (82)	

HCT-CI indicates Hematopoietic Cell Transplantation Comorbidity Index.
* Patients in CR1.
† After processing and before cryopreservation.
‡ Infused cells.

The cumulative incidence of platelet recovery at 60 days was 78%, with a median time of platelet engraftment of 27 days (range, 9-84 days). All patients exhibited PB MC on days +7 and +14, with varying percentages of CB and TPD cells. At 2 months after transplantation, 82% (14 of 17) of evaluable patients exhibited full PB CB chimerism, which was reached in a median of 24 days (range, 13-67 days). All 14 patients had full sustained CB engraftment on the last follow-up. Two of the 3 remaining patients experienced CB graft failure. The third patient exhibited MC during the first 2 months with variable proportions of recipient, CB, and TPD cells; however, TPD was not detected at 2 months post-transplantation, and the patient remained in MC with increasing percentages of recipient cells until relapse (at 4 months posttransplantation).

CB graft failure was documented in 3 patients in the dual SCT group (13%). Two of these patients showed engraftment of TPD cells only. One of these patients died due to toxicity and infection after a second CB transplantation (despite CB myeloid engraftment), and the other patient died due to relapse after multiple infectious complications stemming from poor immune reconstitution. Of note, the 2 patients with persistent TPD neutrophil engraftment experienced severe infectious complications due to poor immune reconstitution. The third patient demonstrated failure of both graft sources and underwent a second dual SCT on day +43, with a favorable outcome. In these 3 patients, CB $CD34^+$ cell and TNC content met cellularity criteria, and retrospective analysis of HLA antibody detection was negative. Postthaw growth of colony-forming units (CFU) was poor in 1 of the 3 patients with CB graft failure cases (23×10^4) and was not detected in the other 2 patients.

In the MUD group, 1 patient (4%) had primary graft failure with early relapse. The remaining 24 patients experienced myeloid engraftment at a median of 16 days post-transplantation (range, 12-28 days) and platelet engraftment

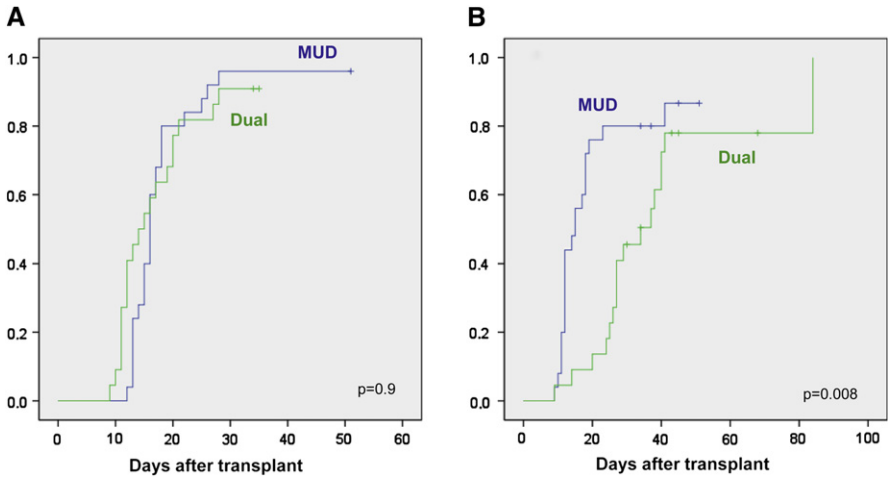

Figure 1. Cumulative incidence of neutrophil (A) and platelet (B) engraftment in dual SCT and MUD SCT recipients.

at a median of 12 days (range, 9-41 days), with a cumulative incidence of 95% at 30 days and 87% at 60 days, respectively.

GVHD

The cumulative incidence of grade II-IV aGVHD at day +100 was 5% in the dual SCT group and 40% in the MUD group (Figure 2). Grade III-IV aGVHD was present in 6 patients in the MUD group but in no patients in the dual SCT group.

cGVHD was documented in 3 of 11 patients (27%) at risk in the dual SCT group and in 8 of 14 patients (57%) in the MUD group. Nevertheless, the 2-year cumulative incidence of moderate-severe cGVHD was 8% in the dual SCT group and 21% in the MUD group ($P = .37$). In addition, 3 patients from the MUD group developed severe GVHD after rapid withdrawal of immunosuppression in 2 patients and donor lymphocyte infusion in 1 patient, which were performed because of relapse and increasing MC.

Survival and Relapse

With a median follow-up of 36 months (IQR, 19-75 months) for the entire cohort, 36 months (IQR, 13-88 months) for the dual SCT group, and 34 months (IQR, 20-68 months) for the MUD group, estimated 3-year OS and DFS were 47% (95% CI, 32%-62%) and 41% (95% CI, 27%-56%), respectively (Figure 3). Differences in OS and DFS between the 2 groups were not statistically significant (Figure 3).

