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Impact of Single-Dose Plerixafor as an Adjunct to Granulocyte Colony-Stimulating Factor–Based Peripheral Blood Stem Cell Mobilization on the Graft Composition and Outcome for T Cell–Replete Haploidentical Peripheral Blood Stem Cell Transplantation with Post-Transplantation Cyclophosphamide: A Comparative Study

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A B S T R A C T

We conducted a prospective study on T and natural killer (NK) cell subset composition of graft and transplant outcomes in T cell–replete haploidentical transplantation with a single dose of subcutaneous plerixafor (Px) added to granulocyte colony-stimulating factor (G-CSF)-based mobilization in allogeneic donors to collect 10×10^6/kg CD34$^+$ hematopoietic stem cells (HSCs) at single apheresis. Twnety-six donors received G-CSF + Px and 25 G-CSF alone for mobilization. Despite significantly lower peripheral blood (PB) CD34$^+$ HSCs on day 4 in the G-CSF + Px group (33 [range, 6-47] cells/μL versus 81 [range, 50-168] cells/μL in the G-CSF group; $P = .0001$), PB CD34$^+$ HSC count (median 136 versus 139 cells/μL) on day 5 as well as that in the graft (2.7 versus 2.3×10^6/mL, $P = .1$) were comparable between the 2 groups. The total nucleated cell count was higher (3.4 versus 3.1×10^8/mL, $P = .05$), but CD4$^+$ T cells (2.3 versus 2.7×10^7/mL, $P = .09$) were lower in the G-CSF group with mobilization of regulatory T cells being similar. NK cells were skewed toward the CD56$^+$/16$^-$ subset in both groups, varying significantly from the steady-state NK subset ratio in PB. The time to engraftment, incidences of acute and chronic graft-versus-host disease, nonrelapse mortality, and overall survival were also similar. Addition of single-dose Px to G-CSF mobilization improves CD34 recovery and does not significantly alter the T and NK cell composition of the graft, including regulatory T cells, with no adverse impact on transplant outcomes.

INTRODUCTION

Haploidentical hematopoietic stem cell transplantation (HSCT) is a viable option for patients without a matched family donor, and post-transplantation cyclophosphamide (PTCy) has indeed broadened the scope of haploidentical HSCT for the entire spectrum of hematologic disorders [1]. The number of CD34$^+$ cells collected and transplanted has been 1 of the important determinants of transplant outcomes for almost 2 decades, especially when researchers have tried to use a mega-dose of CD34-selected cells in the background of haploidentical transplantation [2]. This is most often not achievable with a single collection for an adult recipient. On the other hand, in PTCy-based haploidentical HSCT, the graft has to be administered as a single dose because the administration of PTCy must be timed at 64 to 72 hours from the infusion of the graft [1,3]. Thus, mobilization of adequate graft in terms of CD34$^+$ hematopoietic stem cells (HSCs) by a single apheresis is critical to both these approaches. In addition, certain healthy donors are poor mobilizers (PMs), compounding this problem [4].

Plerixafor (Px) is a bicyclam compound originally synthesized as a drug against HIV. The rapid increase in various blood components in initial studies has led to its use for mobilization of CD34$^+$ HSCs from marrow to peripheral blood (PB) [5,6]. Px is a reversible and selective antagonist of CXCR4 and stromal cell–derived factor 1 alpha interactions, resulting in egress of marrow HSCs to circulation. Px can safely be

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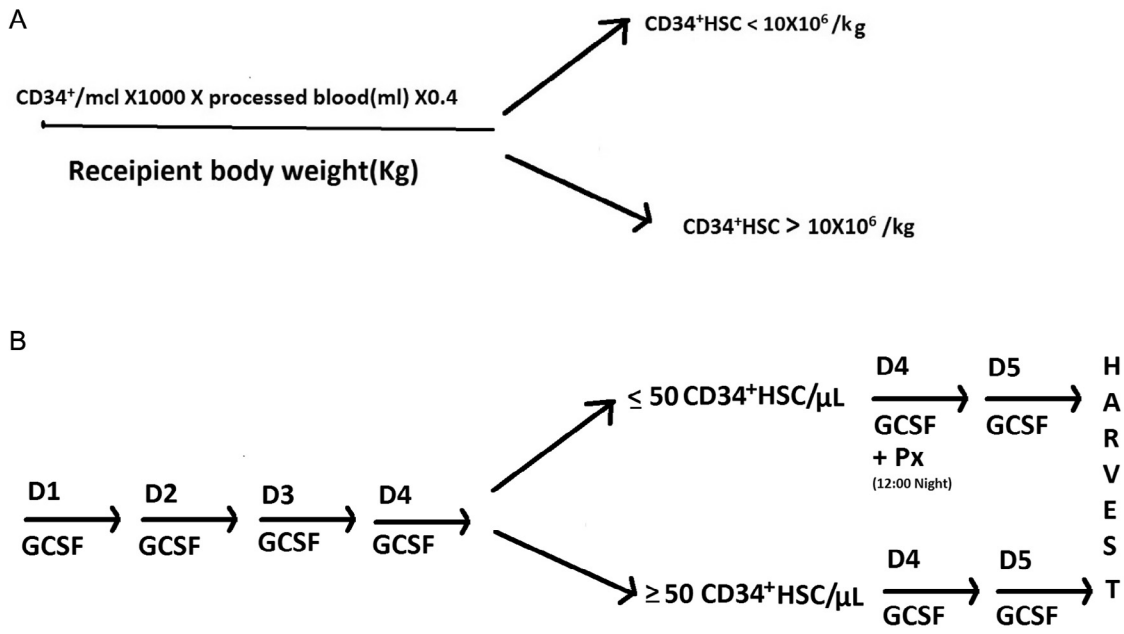

