Transplantation and Cellular Therapy 29 (2023) 181.e1–181.e10

Transplantation and Cellular Therapy

journal homepage: www.tctjournal.org

ASTCT American Society for Transplantation and Cellular Therapy

Full Length Article
Allogeneic – Adult

Effect of CD34$^+$ Cell Dose on the Outcomes of Allogeneic Stem Cell Transplantation with Post-Transplantation Cyclophosphamide

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Article history:
Received 12 October 2022
Accepted 5 December 2022

Key Words:
CD34$^+$ cell dose
Graft
Post-transplantation cyclophosphamide
Haploidentical donor
Allogeneic hematopoietic stem cell transplantation

A B S T R A C T

The impact of infused CD34$^+$ cell dose on outcomes after allogeneic hematopoietic stem cell transplantation (alloHSCT) using standard graft-versus-host disease (GVHD) prophylaxis remains controversial. Information on this subject is scarce for alloHSCT using high-dose post-transplantation cyclophosphamide (PTCy). We aimed to assess the effect of CD34$^+$ cell dose in peripheral blood stem cell (PBSC) grafts on the outcome of alloHSCT using PTCy-based GVHD prophylaxis. To do so, we conducted a single-center retrospective analysis of 221 consecutive adult patients who underwent PTCy alloHSCT from HLA-matched sibling donors (MSDs; n = 22), HLA-matched unrelated donors (MUDs; n = 83), mismatched unrelated donors (MMUDs; n = 73), and haploidentical donors (n = 43). Based on the binary partitioning method, 5 × 10^6/kg was used as the optimal cutoff for CD34$^+$ cell dose. According to our institutional protocol, the maximum CD34$^+$ cell dose was capped at 8 × 10^6/kg. The study cohort was divided into 2 groups based on CD34$^+$ cell dose: high dose (>5 to 8 × 10^6/kg) and low dose (≤5 × 10^6/kg). Patients receiving high-dose CD34$^+$-containing grafts had significantly shorter median times to neutrophil engraftment and platelet engraftment compared to those who received low-dose CD34+ (19 days versus 21 days [*P* = .002] and 16 days versus 22 days [*P* = .04], respectively). There were no differences between the high-dose and low-dose groups in the cumulative incidence of day +100 acute GVHD (grade II-IV: 25% versus 23% [*P* = .7]; grade III-IV: 5% versus 4% [*P* = .4], respectively) or 2-year chronic GVHD (moderate/severe GVHD: 9% versus 6%; *P* = .5). There was no impact of CD34$^+$ cell dose on survival outcomes with the use of MSDs, MUDs, or MMUDs. Recipients of haploidentical alloHSCT using low-dose CD34$^+$ cells had significantly worse overall survival (hazard ratio [HR], 6.01; *P* = .004) and relapse-free survival (HR, 4.57; *P* = .004). In recipients of PBSC PTCy alloHSCT, infused CD34$^+$ cell doses >5 to 8 × 10^6/kg were associated with faster neutrophil and platelet engraftment, independent of donor type. Our study suggests an impact of CD34$^+$ cell dose on survival outcomes only with haploidentical donors, for whom the administration of a CD34$^+$ cell dose ≤5 × 10^6/kg significantly decreased survival outcomes.

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INTRODUCTION

Post-transplantation high-dose cyclophosphamide (PTCy) has become an increasingly popular approach for graft-versus-host disease (GVHD) prophylaxis in allogeneic hematopoietic stem cell transplantation (alloHSCT), not only in the context of haploidentical alloHSCT [1], but also in transplants from HLA-matched sibling donors (MSD), matched unrelated donors (MUDs), and mismatched unrelated donors (MMUDs) [2–6]. As in alloHSCT using other GVHD prophylaxis strategies, peripheral blood stem cells (PBSCs) are considered as the graft source of choice in many PTCy alloHSCTs because of their ease of collection and richness in CD34$^+$ cells. However, the impact of graft cell dose on clinical outcomes after transplantation has not been evaluated in detail in the PTCy setting.

Financial disclosure: See Acknowledgments on page 181.e9.
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https://doi.org/10.1016/j.jtct.2022.12.005
2666-6367/Published by Elsevier Inc. on behalf of The American Society for Transplantation and Cellular Therapy.

The vast majority of analyses reported to date evaluating the influence of graft cell dose on survival outcomes after transplantation has been performed in patients undergoing alloHSCT using standard (ie, non-PTCy) GVHD prophylaxis. These studies have reported inconsistent results that can be explained by differences in disease categories, donor type, conditioning regimen, and GVHD prophylaxis. Low $CD34^+$ cell dose, defined by different thresholds depending on the study, has been associated with higher rates of nonrelapse mortality (NRM) and inferior overall survival (OS) [7,8]. On the other hand, high $CD34^+$ cell dose has been associated with faster neutrophil and platelet engraftment but a higher incidence of clinically significant chronic GVHD and relapse [9–13]. In any case, these findings may not be applied to the PTCy platform, which is associated with a significantly lower risk of GVHD.

The aim of this study was to assess the impact of infused $CD34^+$ cell dose on PBSC PTCy alloHSCT outcomes.

METHODS

Patients and Donors

This study, performed at the Hospital Clínic in Barcelona, Spain between March 2013 and July 2021, analyzed data from 221 consecutive patients who underwent their first peripheral blood alloHSCT for malignant hematologic disease using PTCy-based GVHD prophylaxis. Eligibility criteria for transplantation were age 18 to 69 years, Eastern Cooperative Oncology Group Performance Status ≤2, left ventricular ejection fraction ≥35%, forced expiratory volume in 1 second and forced vital capacity ≥40% of predicted, and adequate hepatic function (defined as total bilirubin ≤3.0 mg/dL or absence of clinically significant liver disease). Patients with previous alloHSCT and indeterminate Disease Risk Index (DRI) were excluded.

All donor-recipient pairs underwent high-resolution typing by allelic level for HLA-A, -B, -Cw, -DRB1, and -DQB1. For patients who did not have a 10/10 HLA MSD or MUD, a search was performed based on a single HLA mismatch at HLA-A, -B, -Cw, or -DRB1 (7/8 HLA mismatch). In the absence of a 10/10 or 9/10 HLA-matched donor, an MMUD was selected. Haploidentical transplantation was performed when an MSD, MUD, or MMUD could not be found. According to our institutional protocol, the absence of anti-HLA antibodies was systematically verified in all patients.

