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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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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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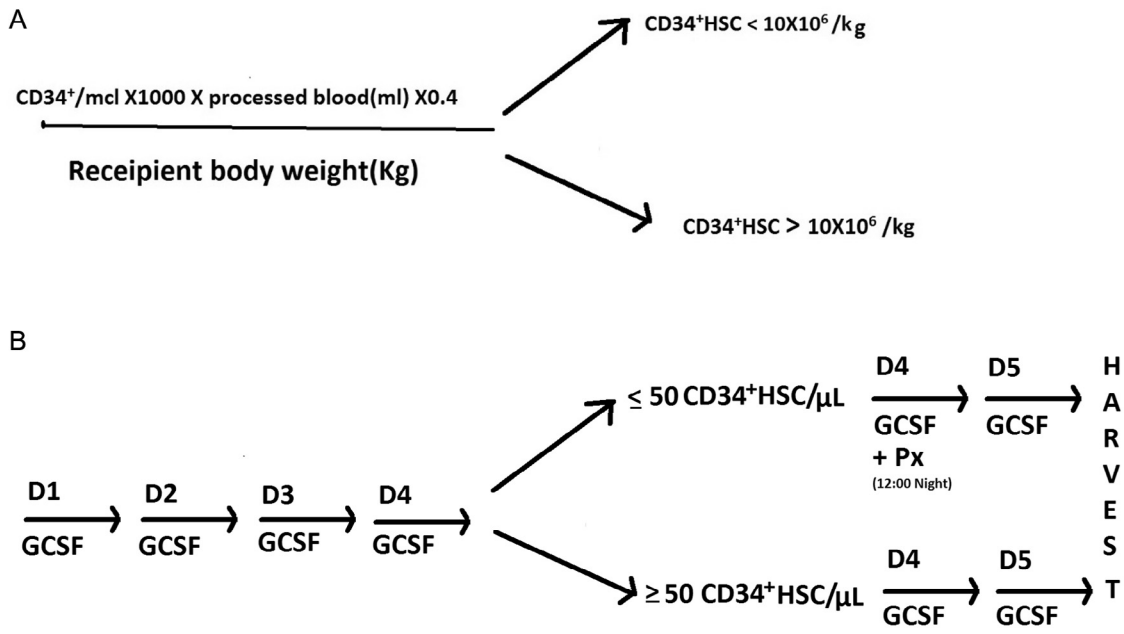

Figure 1. (A) Algorithm for prediction of CD34+ HSC yield based on PB counts on day 4 of mobilization. (B) Graphical presentation of CD34+ HSC mobilization protocol: G-CSF was given subcutaneously at a dose of 12 µg/kg in 2 divided doses. On the fourth day donors received Px at 12:00 midnight at a dose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