Figure 1. (A) Algorithm for prediction of CD34+ HSC yield based on PB counts on day 4 of mobilization. (B) Graphical presentation of CD34+ HSC mobilization protocol: G-CSF was given subcutaneously at a dose of 12 µg/kg in 2 divided doses. On the fourth day donors received Px at 12:00 midnight at a dose of 250 µg/kg if their CD34+ HSCs were less than 50 cells/µL.

used in combination with granulocyte colony-stimulating factor (G-CSF) to increase the mobilization of autologous CD34+ HSCs in patients with myeloma and lymphoma [7]. However, data on safety and efficacy in normal healthy donors are scant [8-10].

Although some studies have also characterized the subsets of lymphocyte subsets after mobilization with Px and G-CSF in the setting of autologous grafts [11], the same for donor grafts remain uncertain. Studies on autologous collections have shown variable impact on T cells. Few data are currently available about the study of natural killer (NK) cell subsets and regulatory T cells (Tregs) in donor grafts after the combination of Px and G-CSF, and fewer still on its impact on transplant outcomes [10,12]. The major focus of our study was to compare the effect of single-dose Px with standard G-CSF mobilization versus G-CSF–based mobilization alone on the graft composition and transplantation outcomes in 51 patients undergoing haploidentical HSCT.

METHODS

In a prospective, nonrandomized, unblended, observational study, between January 2015 and January 2017 patients with hematologic disorders, both malignant and nonmalignant, between ages 2 and 65 years without a matched family donor were enrolled if they possessed a haploidentical family donor. Approval was obtained from Institute Review Committee in accordance with the Declaration of Helsinki (protocol MCF H0401GP), and written informed consents were obtained from all patients and donors. Patients with donors under age 10 years were excluded from the study. For donors under age 18 years, verbal consent from the donor and written consent from the parents were obtained.

Conditioning Regimens and Graft-versus-Host Disease Prophylaxis

All patient's received PTCy-based graft-versus-host disease (GVHD) prophylaxis with cyclosporine and/or sirolimus and mycophenolate mofetil. Conditioning regimens and GVHD prophylaxis for malignant and nonmalignant diseases have been described in detail in our previous publications [13-16].

Mobilization Protocol

The prediction of CD34+ HSC yield in the PB stem cell (PBSC) product in relation to day 4 of mobilization was derived from the calculation shown in Figure 1 [10]. Based on this calculation, it was predicted that for a recipient weight ≥ 20 kg the PB CD34+ HSC has to be above 50 cells/µL to achieve a target dose of CD34+ HSCs of 10×10^6/kg of recipient weight. The mobilization protocol (Figure 1) included subcutaneous G-CSF at 12 µg/kg in 2 divided doses at the gap of 12 hours. On the fourth day of mobilization we routinely performed CD34+ HSC count on PB using flow cytometry. Px (Mozifor; Hetero Healthcare, Solan, India) was administered only to those donors who failed to achieve more than 50 cells/µL CD34+ HSCs on day 4 of mobilization; those donors were considered as PMs. PMs were administered Px at 240 µg/kg as a single dose at midnight (12.00 AM), and leukapheresis was performed 11 hours later on the following morning (G-CSF + Px group). The good mobilizers achieved more than 50 cells/µL CD34+ HSCs on day 4 of mobilization and did not receive Px followed the G-CSF alone protocol (G-CSF group).

On average, a minimum of 3 times the blood volume was processed with an average yield of 200 to 300 mL of final PBSC product. The target dose of CD34+ HSCs was 10×10^6/kg with the minimum cell dose required being 5×10^6 of CD34+ HSC/kg in the graft infused on day 0. The rest were stored as aliquots of donor lymphocyte infusions for malignant diseases as previously described [16]. Those with nonmalignant diseases had the remaining cells cryopreserved as a backup graft in case of graft failure.