The study protocol received Institutional Review Board approval, and all participants provided signed informed consent. Clinical information was collected retrospectively and updated in April 2022. This study was approved by the local Ethics Committee and was conducted following standards set forth by the Declaration of Helsinki.

Graft Information

T cell-replete PBSC grafts were infused in all cases. The PBSC doses were calculated based on patients' actual body weight. According to our institutional protocol, the maximum $CD34^+$ cell dose was capped at 8×10^6/kg. Stem cells were mobilized with s.c. infused granulocyte colony-stimulating factor daily for 5 days. After progenitor stem cell collection, the $CD34^+$ and $CD3^+$ cell counts were determined. The products were cryopreserved in all cases after March 2020 because of the Coronavirus disease 2019 pandemic; cellularity was not reevaluated after thawing.

Based on the binary partitioning method, 4.96×10^6/kg was the optimal cutoff of $CD34^+$ cell dose to separate patients in 2 groups in terms of our main outcome variable (OS). To make the cutoff applicable to regular clinical practice, high dose was defined as >5 to 8×10^6/kg $CD34^+$ cells, and low dose was defined as ≤5 $\times 10^6$/kg $CD34^+$ cells. We evaluated the transplantation outcomes for the complete cohort as well as separately according to type of donor selected.

Conditioning Regimen, GVHD Prophylaxis, and Supportive Care

The specific conditioning regimens used were based on the type of hematologic disease and patient characteristics in accordance with institutional protocols. Patients age >50 years or who had undergone previous autologous HSCT received a reduced-intensity conditioning (RIC) regimen; otherwise, a myeloablative conditioning (MAC) regimen was administered. All patients received fludarabine-based conditioning (fludarabine 30 to 40 mg/m^2/day for 4 days) in combination with busulfan (3.2 mg/kg/day for 4 days for MAC or 3 days for RIC), total body irradiation (TBI) (12 Gy for MAC or 8 Gy for RIC), melphalan (70 mg/m^2 for 2 days), cyclophosphamide (14.5 mg/kg for 2 days) plus 2 Gy TBI, or a sequential conditioning regimen consisting of fludarabine 30 mg/m^2/day for 5 days, cytosine arabinoside 2 g/m^2 for 5 days, idarubicin 12 mg/m^2 for 3 days, and melphalan 70 mg/m^2 for 2 days.

The institutional standard GVHD prophylaxis consisted of PTCy 50 mg/kg/day administered on days +3 and +4, followed by tacrolimus started on day +5 at a dose of .04/kg/day i.v., titrated to a therapeutic level of 5 to 15 ng/L, maintained until day +90 and tapered progressively up to day +180 in the absence of GVHD. In addition, mycophenolate mofetil was administered from day +5 to day +28 for haploidentical transplant recipients. Antithymocyte globulin (ATG) and alemtuzumab were not administered. Colony-stimulating factor and cytomegalovirus prophylaxis were not provided routinely. Supportive care and infectious prophylaxis have been described previously [6].

Definitions

Neutrophil recovery was defined as the first of 3 consecutive days with an absolute neutrophil count >.5 $\times 10^9$/L after transplantation. Platelet recovery was defined as a platelet count >20 $\times 10^9$/L without transfusion in the 7 preceding days. Primary graft failure was defined as the absence of absolute neutrophil count recovery (>.5 $\times 10^9$/L) before day +28, which was maintained for 3 consecutive days with a platelet count <20 $\times 10^9$/L, hemoglobin level <80 g/L, and the need for transfusion support. Immune reconstitution was defined as $CD3^+/CD4^+$ and $CD3^+/CD8^+$ lymphocyte counts >200 cells/L.

Toxicity was scored using Common Terminology Criteria for Adverse Events version 5. Acute GVHD (aGVHD) was scored using the Glucksberg criteria, and chronic GVHD (cGVHD) was defined and scored according to the current National Institutes of Health consensus criteria [14].

Statistical Analysis

$CD34^+$ cell dose was considered the primary explanatory variable of interest, and OS and NRM were the primary outcome variables of the study. The primary variable of interest was treated as continuous variable and transformed into a dichotomous variable after calculating the optimal cutoff value (5×10^6/kg $CD34^+$ cells) for OS prediction. This cutoff value was calculated based on the binary partitioning method [15], which uses the likelihood ratio statistic to evaluate the performance of individual splits. This test permits partitioning of a set of longitudinal data into 2 mutually exclusive groups based on an optimal split of a continuous prognostic variable. Relapse-free survival (RFS), cumulative incidence of relapse (CIR), and cumulative indices of acute and chronic GVHD and primary GF were considered relevant variables of interest for exploring the study's secondary endpoints.

Time to event was calculated from the date of transplantation to the date of the event or last follow-up. OS and RFS were calculated using the Kaplan-Meier method. NRM and CIR were estimated using competing-risk analysis and considering relapse as a competing event for NRM and death without relapse as a competing event for CIR. The cumulative incidence of GVHD was calculated by accounting for death and relapse as competing events, and the cumulative incidence of primary GF was estimated considering death as the competing event. Univariate and multivariate regression analyses were conducted to explore the impact of $CD34^+$ cell dose on post-transplantation outcomes. Those variables found to be significant in the univariate model or considered clinically relevant were included in the multivariate analysis. The primary explanatory variable was always included in the multivariate model. All P values were 2-sided, and $P <$ .05 was considered to indicate statistical significance. Statistical analyses were performed using EZR software [16].

RESULTS

Patient and Donor Characteristics

Baseline characteristics of the entire cohort and of 2 groups stratified according to $CD34^+$ cell dose are provided in Table 1. Acute leukemia and myelodysplastic syndrome were the most frequent indications for transplantation (72%). Most patients (79%) had low- to intermediate-risk disease as defined by the refined DRI. The most frequently selected donor type was MUD/MMUD (71%). One hundred forty-four patients (65%) received a high $CD34^+$ cell dose, and 77 (35%) received a low $CD34^+$ dose. No differences between the 2 groups were seen in the main characteristics such as patient age, hematologic diagnosis, DRI, Karnofsky Performance Status (KPS), Hematopoietic Cell Transplantation Comorbidity Index, and conditioning regimen. The median duration of follow-up was 25 months (IQR, 11 to 55 months) for patients in the high-dose group and 21 months (IQR, 6 to 41 months) in the low-dose group (P = .06).