The cumulative incidence of relapse was 44% at both 1 year and 3 years in the dual SCT group and 35% at 1 year and 41% at 3 years in the MUD group ($P = .92$). For the entire cohort, the cumulative incidence of relapse at 3 years was 34% for patients undergoing SCT in CR1 and 57% in those undergoing SCT with active disease. No patient in either group experienced relapse beyond 2 years posttransplantation (Figure 3).

NRM, Infections, and Hospital Stay

Most of the transplantation-related deaths occurred within the first year after transplantation, with no significant differences in the cumulative incidence of NRM between the 2 groups ($P = .79$) (Figure 3). A tendency toward a higher incidence of viral hemorrhagic cystitis was seen in the dual SCT group compared with the MUD group (31% versus 8%; $P = .08$). The remaining infectious events in the first 100 days after SCT showed similar incidence rates in the dual SCT and MUD groups, including probable and proven fungal infections (10% versus 12%) and CMV reactivation (77% versus 76%). Using a preemptive therapy approach, no significant CMV disease incidence or CMV-related deaths

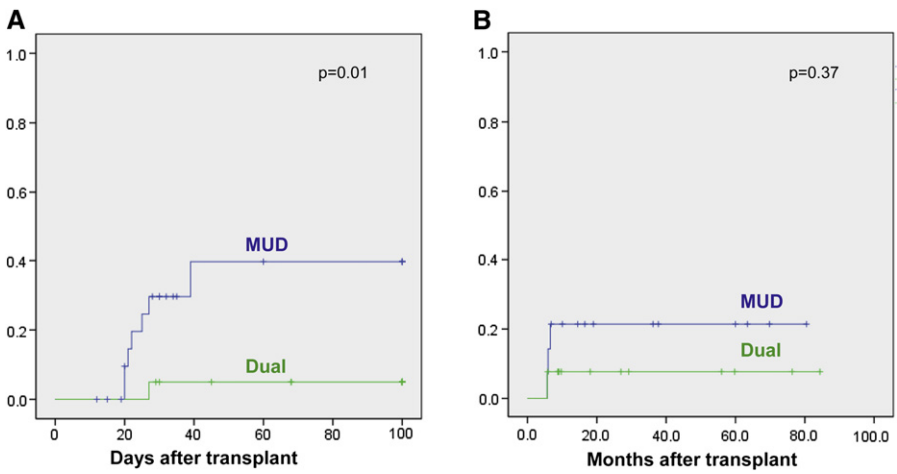

Figure 2. Cumulative incidence of grade II-IV aGVHD (A) and moderate to severe cGVHD (B) in dual SCT and MUD SCT recipients.