Supportive Care

All patients were treated in protective isolation rooms provided with high efficiency particle air filters. Antimicrobial prophylaxis was instituted as per the departmental guidelines. Cytomegalovirus prophylaxis was guided by preemptive monitoring of viral cytomegalovirus load by quantitative PCR twice a week until day 100. Viral loads of cytomegalovirus, Epstein-Barr virus, and adenovirus were monitored twice weekly.

Acute GVHD was graded according to modified Glucksberg criteria [17], and chronic GVHD was scored based on the National Institutes of Health global severity criteria [18]. The details of HLA typing and NK KIR haplotype assignment have been previously described [16].

Assessment of CD34+ Cells on Days 4 and 5

CD34+ HSCs were assessed on days 4 and 5 of mobilization by 6 color flow cytometry in Navios (Beckman Coulter Inc, Marseille, Cedex.) using the following mouse anti-human mAbs from Beckman Coulter as per the ISHAGE protocol [19].

Assessment of T and NK Cells on Leukapheresis Product

The assessment of T and NK cells on the leukapheresis product has been described previously [20,21]. In brief, assessment was carried out on the PB on donors before starting G-CSF and on the leukapheresis products. The cell surface staining procedure was performed in 5 mL propylene tubes

containing 1.5×10^6 cells in 100 μL of the PB. The NK cell and T cell immunophenotypes were carried out by 6 color flow cytometry in Navios using the following mouse anti-human mAbs from Beckman Coulter and Immunotech (Marseille, France): CD45 (J33), CD3 (UCHT1), CD4 (13B8.2), CD8 (B9.11), CD56 (N901), and CD16 (3G8). The samples were incubated with antibodies for 20 minutes at room temperature in the dark followed by incubation with RBC lyses buffer Optimise C (Beckman Coulter) for 10 minutes and 2 washes and resuspension of the pellet in 500 μL of PBS buffer. Data were analyzed with Kaluzaver 1.3 (Beckman Coulter) in analysis software.

Tregs were analyzed from PB using mouse anti-human mAbs from BD Biosciences (San Jose, CA): CD4 (SK3), CD25 (2A3), and CD127 (HIL-7R-M21). Tregs were defined as the population of lymphocytes expressing $CD4^+CD25^+CD127^{dim/-}$ phenotype with expression of FoxP3. Intracellular staining for FoxP3 was carried out with mouse anti-human FoxP3 (259D/C7; BD Biosciences).

Statistics

This was a prospective, nonrandomized, unblended, observational study. The primary endpoint of the study was achievement of the target dose of $CD34^+$ HSCs of 10×10^6/kg with a single apheresis. The secondary endpoints were engraftment, GVHD, nonrelapse mortality (NRM), and overall survival at 12 months. These endpoints were compared between the G-CSF and G-CSF + Px groups. The T and NK cell subsets were compared between the 2 groups as well.

Binary variables were compared between the 2 groups using the chi-square test. Continuous variables were presented as median with range and were analyzed using independent sample *t*-test, taking into account Levenes test for equality of variances. Probabilities of survival were estimated using the Kaplan-Meier product-limit method. The cumulative incidence rates of NRM, graft failure, acute GVHD, chronic GVHD, and relapse were computed to take account of the presence of competing risks. An outcome was determined to be significantly different if the observed $P < .05$. All analyses were performed using statistical software IBM SPSS statistics version 20 (Armonk, NY).

RESULTS

Patient and Donor Characteristics

Fifty-one patients were enrolled in this study. Thirty-eight were transplanted for malignant conditions (acute myelogenous leukemia, 19; acute lymphoblastic leukemia, 10; lymphoma, 9) and 13 for nonmalignant conditions (aplastic anemia, 10; hemoglobinopathies, 3). All patients were high/very high risk per the Disease Risk Index [22]. None of the patients with lymphoma was in complete remission at transplant, and 10 of 29 patients with acute leukemia were not in morphologic complete remission. The other 19 patients with acute leukemia were in morphologic complete remission but were Minimal Residual Disease positive at transplant. All patients with aplastic anemia were high risk as defined previously [13].

Overall, 26 donors (PM) did not achieve the desired $CD34^+$ HSC yield of more than 50 cells/μL. Hence, they were enrolled in the G-CSF + Px group. The remaining 25 patients (good mobilizers) were administered G-CSF alone as a mobilizing agent.

The characteristics of both the groups are detailed in Table 1. The median age was 24.5 years (range, 2 to 61) in the G-CSF group versus 18 years (range, 2 to 63) in the G-CSF + Px group ($P = .05$). The median donor age was 34 years (range, 12 to 57) in the G-CSF group versus 43 years (range, 17 to 60) in the G-CSF + Px group ($P = .08$).