Engraftment and Immune Reconstitution

Engraftment information is summarized on Table 2. Overall, 217 patients (98%) achieved primary engraftment. Patients receiving high-dose $CD34^+$ cell-containing grafts had significantly

Table 1
Patient, Donor, and Transplantation Characteristics According to $CD34^+$ Cell Dose

Characteristic	All Patients (N = 221)	$CD34^+$ Cell Dose, $\leq 5 \times 10^6$ (N = 77)	$CD34^+$ Cell Dose 5-8 $\times 10^6$ (N = 144)	*P* Value
Patient information				
Age, yr, median, (range)	52 (18-70)	53 (19-69)	51 (18-70)	.20
Male sex	121 (54)	48 (62)	73 (51)	.09
Baseline diagnosis, n (%)				—
AML	81 (37)	32 (42)	49 (34)	
ALL	39 (18)	10 (13)	29 (20)	
MDS/CMML	38 (17)	17 (22)	21 (15)	
MPN	13 (6)	4 (5)	9 (6)	
NHL/CLL	30 (13)	5 (6)	25 (17)	
Other	20 (9)	9 (12)	11 (7)	
DRI high-very high, n (%)	47 (21)	18 (23)	29 (20)	.57
KPS 60-80, n (%)	51 (23)	17 (22)	34 (24)	.77
HCT-CI score >3, n (%)	49 (22)	15 (19)	34 (24)	.48
Donor information				
Age, yr, median (IQR)	34 (26-44)	34 (26-45)	35 (26-43)	.44
Male sex, n (%)	121 (55)	48 (62)	73 (51)	.03
Female donor to male recipient, n (%)	37 (17)	20 (28)	17 (12)	.007
Donor type, n (%)				
HLA MSD	22 (10)	6 (8)	16 (11)	.52
10/10 HLA MUD	83 (38)	28 (36)	55 (38)	
7/8 HLA MMUD	73 (33)	30 (39)	43 (30)	
Haploidentical	43 (19)	13 (17)	30 (21)	
Donor/recipient CMV status, n (%)				
Positive/positive	106 (48)	35 (45)	71 (49)	.59
Negative/positive	76 (34)	29 (38)	47 (32)	
Positive/negative	19 (8)	5 (6)	14 (10)	
Negative/negative	20 (9)	8 (10)	12 (8)	
Graft information				
$CD34^+$ cell dose, $\times 10^6$, median (IQR)	5.7 (4.5-7.0)	4 (3.6-4.5)	7.5 (5.8-7.5)	—
$CD3^+$ cell dose, $\times 10^6$, median (IQR)	252 (184-340)	254 (184-359)	248 (184-327)	.45
Type of stem cell product, n (%)				
Fresh	169 (76)	58 (75)	111 (77)	.76
Cryopreserved	52 (23)	19 (25)	33 (23)	
Transplantation information, n (%)				
Conditioning regimen				
MAC	90 (41)	28 (36)	62 (43)	.49
FluBu 4 days	51 (23)	21 (27)	30 (21)	
FluTBI 12 Gy	39 (18)	7 (9)	32 (22)	
RIC	131 (59)	49 (64)	82 (57)	
FluBu 2-3 days	74 (33)	30 (39)	44 (31)	
FluBu 2-8 Gy	22 (10)	7 (9)	15 (10)	
FluMel	14(6)	6 (8)	8 (6)	
IDA-FLAG/Mel (sequential)	8 (4)	4 (4)	4 (3)	
Flu/CFM/TBI 2 Gy (Baltimore)	13 (6)	2 (3)	11 (7)	
Follow-up, mo, median (IQR)	24 (9-50)	21 (6-41)	25 (11-55)	.06

AML indicates acute myelogenous leukemia; ALL, acute lymphoblastic leukemia; MDS, myelodysplastic syndrome; CMML, chronic myelomonocytic leukemia; MPN, myeloproliferative neoplasms; NHL, non-Hodgkin lymphoma; CLL, chronic lymphoblastic leukemia; HCT-CI, Hematopoietic Cell Transplantation Comorbidity Index; CMV, cytomegalovirus; Flu, fludarabine; Bu busulfan; TBI, total body irradiation; Mel, melphalan; IDA-FLAG, idarubicin, fludarabine, cytarabine, granulocyte colony-stimulating factor; CFM, cyclophosphamide.

shorter median times to neutrophil engraftment (19 days versus 21 days; $P = .002$) and platelet engraftment (16 days versus 22 days; $P = .04$) compared with those receiving low-dose grafts (Table 2, Figure 1). Four alloHSCT recipients (1.8%) experienced primary GF, including 1 patient with an HLA-matched donor, 2 patients with an MMUD, and 1 patients with a haploidentical donor. The cumulative incidence of GF was 4% for patients in the low-dose group and .7% for those in the high-dose group ($P = .08$) (Table 2).

The cumulative incidences of $CD4^+$ and $CD8^+$ lymphocyte recovery at 1 year were similar in the high-dose and low-dose groups (58% versus 55% [$P = .2$] and 64% versus 63% [$P = .4$], respectively). The infusion of $\leq 5 \times 10^6$/kg $CD34^+$ cells was not associated with worse $CD4^+$ and $CD8^+$ cell recovery (hazard ratio [HR], .83 [$P = .36$] and .88 [$P = .53$], respectively). The $CD3^+$ cell dose had no significant effect on hematopoietic recovery or immune reconstitution.

Table 2
Main Post-Transplantation Outcomes According to $CD34^+$ Cell Dose

Post-Transplantation Outcome	$CD34^+$ Cell Dose $\leq 5 \times 10^6$	$CD34^+$ Cell Dose 5-8 $\times 10^6$	*P* Value
Engraftment			
Days to neutrophil engraftment, median (IQR)	21 (18-25)	19 (16-22)	**.002**
Days to platelet engraftment, median (IQR)	22 (13-30)	16 (12-24)	**.04**
Cumulative incidence of primary graft failure, % (95% CI)	4 (1-10)	.7 (.1-3)	.08
Cumulative incidence of GVHD, % (95% CI)			
Day +100 grade II-IV aGVHD	23 (15-33)	25 (18-32)	.70
Day +100 grade III-IV aGVHD	4 (1-10)	5 (2-10)	.45
2-year moderate/severe cGVHD	6 (2-14)	9 (5-16)	.55
Infections, n (%)			
Bacterial infection	41 (53)	73 (50)	.71
CMV reactivation	43 (56)	77 (53)	.66
Other viral infections	30 (39)	66 (46)	.32
Fungal infection	12 (15)	12 (8)	.09
1-year NRM, % (95% CI)*	21 (13-31)	13 (8-20)	.18
1-year cumulative incidence of relapse, % (95% CI)	21 (13-31)	19 (13-26)	.50
Survival outcomes, % (95% CI)*			
2-year OS	64 (52-74)	71 (62-78)	**.04**
2-year RFS	52 (40-62)	59 (50-66)	.07

Significant *P* values are in bold type.
* Estimated probability.