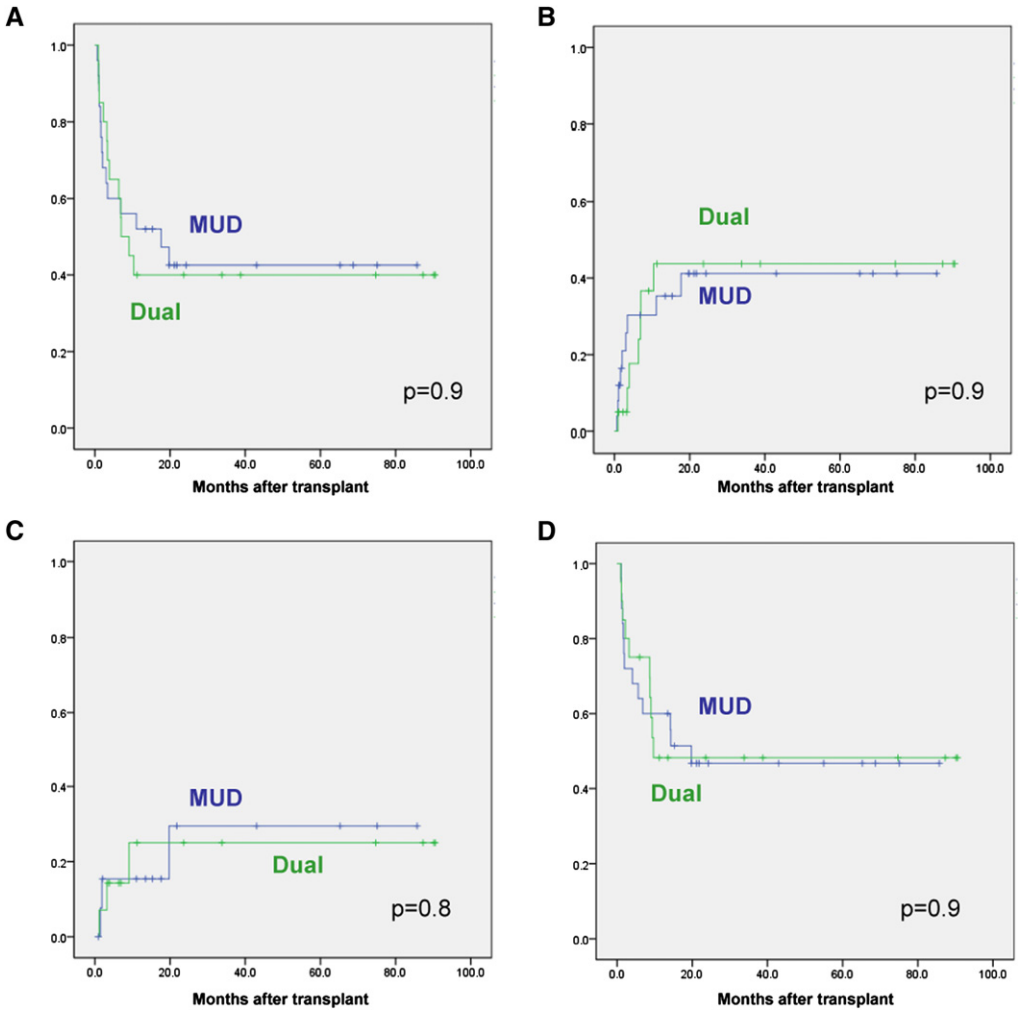

Figure 3. DFS (A), cumulative incidence of relapse (B), cumulative incidence of NRM (C), and OS (D) in dual SCT and MUD SCT recipients.

were identified. Two cases of EBV posttransplantation lymphoproliferative disease (PTLD) were documented, 1 treated successfully with rituximab in the dual SCT group and the other requiring chemotherapy with R-CHOP (rituximab, cyclophosphamide, hydroxydaunorubicin, ocovin, and prednisone) and immunosuppression withdrawal with fatal evolution in the MUD group. In addition, 1 case of EBV reactivation without PTLD documentation occurred and was well controlled in the MUD group.

The median number of hospitalization days during the first 6 months after transplantation was 64 days (IQR, 39-82 days) for the dual SCT group and 49 days (IQR, 32-92 days) for the MUD group ($P = .59$).

DISCUSSION

Umbilical CB from unrelated donors is being increasingly used as an alternative stem cell source for adult patients with hematologic malignancies lacking an HLA-matched adult donor [2-5]. Comparisons of standard single CB SCT with MUD SCT in registry studies have shown comparable DFS in the 2 groups, with lower GVHD rates but higher NRM in the CB recipients [5].

Several strategies are being explored in the setting of CB transplantation to overcome the complications derived from delayed myeloid engraftment. In our institution, we have adopted the dual strategy of coinfusion of mobilized and selected $CD34^+$ cells from a third party HLA-mismatched donor together with a single CB unit for allogeneic SCT candidates without a suitable HLA-matched adult donor [11-15]. This approach offers the advantage of rapid engraftment of the TPD cells, reducing the risk of early infections, followed by sustained hematopoietic engraftment of the CB. In fact, it provides the most rapid neutrophil engraftment reported among different CB transplantation modalities [3,7-10]. A single-center comparative study including dual SCT for patients with acute leukemia and myelodysplastic syndromes showed comparable DFS and OS as myeloablative SCT from HLA-identical related donors [15]. The dual platform also has been recently adopted by other groups for patients with aplastic anemia [23] and patients with high-risk hematologic disorders using reduced-intensity conditioning [24], with similar results in terms of both engraftment and survival. Nevertheless, cumulative experience with this CB transplantation modality remains scarce. Our single-center study provides 2 unique aspects compared with previous reports: (1) a comparison with myeloablative MUD transplants, considered the standard of care for patients with indications for allogeneic transplantation lacking an HLA-identical sibling, and (2) a long-term follow-up of the series (3 years).

Patient features were well balanced between the 2 groups in terms of demographic features, previous transplantation, comorbidities, and time from diagnosis to transplantation

(Table 1). Among clinical variables, no eventual differences regarding specific diagnosis and disease status at transplantation were noted, possibly owing to the limited sample size. Nevertheless, these potential specific differences are not critical, given that the vast majority of cases involve high-risk patients with acute leukemia in both groups, making pretransplantation characteristics comparable in the 2 groups.