Adverse Effects of Mobilization Protocols

All donors receiving G-CSF experienced mild to moderate body aches on days 4 and 5 of mobilization. In addition, 20 of 26 donors receiving Px experienced gastrointestinal symptoms in the form of mild to moderate nausea and self-limiting diarrhea. This was not seen the G-CSF group.

Mobilization of $CD34^+$ HSCs When Px Was Administered in Addition to G-CSF

The PB $CD34^+$ HSCs on day 4 in the 26 patients of the G-CSF + Px group was a median of 33 cells/μL (range, 6 to 47) versus 81 cells/μL (range, 50 to 168) in the G-CSF group ($P = .0001$). The PB $CD34^+$ HSCs on day 5 in the G-CSF + Px group was a median of 136 cells/μL (range, 52 to 240). This was similar to that of the G-CSF group at 139 cells/μL (range, 72 to 272; $P = .7$) (Figure 2).

None of the donors required more than a single apheresis to achieve the desired number of $CD34^+$ HSCs in the infused and the stored grafts. $CD34^+$ HSCs were similar in the apheresis product as well (2.7 [range, 1.1 to 6.0] versus 2.3 [range, .5 to 5.3] $\times 10^6$/mL [$P = .09$] in G-CSF + Px and G-CSF groups, respectively). Both groups received similar numbers of $CD34^+$ HSCs in the graft (median 7.6 versus 7.9×10^6/kg in G-CSF + Px and G-CSF groups, respectively; $P = 1.0$).

Assessment of T Cell Subsets in the Graft

$CD3^+$, $CD4^+$, and $CD8^+$ T cells in the graft

The total nucleated cell count was higher in the G-CSF group (3.4 [range, 1.7 to 5.0] versus 3.1 [range, 1.15 to 4.73] $\times 10^8$/mL, $P = .05$) (Figure 3A). On assessment of $CD3^+$ cells in apheresis product, this was similar in both groups. The median absolute $CD3^+$ cell count was 4.3 versus 5.3×10^7 cells/μL with a median $CD8^+$ cell count of 1.5 versus 2.0 cells/μL in the G-CSF group and G-CSF + Px group, respectively ($P = .3$). $CD4^+$ T cells tended to be higher in the G-CSF + Px group (2.7 [range, 1.8 to 3.7] versus 2.3 [range, .3 to 4.2] $\times 10^7$/mL in the G-CSF group, $P = .09$).

The steady-state CD4/CD8 ratio in the PB was 1.8 to 2.6 (median, 1.95) in the donors, and this was not significantly different in the mobilized grafts with a median ratio of 1.61 ($P = .8$). This was similar in both groups as well with a median ratio of CD4/CD8 of 1.57 in the G-CSF + Px group and 1.67 in the G-CSF group ($P = .4$; Table 2).

Table 1
Patient and Donor Characteristics

	G-CSF Group (n = 25)	GCSF + Px Group (n = 26)	*P*
Recipient age, yr	24.5 (2-61)	18 (2-63)	.05
Diagnosis, malignant/nonmalignant	21/5	17/8	.3
Recipient gender, F/M	11/15	8/17	.5
Donor age, yr	34 (12-57)	43 (17-60)	.08
Donor gender, F/M	10/16	9/16	1.0
Donor weight	67.5 (40-93)	66 (40-90)	.7
Median CD34 count on day 4, cells/μL	81 (50-168)	33 (6-47)	.0001
Median CD34 count on day 5, cells/μL	139 (72-272)	136 (52-351)	.7

Values in parentheses are ranges.