GVHD

The cumulative incidence of aGVHD by day +100 was similar in high-dose and low-dose groups: grade II-IV, 25% versus 23% (HR, .89; *P* = .7); grade III-IV, 5% versus 4% (HR, .6; *P* = .4) (Table 2, Figure 2). There was no between-group difference in the cumulative incidence of 2 year moderate-severe cGVHD (9% versus 6%; HR, .7; *P* = .5) (Table 2, Figure 2). There was no statistically significant correlation between infused $CD3^+$ cell dose and the development of GVHD.

NRM, Relapse, and Infections

The cumulative incidence of NRM at 1 year was 13% for patients in the high-dose group compared with 21% for those in the low-dose group (*P* = .18) (Table 2, Figure 3). The analysis of NRM according to donor type and $CD34^+$ cell dose showed no differences between the high-dose and low-dose groups in MSD, MUD, and MMUD alloHSCT, whereas a trend toward a higher NRM was observed in recipients of haploidentical donor alloHSCT receiving low-dose $CD34^+$ cell grafts (39% versus 13%; *P* = .05) (Table 3, Figure 4). Only older age and a KPS of 60 to 80 were identified as significant risk factors for NRM in the univariate and multivariate analyses (Table 3). In the high-dose group, the causes of death without relapse were bacterial septic shock (n = 8), aGVHD (n = 4), infectious or toxic leukoencephalopathy (n = 2), thrombotic microangiopathy (n = 1), melanoma (n = 1), and lung neoplasia (n = 1). In the low-dose group, 18 non-relapse-related deaths were recorded due to bacterial septic shock (n = 12), neurotoxoplasmosis

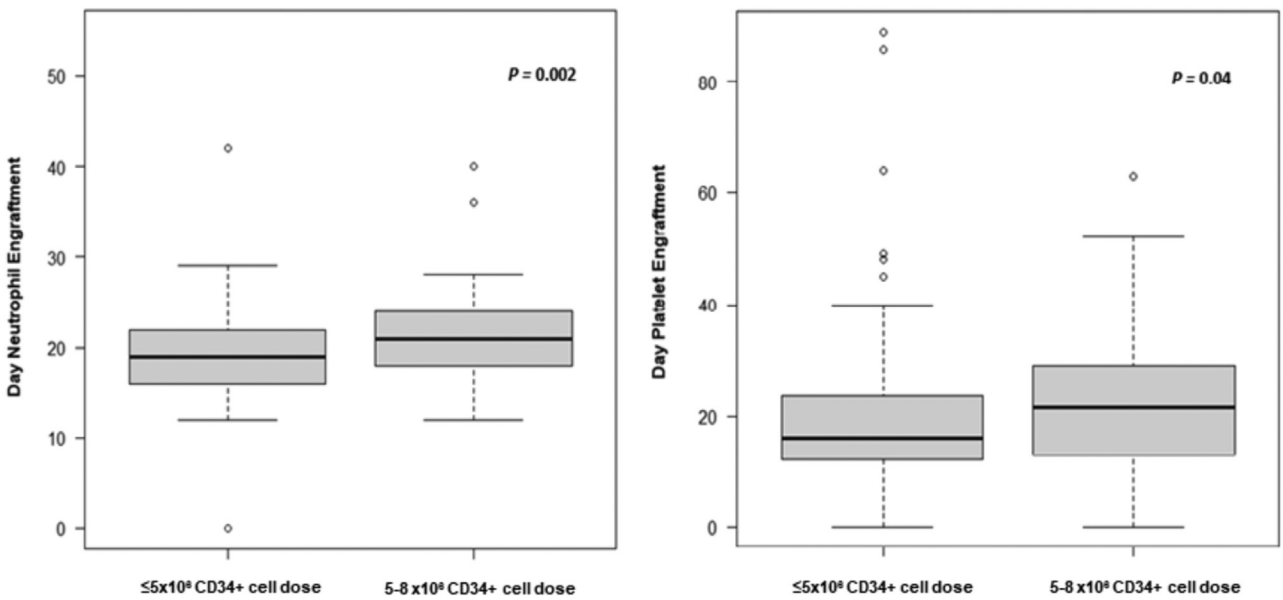

Figure 1. Times to neutrophil and platelet engraftment.

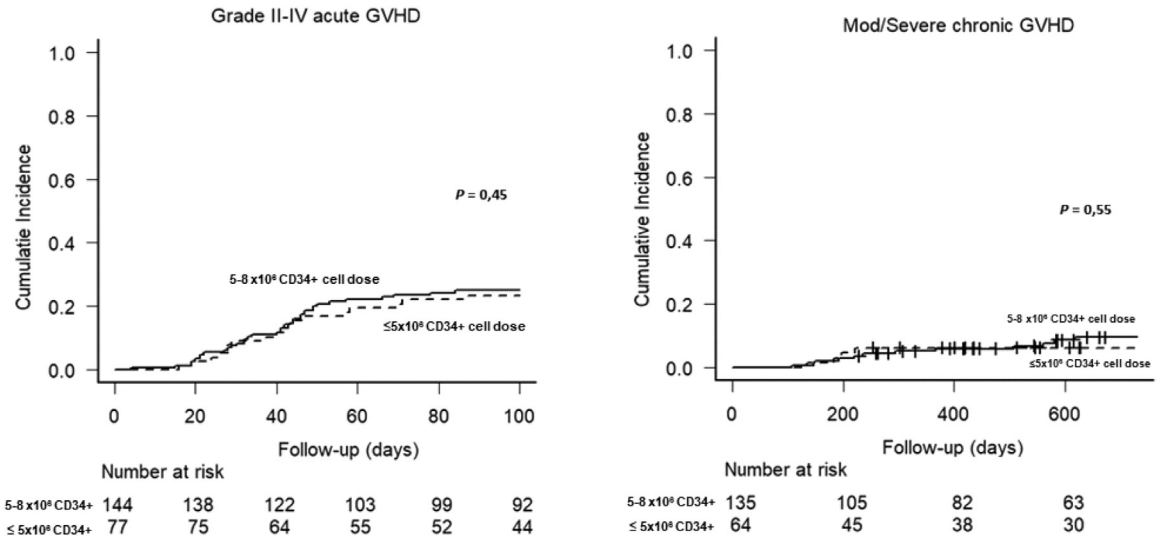

Figure 2. Cumulative incidences of acute and chronic GVHD according to CD34$^+$ cell dose.