The 47% OS and 41% DFS at 3 years derived from our experience in high-risk patients with hematologic disorders (including 38% of patients with relapsed/refractory disease at transplantation), with similar results in the dual SCT and MUD SCT groups, are comparable to those reported in previous registry studies [5,6,25,26]. The incidence of relapse was comparable in the 2 groups. The relapse rate in our MUD group was similar to that reported in 1 study of high-risk patients [26], but that in our dual SCT group was higher than reported in another study [14]. Two factors must be considered related to the risk of relapse in the present study: (1) a significant incidence of active disease at the time of transplantation (37% in the whole series, 30% in the MUD SCT group, and 40% in the dual SCT group) and (2) a low incidence of cGVHD, eventually related to the use of ATG as part of the conditioning regimen in both groups.

As reported previously [11-15], rapid neutrophil and platelet recovery (14 and 27 days, respectively) at the expense of variable proportions of TPD cells superseded by permanent CB engraftment was seen after dual SCT. Furthermore, days to engraftment were similar in the 2 groups.

Using our current umbilical CB unit selection criteria based primarily on cell dose, our CB graft failure rate (13%) was similar to or even lower than rates reported previously [27,28]. Nevertheless, other series with dual SCT reported CB failure rates below 5% [12]. From the extensive experience with CB transplantation, various mechanisms have been associated with CB graft failure, including unit cell content, degree of HLA mismatch, clonogenic capacity of CB progenitors, presence of host anti-HLA antibodies directed to CB antigens, and host disease status [27-31]. In our series, the poor postthaw CB viability was most likely related to CB graft failure in the 3 cases, given that no other known causes for CB failure were documented. Therefore, we believe that postthaw CB colony-forming unit assays should be used as criteria for unit selection, as has been suggested previously [31-33]. TPD myeloid engraftment in 2 of these cases allowed a second CB transplantation in 1 patient; however, both cases had a dismal outcome with multiple infectious complications most likely related to poor immune reconstitution. Rapid rescue measures should be started as soon as CB failure is detected in this setting.

Early toxicity was associated with infectious complications independent of neutrophil engraftment, most frequently viral hemorrhagic cystitis was apparently higher in the dual SCT group compared with the MUD group (31% versus 8%). The dual SCT group had a longer duration of inpatient hospitalization in the first 6 months, which could be related to this viral complication, although the difference between the 2 groups was not statistically significant, likely owing to the limited number of evaluable patients. The use of high-dose steroids for GVHD prophylaxis is the most likely cause of the higher rate of hemorrhagic cystitis in the dual SCT group. Nonetheless, mortality associated with viral complications was not significantly higher in the dual SCT group compared with the MUD group. The rates of other significant infectious complications also were similar in the 2 groups; of note, there were no differences in CMV reactivation and fungal infections. A high incidence of EBV PTLD was recently reported in the dual SCT reduced-intensity conditioning setting [29]; however, only 1 in 24 cases was documented in our series of dual SCT recipients. The higher ATG doses used in the aforementioned study could be a factor in this result.

GVHD is a major complication after allogeneic SCT, resulting in variable degrees of morbidity and compromised quality of life after transplantation, as well as higher rates of NRM, especially after unrelated donor transplantation [5,34,35]. We found significantly lower rates of grade II-IV aGVHD in the dual SCT group compared with the MUD group, with similar DFS and OS in the 2 groups. Thus, this strategy may be associated with significantly less morbidity from GVHD and its therapy, maintaining disease control in surviving patients. This finding is of special interest for patients with nonmalignant conditions, such as aplastic anemia [23]. The observation of a graft-versus-tumor effect and a low incidence of serious GVHD is consistent with previous data on early and sustained recovery of natural killer cells after CB transplantation and the recognition of the eventual role of these cells as a potent antitumor effector [15,36,37].

In the search for the ideal alternative donor source and transplantation modality for patients lacking an HLA-matched adult donor, procedures such as reduced-intensity conditioning with unrelated double CB and partially HLA-mismatched related BM are currently being evaluated [38,39]. In our experience, single CB transplantation together with the coinfusion of $CD34^{+}$ cells from a third party HLA-mismatched donor in high-risk patients offers time to engraftment and survival rates comparable to those seen with myeloablative 8/8 HLA-matched MUD transplantation, with significantly lower GVHD rates. The relatively small number of patients included in this analysis requires that our results be interpreted with caution. However, the homogeneous patient management in terms of both inclusion criteria and clinical care, with all patients undergoing transplantation in the same center at the same time frame with the same follow-up, should outweigh the effect of small sample size as a potential limitation. In contrast to previous comparative studies including single CB transplantations, the dual approach offers similar NRM rates as MUD transplantation. Our results show that dual SCT provides a valuable option for high-risk patients who lack a matched adult donor or who require urgent transplantation. Thus, this CB transplantation approach merits broader exploration and validation in different hematologic diseases and centers.

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