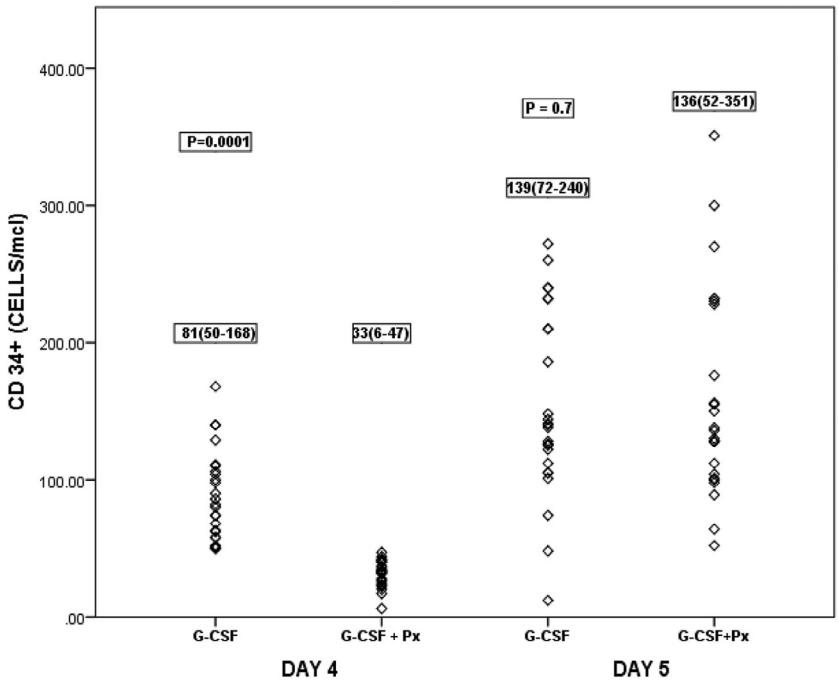

Figure 2. CD34⁺ HSCs in the PB on days 4 and 5 of mobilization in the G-CSF and G-CSF + Px groups.

Table 2
Comparison of Graft Composition

	G-CSF Group (n = 25)	GCSF + Px Group (n = 26)	*P*
PBSC harvest CD34⁺ count, $\times 10^6$ cells/mL	2.3 (.5-5.3)	2.7 (1.1-6.0)	.1
TNC count, $\times 10^8$ cells/mL	3.4 (1.7-5.0)	3.1 (1.15-4.73)	.05
CD3⁺, $\times 10^7$ cells/mL	4.3 (.31-9.7)	5.3 (.93-10.5)	.2
CD4⁺, $\times 10^7$ cells/mL	2.3 (.3-6.8)	2.7 (.53-6.8)	.09
CD8⁺, $\times 10^7$ cells/mL	1.5 (.05-3.9)	2.0 (.04-4.5)	.3
CD4⁺CD8⁺ ratio	1.67 (.5-5.54)	1.57 (.59-3.17)	.4
Tregs absolute, $\times 10^4$ cells/mL	150.3 (18.0-296.7)	181.7 (18.0-405)	.3
Tregs, %	7.1 (3.7-9.7)	6.9 (3.9-10.9)	.1
CD56⁺CD3⁻, $\times 10^6$ cells/mL	5.7 (1.7-12.9)	5.3 (.8-11.6)	.9
CD56⁺CD16⁺, $\times 10^6$ cells/mL	3.2 (.9-9.0)	2.9 (.2-8.1)	.32
CD56⁺CD16⁻, $\times 10^6$ cells/mL	2.0 (.8-6.6)	2.0 (.3-5.7)	.3
CD56⁺CD16⁺/CD16⁻ ratio	1.8 (.25-5.25)	1.22 (.32-4.18)	.4

Values are medians with the ranges in parentheses. TNC indicates total nucleated cell.

Assessment of Tregs

Tregs represented by CD4⁺CD25⁺CD127^{dim} FoxP3 positive cells accounted for 7.1% of CD4⁺ cells in the G-CSF group compared with 6.9% in G-CSF + Px group ($P = .1$) (Figure 3B). The absolute number of Tregs (150.3 [range, 18.2 to 296.7] in the G-CSF group versus 181.7 [range, 18 to 405] $\times 10^4$ cells/mL in the G-CSF + Px group) was similar ($P = .3$).

Assessment of NK cell subsets in the graft

NK cells were mobilized in a similar pattern in both groups (Figure 3C). The median values of CD56⁺CD3⁻ cells in the graft were 5.3×10^6/mL in the G-CSF + Px group compared with 5.7×10^6/mL in the G-CSF group. Similar numbers of CD56⁺16⁺ and CD56⁺16⁻ NK cells were mobilized in both groups. However, the fraction of CD56⁺16⁻ NK cells mobilized in the graft was 3-fold higher than that detected in the PB of the donors.

The CD56⁺16⁺/CD56⁺16⁻ NK cell ratio in PB at steady state in the donors varied between 4.5 and 10.1 (median, 6.9). This was significantly lower in the mobilized grafts at a median ratio of 1.39 ($P = .01$), indicating a greater mobilization of CD56⁺16⁻ NK cells with G-CSF with or without Px. The ratios of CD56⁺16⁺/CD56⁺16⁻ NK cells were 1.8 in the G-CSF group and 1.2 in the G-CSF + Px group ($P = .4$). There was also no difference in the T and NK cell subsets infused between the 2 groups, measured as cells/kg body weight, between the 2 groups (data not shown).

Comparison of Transplant Outcomes

Table 3 shows a comparison of transplant outcomes.

Engraftment and Chimerism

All 51 patients engrafted both neutrophils and platelets at a median of 15 days (range of 9 to 19 days for neutrophils and 9 to 23 days for platelets), which was similar in both groups. All had >95% donor chimerism at day +30 post-transplant.