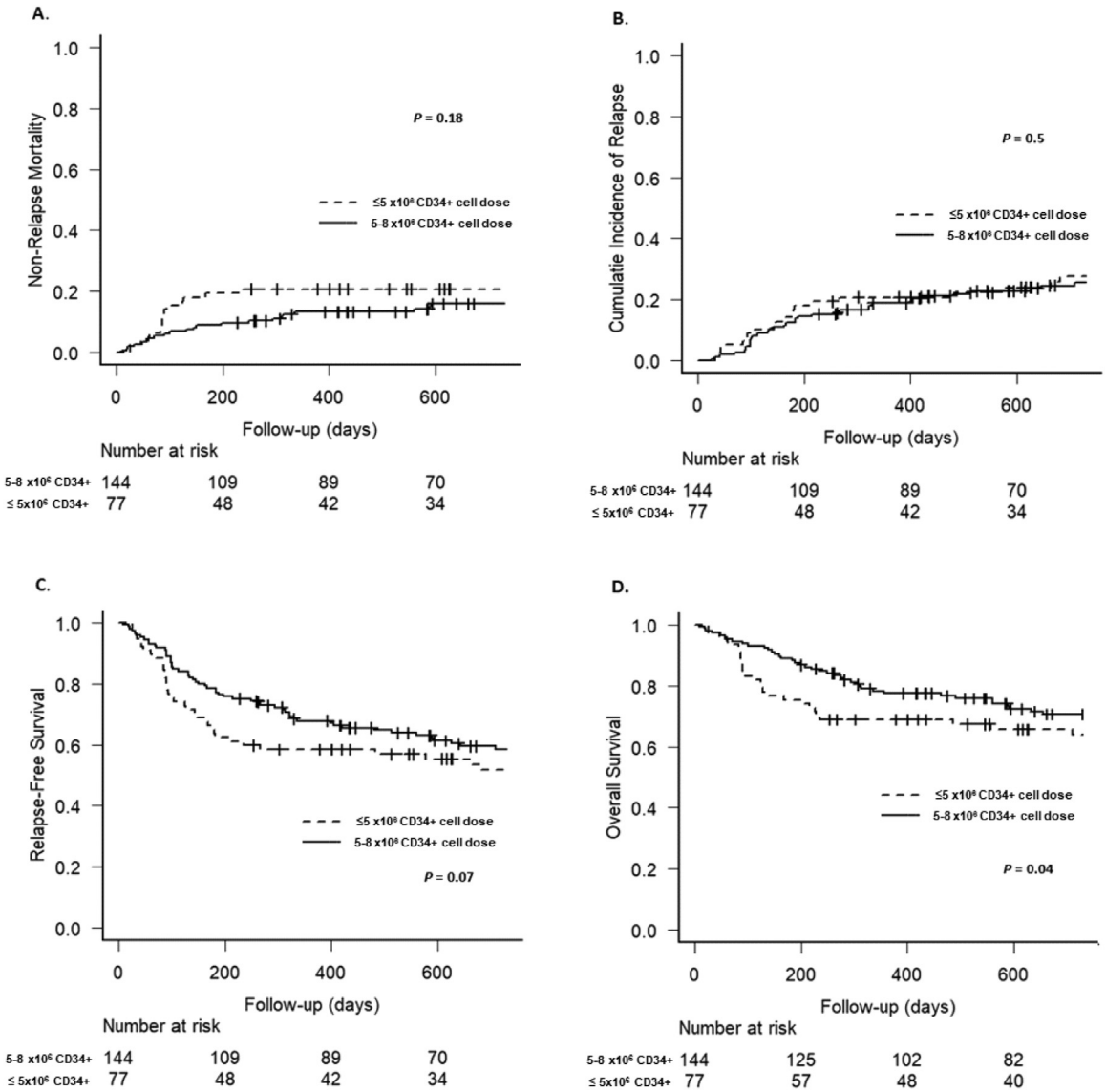

Figure 3. Main post-transplantation outcomes according to CD34$^+$ cell dose. (A) NRM; (B) CIR; (C) RFS; (D) OS.

Table 3
Main Post-Transplantation Outcomes According to Donor Type and $CD34^+$ Cell Dose

Post-Transplantation Outcomes	$CD34^+$ Cell Dose $\leq 5 \times 10^6$	$CD34^+$ Cell Dose 5-8 $\times 10^6$	P Value
MSD and MUD transplantation, % (95% CI)			
1-yr NRM	15 (5-29)	11 (5-20)	.39
1-yr relapse rate*	21 (9-36)	26 (16-36)	.45
2-yr OS	70 (50-84)	69 (56-79)	.72
2-yr RFS	57 (38-72)	52 (39-64)	.91
MMUD transplantation, % (95% CI)			
1-yr NRM	20 (8-36)	16 (7-29)	.83
1-yr relapse rate*	17 (6-32)	16 (7-29)	.56
2-yr OS	67 (47-81)	71 (55-83)	.98
2-yr RFS	63 (44-78)	67 (51-79)	.69
Haploidentical transplantation, % (95% CI)			
1-yr NRM	39 (13-64)	13 (4-28)	**.05**
1-yr relapse rate*	46 (17-72)	11 (3-25)	**.02**
2-yr OS	39 (14-63)	83 (64-92)	**<.001**
2-yr RFS	15 (3-39)	76 (56-88)	**<.001**

Significant *P* values are in bold type.
* Cumulative incidence.

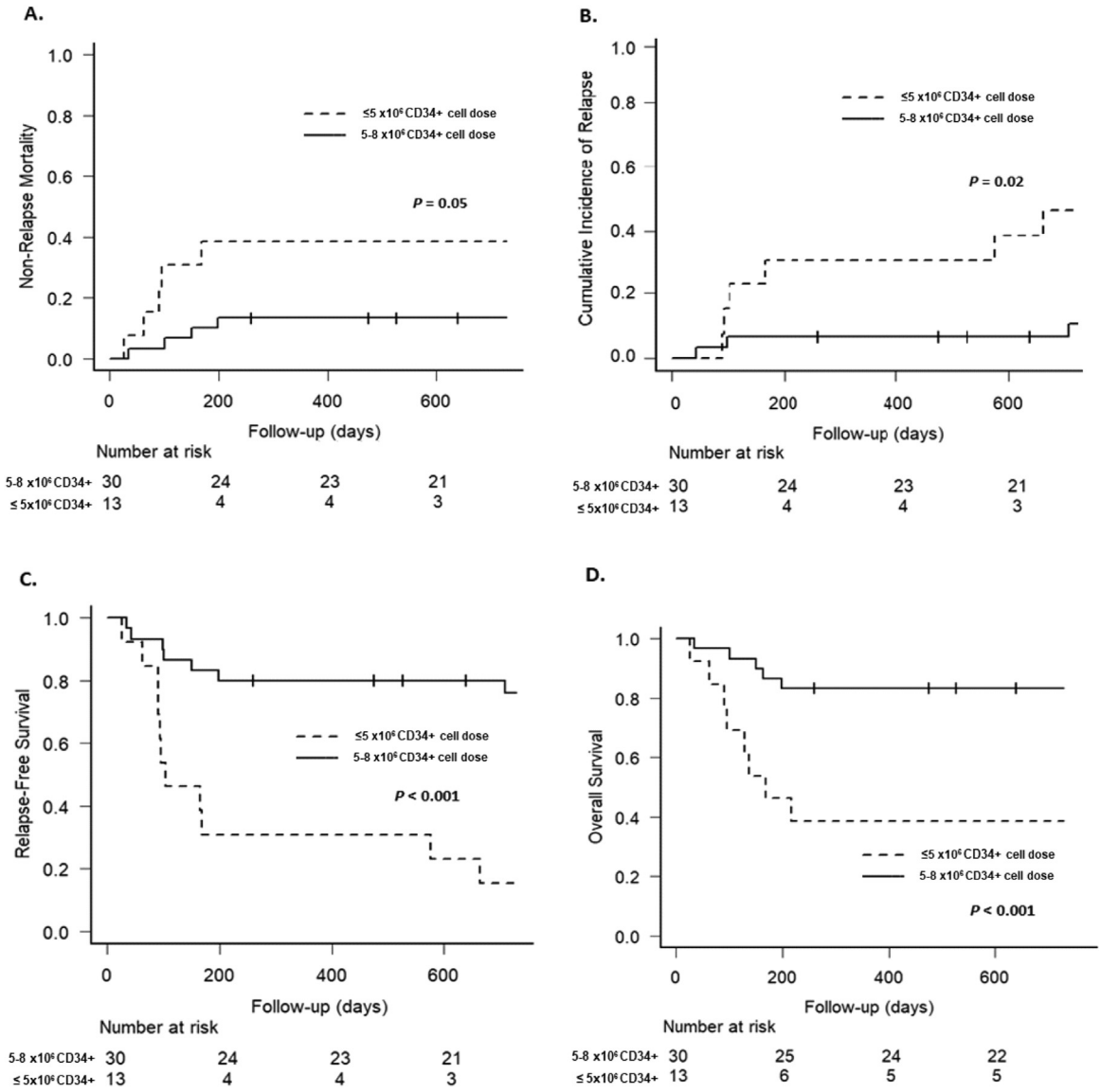

Figure 4. Main outcomes after haploidentical alloHSCT according to $CD34^+$ cell dose: (A) NRM; (B) CIR; (C) RFS; (D) OS.

(n = 1), severe aGVHD (n = 2), cryptogenic pneumonia (n = 2), and myocardial infarction (n = 1).

Cumulative incidence of relapse (CIR) rate was similar between groups (19% for high-dose vs. 21% for low-dose, P=.5) (Table 2, Figure 3). The analysis of CIR according to donor type and CD34+ cell dose showed that, on the contrary to other type of donors, the use of haploidentical donors with low-dose CD34+ cells was associated with higher CIR (46% vs. 11%, P=.02) (Table 3, Figure 4). In univariate analysis, older age, KPS of 60-80%, and high-very high DRI were associated with higher risk of relapse (Table 4). The impact of age and performance status on CIR was confirmed in multivariate analysis (Table 4).

Cytomegalovirus reactivation occurred in 40 of 83 (48%) seropositive patients in the high-dose group and in 18 of 40 (45%) seropositive patients in the low-dose group (P = .6). The incidence of other viral infections was also similar in the 2 groups (55% versus 54%; P = .5). There also were no significant differences between the groups in the rate of bacterial infections (50% versus 52%; P = .7) or fungal infections (12% versus 22%; P = .6).

OS and RFS

One hundred and forty-three patients (65%) were alive at the time of this report, with a median follow-up for all patients of 2 years (IQR, .7 to 4.1 years). At 2 years post-transplantation, OS and RFS for the entire cohort were 68% (95% CI, 61% to 74) and 56% (95% CI, 49% to 63%), respectively. OS was significantly higher in the high-dose group compared with the low-dose group (71% versus 64%; P = .04) (Table 2, Figure 3). Nevertheless, when this analysis was conducted according to donor type, the negative impact of low $CD34^+$ cell dose on post-transplantation outcomes was observed only in the haploidentical alloHSCT setting (83% versus 38%; $P < .001$) (Table 3, Figure 4). Considering these results, a multivariate regression model was created using $CD34^+$ cell dose ($\leq 5 \times 10^6$ versus >5 to 8×10^6), donor type (haploidentical versus others), and the product of the 2 ($CD34^+$ cell dose and donor type) as explanatory variables. The estimated coefficient of the product variable compared the effect on the risk of OS from receiving grafts with low cellularity between recipients of haploidentical donor grafts and recipients of grafts from other donor types. As reported in Table 4, haploidentical alloHSCT with a low $CD34^+$ cell dose resulted in a statistically significant worse OS (HR, 6.01; P = .006). However, the infusion of low $CD34^+$ cell dose-containing grafts in alloHSCT performed from MSDs, MUDs, and MMUDs did not have a statistically significant effect on OS (HR, 1.15; P = .59) (Supplementary Tables S1 and S2). In addition, older age, KPS 60 to 80, Hematopoietic Cell Transplantation Comorbidity Index >3, and high-very high DRI also were associated with lower OS.