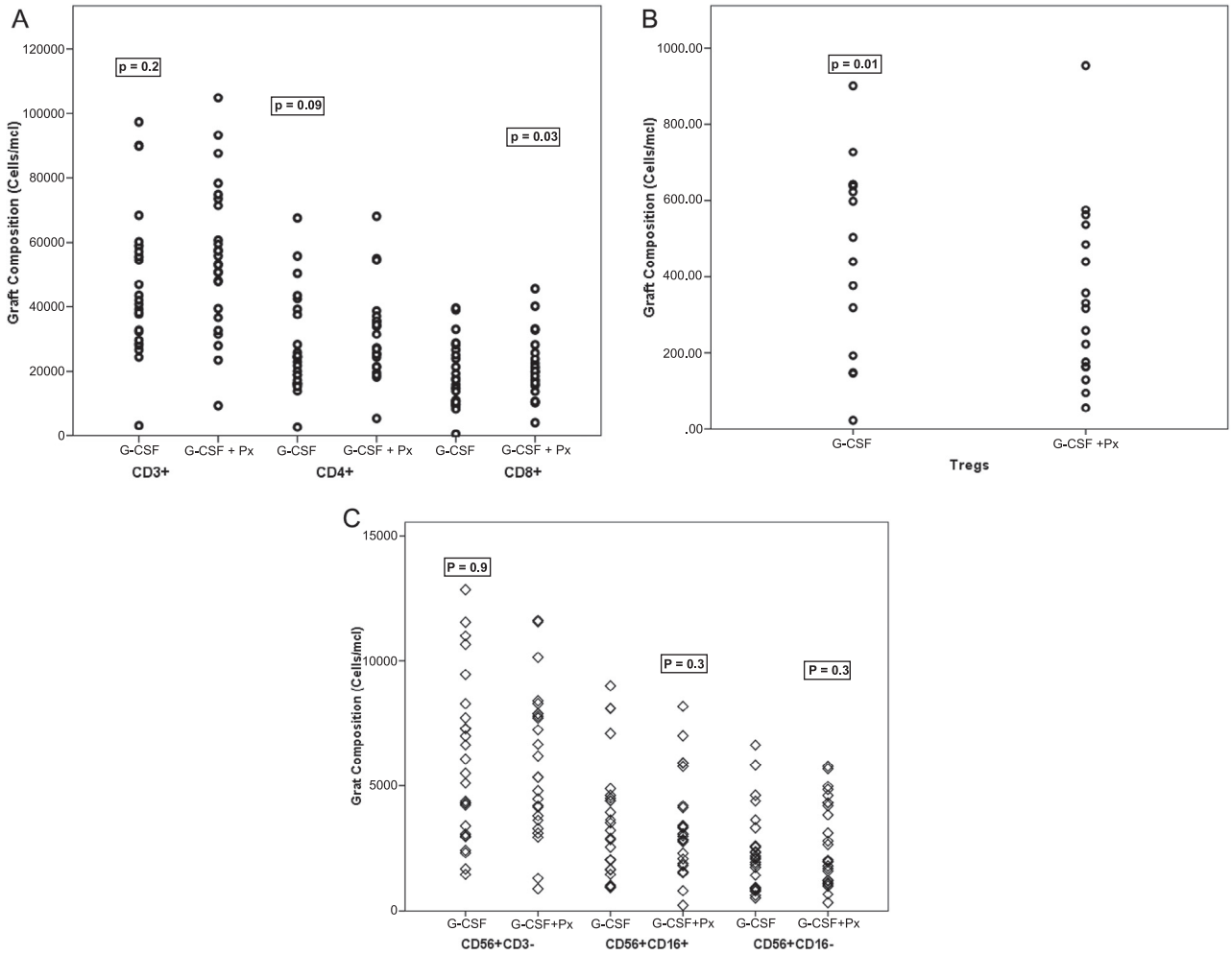

Figure 3. (A) Graft composition of T cell subsets. The *y* axis represents number of cells per microliter ($\times 10^7$ cells/μL), whereas the *x* axis represents $CD3^+$, $CD3^+CD4^+$, and $CD3^+CD8^+$ (individual population in G-CSF and G-CSF + Px groups) enumerated in the grafts. (B) Graft composition of Tregs in G-CSF and G-CSF + Px groups ($\times 10^4$ cells/μL) enumerated in the grafts. (C) Graft composition of NK cell subsets. The *y* axis represents number of cells per microliter ($\times 10^6$ cells/μL), whereas the *x* axis represent $CD56^+CD3^-$,$CD56^+CD16^+$, and $CD56^+16^-$ subsets (individual population in G-CSF and G-CSF + Px groups) enumerated in the grafts.