A trend toward a better RFS also was observed in the high-dose group (59% versus 52%; P = .07) (Table 2, Figure 3). Similar to OS, the adverse effect of low doses of $CD34^+$ cells on RFS was observed only with the use of haploidentical donors (HR, 4.57; P = .004), and these differences were not statistically significant when low-dose grafts were included outside of the haploidentical alloHSCT setting (HR, 1.04; P = .85) (Table 3, Figure 4, Supplementary Figures 1 and 2). Other variables statistically significantly associated with worse RFS were old age, KPS 60 to 80, high-very high DRI, and nadir of neutropenia >19 days (Table 4). We observed no significant effect of infused $CD3^+$ cell dose on OS or RFS outcomes.

DISCUSSION

Our present results show that the impact of $CD34^+$ cell dose on PBSC PTCy alloHSCT survival outcomes depends on the type of donor. Administration of a $CD34^+$ cell dose $\leq 5 \times 10^6$/kg with haploidentical PBSC PTCy HSCT was associated with decreased OS and RFS. This negative effect was not observed when other types of donors were used. A high $CD34^+$ cell dose was associated with faster neutrophil and platelet recovery in all alloHSCT recipients independent of donor type, consistent with prior studies [17].

Several studies have analyzed the effect of $CD34^+$ cell dose on the outcome after PBSC alloHSCT using standard, non-PTCy GVHD prophylaxis and have reported inconsistent conclusions [18–25]. Some of these studies, using different $CD34^+$ cell dose thresholds, showed that higher doses are associated with higher rates of cGVHD, NRM, and relapse [9–13]. More recently, several studies evaluated the effect of $CD34^+$ cell dose on outcomes after PTCy alloHSCT, including 2 studies of haploidentical transplantation [26,27]. The European Society for Blood and Marrow Transplantation (EBMT) published a retrospective registry-based study of 414 haploidentical alloHSCT for acute myelogenous leukemia, including PTCy-based or ATG-based GVHD prophylaxis [26]. As in our study, patients were divided into 2 cohorts based on $CD34^+$ dose with a cutoff point of 4.96×10^6/kg. The median $CD34^+$ cell dose was 6.58×10^6/kg (range, 2.2 to 31.2×10^6). Patients in the high-dose group ($>4.96 \times 10^6$/kg) experienced higher rates of neutrophil and platelet engraftment and a shorter median time to engraftment compared with those in the low-dose group. $CD34^+$ cell dose did not impact the development of aGVHD or cGVHD. Patients who received $>4.96 \times 10^6$/kg $CD34^+$ cells had prolonged survival, owing mainly to a reduced NRM rate. There was no impact of the type of GVHD prophylaxis, PTCy versus ATG, on survival outcomes in this study. Our results using a virtually identical cutoff were similar to those of the EBMT study for haploidentical transplant recipients. Salas et al. [27] explored the impact of $CD34^+$ cell dose in 68 PBSC haploidentical HSCT recipients using RIC and PTCy plus ATG. Patients were divided into 2 groups using a $CD34^+$ cell dose $\geq 9 \times 10^6$/kg as a threshold. In this study, the median infused $CD34^+$ cell dose was 9.32×10^6/kg (range, 3.4 to 28.6×10^6/kg). The authors reported that the infusion of higher-cellularity grafts ($\geq 9 \times 10^6$/kg $CD34^+$ cells) had a significant adverse impact on OS and nonsignificant trends toward worse NRM and RFS. No statistically significant differences in outcomes were seen when other exploratory cutoff values of $CD34^+$ cells were considered (7×10^6/kg, 8×10^6/kg, and 10×10^6/kg); however, the impact of low $CD34^+$ cell doses ($<5 \times 10^6$/kg) was not specifically analyzed. In our study, the maximum $CD34^+$ cell dose was capped at 8×10^6/kg in accordance with our institutional protocol based on previously published data that associated high $CD34^+$ cell dose with worse post-transplantation outcomes in alloHSCT using conventional GHVD prophylaxis [9,10,13,28], and thus we could not analyze the impact of doses $>8 \times 10^6$/kg on outcomes of transplantation.

Regarding other type of donors, Elmariah et al. [17] retrospectively analyzed the effect of cell dose on outcomes of 144 adult recipients of PBSC alloHSCT with PTCy-based GVHD prophylaxis for a hematologic malignancy. Infused $CD34^+$ cell doses were stratified into low-dose ($\leq 5 \times 10^6$/kg), intermediate-dose (5 to 10×10^6/kg), and high-dose ($>10 \times 10^6$/kg) groups. Similar to our study, $CD34^+$ cell dose had no significant impact on aGVHD, cGVHD, or relapse. However, in contrast to our findings, their multivariate analysis showed worse NRM,

Table 4
Univariate and Multivariate Models: Risk Factors for OS, NRM, RFS, and CIR

Variables	OS, Cox Regression Model		NRM, Fine-Gray Proportional Hazards Regression Model		RFS, Cox Regression Model		CIR, Fine-Gray Proportional Hazards Regression Model	
	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Univariate regression analysis								
$CD34^+$ cell dose								
Continuous	.91 (.79-1.05)	.21	.89 (.71-1.16)	.32	.90 (.79-1.23)	.12	.89 (.72-1.12)	.32
$\leq 5 \times 10^6$ (vs 5-8 $\times 10^6$)	1.56 (.99-2.44)	**.05**	1.53 (.81-2.88)	.18	1.43 (.95-2.13)	**.08**	1.53 (.81-2.88)	.18
CD3 cell dose								
Continuous	1.01 (.99-1.01)	.46	1 (.99-1.01)	.88	1.0 (.99-1.01)	.94	1.00 (.99-1.00)	.88
Age								
Continuous	1.02 (1.01-1.03)	**.01**	1.03 (1.01-1.05)	**.02**	1.02 (1.01-1.03)	**.008**	1.03 (1.00-1.06)	**.03**
Age >60 yr	2.47 (1.58-3.86)	<**.001**		**.001**		<**.001**	2.82 (1.49-5.33)	**.001**
HCT-CI score			2.82 (1.49-5.36)		2.13 (1.43-3.16)			
>3 (vs 0-3)	1.76 (1.08-2.87)			.57			1.23 (.61-2.48)	.57
KPS		**.02**	1.22 (.60-2.48)		1.49 (.95-2.34)	.080		
80-60 (vs 100-90)	2.07 (1.29-3.33)			<**.001**			2.97 (1.58-5.60)	<**.001**
DRI		**.002**				**.002**		
High-very high (vs low-intermediate)	2.19 (1.35-3.54)	**.001**	2.97 (1.57-5.60)	**.05**	1.94 (1.26-2.97)	**.004**	1.89 (.99-3.62)	**.05**
Conditioning intensity								
RIC (vs MAC)	1.22 (.78-1.90)						1.06 (.57-1.98)	
Grouped donors		.37	1.89 (.99-3.62)	.86	1.88 (1.22-2.91)	.20		.86
Haplo (vs others)	.95 (.54-1.67)						1.25 (.59-2.66)	
Donor age		.87		.55		.79		.55
Continuous	1.00 (.98-1.02)						.99 (.96-1.02)	
≥50 yr	.81 (.41-1.58)	.60	1.05 (.56-1.97)	.41	1.29 (.87-1.91)	.06	.66 (.23-1.87)	.41
Female donor to male recipient		.54		.43		.54		.43
Yes (vs no)	1.21 (.69-2.13)		1.25 (.59-2.66)		.93 (.56-1.54)		.72 (.28-1.85)	
Type of stem cell product		.49		.30		.77		.49
Cryopreserved (vs fresh)	.70 (.36-1.35)						.63 (.26-1.51)	
Nadir neutropenia >19 d		.29	.98 (.96-1.01) .65 (.23-1.86)	.49	1.01 (.99-1.03) 1.18 (.69-2.02)	.41		.30
Yes (vs no)	1.58 (1.01-2.48)	.60	.67 (.26-1.51) .71 (.28-1.84) 1.38 (.73-2.59)	.31	1.08 (.64-1.82) .79 (.47-1.35) 1.76 (1.18-2.63)	**.005**	1.38 (.74-2.59)	.31
Multivariate regression analysis								
CD34 cell dose					1.01		1.01	
$\leq 5 \times 10^6$ (vs 5-8 $\times 10^6$)	1.15 (.67-1.98)	.59	1.34 (.64-2.82	.43	1.04 (.65-1.68)	.85	1.27 (.60-2.68)	.52
Donor type					1.01		1.01	
Haploidentical (vs others)	.62 (.24-1.62)	.33		.56	1.01	.31	1.37 (.43-4.35)	.59
Donor type and cell dose count			1.40 (.44-4.43)		.67 (.31-1.44) 1.06		1.01	
Haplo receiving $\leq 5 \times 10^6$ (vs others)	6.01 (1.76-2.49)	**.004**	1.91 (.43-8.36)	.39	4.57 (1.59-3.14)	**.004**	1.90 (.42-8.40) 1.01	.40
Age					1.01		1.01	

(*continued*)

progression-free survival, and OS in the low-dose group compared with the intermediate-dose group for the entire series of patients independent of donor type. Although the proportion of nonhaploidentical alloHSCT recipients with a low $CD34^+$ cell dose was clearly higher in our study than in the Elmariah et al. study (58% versus 8%), we found no negative effect on survival outcomes in that group of patients. Other differences between the studies, such as the type and intensity of conditioning regimens or GVHD prophylaxis schedule, might have contributed to these discrepant findings.

The main limitation of our study is its retrospective nature and the limited number of haploidentical alloHSCT recipients in the low-dose group. Nonetheless, we emphasize that although the statistical impact of this small sample size would be a higher likelihood of type II error (false-negative), we were still able to detect significant differences in outcomes between the low-dose and high-dose group. Moreover, our results are in line with those published by Maffini et al. [26] in a large series of haploidentical transplantations from the EBMT database. Finally, the other available study on this subject showed similar results even with a haploidentical population with a lower $CD34^+$ cell dose than ours (n = 5) [17]. Our statistical analysis supports the findings of these 2 previous studies suggesting that the $CD34^+$ dose should not be $<5 \times 10^6$/kg when using haploidentical donors. We also have shown that in MSD, MUD, and MMUD transplantations, the infused $CD34^+$ cell dose had no impact on survival after transplantation. However, considering the benefit of the infusion of a high $CD34^+$ cell dose on engraftment, we also suggest that administration of no less than 5×10^6/kg $CD34^+$ cells could be beneficial in all PBSC PTCy alloHSCT recipients regardless of donor type.

ACKNOWLEDGMENTS

The authors acknowledge the kind support of all physicians and data managers who participated in this study.

Financial disclosure: There are no financial disclosure.

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

Authorship statement: C.M., A.P., and M.Q.S. conceived and designed the study; A.P. collected and assembled data; C.M., A.P., and M.Q.S. analyzed and interpreted the data; and A.P. and C.M. wrote the manuscript. All coauthors are physicians at our center, who performed the transplantations, took care of the patients, made significant contributions to the discussion of the results, and participated substantively in the writing of the manuscript.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.jtct.2022.12.005.

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Table 4 (*Continued*)

Variables	OS, Cox Regression Model		NRM, Fine-Gray Proportional Hazards Regression Model		RFS, Cox Regression Model		CIR, Fine-Gray Proportional Hazards Regression Model	
	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
≥60 yr	1 (1.01 - 1.03)	**.03**	1.02 (1.0-1.05)	**.01**	1.01(1.01-.03)	**.041**	1.02(1.01-.01)	**.02**
KPS					1.01			
80-60 (vs 100-90)	1.97 (1.21-3.21)	**.006**		**.001**		**.007**	2.87 (1.51-.47)	**.001**
DRI			2.92 (1.53-5.55)		1.81 (1.17-2.82)			
High-very high (vs low-intermediate)	1.95 (1.20-3.18	**.007**		-		**.014**	1.59 (.80-3.15)	.18
HCT-CI score								
>3 (vs 0-3)	1.72 (1.04-2.85)	**.03**	-	-	1.73 (1.11-2.70)	.100	-	-
Nadir neutropenia >19 d	-	-	-	-	1.46 (.92-2.32)	**.032**	-	-
Yes (vs no)			.		1.58 (1.04-2.40)			

Significant *P* values are in bold type.

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