Table 3
Comparison of Transplant Outcomes

	G-CSF Group (n = 25)	GCSF + Px Group (n = 26)	*P*
Neutrophil engraftment, days	14 (9-16)	15 (11-19)	.5
Platelet engraftment, days	13 (9-23)	15 (11-19)	.9
Acute GVHD	5	4	1.0
Chronic GVHD	6/19	4/21	.7
NRM	4	2	.2
Relapse	3	4	.2

Values are medians with the ranges in the parentheses.

Graft-versus-host disease

Four patients in G-CSF + Px group developed grades II to IV acute GVHD compared with 5 patients in the G-CSF group ($P = 1.0$). Four patients developed de novo chronic GVHD in the G-CSF + Px group as compared with 6 in G-CSF group ($P = .7$).

NRM, relapse, and overall survival

The NRM was only 11.9% ± 4.6% in the entire cohort, with 6 patients dying at a median of 61 days (range, 15 to 148). All but 1 patient died of infection-related causes. NRM was 8.4% ± 5.7% in the G-CSF + Px group compared with 15.4% ± 7.1% in the G-CSF group (log rank $P = .4$, Figure 4A).

Seven patients relapsed at a median of 167 days (range, 76 to 196), with a cumulative incidence of 15.6% ± 5.4%. This was 17.5% ± 8.0% in the G-CSF + Px group and 13.5 ± 7.3% in the G-CSF group (log rank $P = .7$, Figure 4B). The overall survival was 77.9% ± 5.9%: 83.1% ± 7.7% in the G-CSF + Px group and 73.1% ± 8.7% in the G-CSF group ($P = .9$, Figure 4C).

DISCUSSION

PTCy-based haploidentical HSCT is based on the premise that high-dose cyclophosphamide would eliminate alloreactive T cells thta proliferate between 24 and 72 hours to their optimal capacity, provided it is administered between 64 and 72 hours and neither earlier nor later. PBSC grafts contain 2 logs more T cells, and the timing of PTCy is critical in moderating the incidence of GVHD without compromising the relapse risk [3]. Thus, it is important that the graft is mobilized in a single apheresis to maintain the critical component of such transplants within the planned schedule.

We had observed that those donors mobilizing more than 50 cells/μL of $CD34^+$ HSCs on day 4 would successfully mobilize 10 million/kg $CD34^+$ HSCs in a single apheresis for

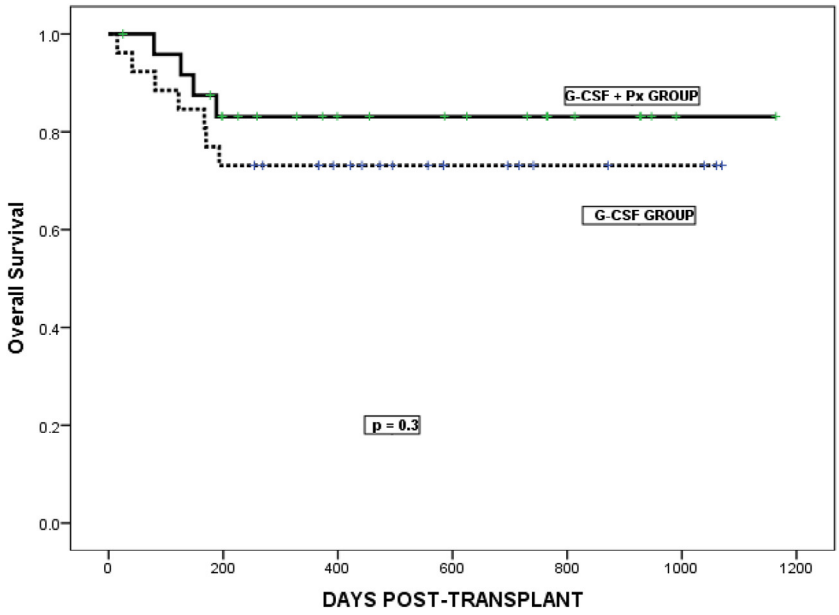

Figure 4. Transplant outcomes: overall survival. The solid line (—) represents the G-CSF + Px group and the broken line (- - -) represents the G-CSF group.

patients weighing > 20 kg, similar to that suggested by Rutella et al [10]. Mobilization of donors with G-CSF is the standard procedure for PBSC grafts in allogeneic HSCT. However, certain healthy donors fail to mobilize adequately with G-CSF alone [4,9,10]. Previous studies have demonstrated that addition of single-dose Px as an adjunct to G-CSF–based mobilization augments $CD34^+$ HSC yield in donors having less than required $CD34^+$ cells mobilized on day 4 of the mobilizing protocol. Most such studies have been carried on patients who had received chemotherapy and are planned for autologous PBSC transplantation [10,23,24]. There are only few data available on healthy donors and the impact of this intervention on the other components of graft composition.

Of 51 donors, 50.9% were PMs by our criteria. It is interesting that the PMs were mainly older donors for younger patients who in this context were parents. They all received the planned dose of Px 11 hours before apheresis with self-limiting gut-related side effects in most of them. There was a 4- to 8-fold increase in $CD34^+$ HSCs in the PB 11 hours after a single dose of Px. This is in keeping with 3- to 10-fold rises of $CD34^+$ HSCs described by others in the context of healthy donors [8-10]. As a result, the desired $CD34^+$ HSC numbers were achieved in both groups in a single apheresis.

Apart from achievement of the primary endpoint, we aimed to study the impact of single-dose Px on the graft composition. T and NK cell subsets of the graft and their impact on the outcome of PTCy-based haploidentical PBSCT have not been studied. In addition, the literature is divided on the impact of Px on the T cell subsets mobilized, even though most studies are in animal models. Devine et al. [12] studied the impact of single-dose Px versus standard G-CSF mobilization in a cohort of 20 patients and found a greater mobilization of both $CD3^+$ T cells and $CD4^+$ T cell subset. Although we did not find any impact on $CD3^+$ T cells or the $CD8^+$ T cell component, $CD4^+$ T cells were mobilized in greater numbers in the G-CSF + Px group, albeit bordering on statistical significance. It was important to observe in our cohort that CD4/CD8 ratios were maintained in both groups as found in steady-state PB of the donors. Keane et al. [25] in a rhesus macaque model demonstrated a several-fold higher T cell subset mobilization with Px, particularly that of Tregs. The literature is scant regarding the impact of Px on Tregs in human subjects. Devine et al. [12] did not find any impact of Px on PB Treg composition. Despite a higher $CD4^+$ T cell mobilization in the G-CSF + Px group, we did not find any impact of Treg component of $CD4^+$ T cell population. Patients transplanted with grafts mobilized with G-CSF + Px had a similar pattern of engraftment and no increase in either acute or chronic GVHD. This is similar to the limited data reported in clinical studies, although studies in mice showed a higher incidence of GVHD in recipients of Px-treated grafts [26].

There is even scantier data on the impact of the same on NK cell subsets. The only study exploring the impact of single-dose Px in a similar protocol reported lesser numbers of NK cells mobilized by the addition of Px [10]. We did not find any significant difference in absolute NK cell numbers or their subsets. However, we did observe skewing of the ratio of $CD56^+16^+/CD^-$ subsets in G-CSF–mobilized graft compared with that observed in steady-state PB of the donors. There was no significant alteration in the subsets or the ratio after the addition of Px. In the steady state, $CD56^+16^+$ cells are the dominant NK cell population, averaging 4- to 10-fold higher than that of $CD56^+16^-$ cells, reflecting a greater number of mature and cytotoxic NK cells in the PB. G-CSF mobilizes more immature NK cells, skewing this ratio and suggesting that these subsets of NK cells are marrow/tissue residents. The adverse impact of G-CSF on NK cell cytotoxicity and proliferation has been reported by Su et al [27]. A similar observation was described by Rutella et al. [10] in terms of mature and immature NK cells, although they found this phenomenon to be further exaggerated in Px-mobilized grafts. The finding of a higher total nucleated cell in the G-CSF–mobilized grafts is commensurate with greater mobilization of neutrophils and monocytes in this cohort.

One limitation of our study is exclusion of dendritic cell assessment. Cashen et al. [28] showed an increased mobilization of plasmacytoid dendritic cells with Px, but why and

how this might affect the transplant outcome are not clear. Others have shown a greater mobilization of regulatory myeloid dendritic cells with Px with less of proinflammatory subtypes [10,29].

Although Px alone might mobilize a graft with differing composition than that mobilized with G-CSF alone, addition of a single dose of Px to PMs on G-CSF–based mobilization did not seem to alter the graft composition significantly with respect to T and NK cell subsets. However, our study also demonstrates a skewed mobilization of NK cells in grafts mobilized by G-CSF that is augmented in those receiving a single dose of Px. Importantly, a single dose of Px was sufficient to mobilize greater numbers of $CD34^+$ HSCs, sufficing a single apheresis in all cases. Animal studies had raised concerns regarding the impact of Px on GVHD or graft-versus-leukemia effect.

Our findings affirm the safety of G-CSF + Px–mobilized grafts without any adverse impact on short- and intermediate-term transplant outcomes. However, despite our and other studies demonstrating the short-term safety of Px in normal donors, its long-term safety is yet to be confirmed. We hope our findings encourage further clinical trials in this area to shed more light on various approaches of PBSC mobilization in healthy donors using Px with or without G-CSF with respect to graft composition and, more importantly, transplant outcomes.

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