

Progenitor cell mobilisation

GM-CSF-based mobilization effect in normal healthy donors for allogeneic peripheral blood stem cell transplantation

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Summary:

It is important to optimize methods to mobilize hematopoietic stem cells into peripheral blood (PB) for successful allogeneic peripheral blood stem cell (PBSC) transplantation. Our primary intent was to investigate the role of GM-CSF for mobilization in normal healthy donors and to compare its efficacy in mobilizing stem cells alone, in concurrent combination and in sequential combination with G-CSF in this study. We analyzed the results of the PBSC harvest through large volume leukapheresis from 48 normal healthy donors mobilized by three different regimens including GM-CSF. Donors were assigned sequentially to one of the following regimens for mobilization: GM-CSF 10 µg/kg/day alone (group 1, *n* = 9); concurrent combination (group 2, *n* = 20) of G-CSF 5 µg/kg/day and GM-CSF 5 µg/kg/day; sequential combination (group 3, *n* = 19) of GM-CSF alone 10 µg/kg/day for 3 days followed by G-CSF alone 10 µg/kg/day for 2–3 days. The harvested CD34⁺ cell count (*P* < 0.05) was statistically higher in group 3 than in group 1 or 2. Pre-collection WBC count in donors (*P* < 0.05), harvested MNC (*P* < 0.05) and CD3⁺ cell count (*P* < 0.05) of group 2 or 3 were significantly higher than those of group 1. Recipients who received stem cells mobilized with combination regimens showed an earlier recovery of WBC and platelets count than those with GM-CSF alone. The incidence of acute graft-versus-host disease was not statistically different among three recipient groups. GM-CSF-based mobilization was well tolerated in normal healthy donors. The sequential combination regimen appears to be an excellent mobilization strategy and might be preferred as the optimal method in some clinical situations that need a higher number of stem cells.

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Peripheral blood stem cells (PBSC) are being used increasingly as a source of marrow repopulating cells in allogeneic stem cell transplantation. The advantages for the donors are no major sequelae of the harvest procedure and no need for general anesthesia. The most important factor for successful engraftment is to harvest a sufficient number of stem cells. It is therefore important to optimize methods to mobilize hematopoietic stem cells into the peripheral blood in allogeneic settings. Most centers have used recombinant granulocyte colony-stimulating factor (G-CSF) to mobilize stem cells.^{1–3}

Recently, new mobilization protocols with other cytokines have been introduced to increase the mobilization yield of stem cells in normal healthy donors.⁴ It is not yet evident that mobilization with other cytokines in donors will lead to a higher yield of stem cell harvest. However, a recent study⁵ suggested that GM-CSF-containing regimens for mobilization might augment mobilization of pure primitive stem cells when used in combination with G-CSF. Corringham *et al*⁶ also previously showed that mobilization might proceed well with a combination of GM-CSF and G-CSF after a mobilization failure with G-CSF alone in some individuals. Based on these observations, we adopted the mobilization protocol with GM-CSF-based cytokine treatment in normal healthy donors. Our primary intent was to investigate the role of GM-CSF for mobilization in normal healthy donors and to compare its efficacy in mobilizing stem cells alone, and in concurrent combination and in sequential combination with G-CSF.

Materials and methods

Study design

Allogeneic PBSCs were harvested from 48 healthy HLA-matched sibling donors. Donors were assigned sequentially to one of the following regimens for mobilization: GM-CSF (Sargramostim, Leucogen[®], LG CI, Seoul, Korea) 10 µg/kg/day alone for 5–6 days (group 1, *n* = 9); concurrent combination regimen (group 2, *n* = 20) with G-CSF (Filgrastim, Leukostim[®], Donga, Seoul, Korea) 5 µg/kg/day and GM-CSF 5 µg/kg/day for 5–6 days; sequential combination regimen (group 3, *n* = 19) with

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GM-CSF alone 10 $\mu\text{g}/\text{kg}/\text{day}$ for 3 days followed by G-CSF alone 10 $\mu\text{g}/\text{kg}/\text{day}$ for 2–3 days. In group 3, filgrastim (non-glycosylated G-CSF) was administered for G-CSF in group 3-1 and lenograstim (glycosylated G-CSF, Neutrogin[®], Choongwae, Seoul, Korea) for G-CSF in group 3-2. Donors were healthy, met the American Association of Blood Banks health history standards for blood donors. Donors were tested for antibody to human immunodeficiency virus, hepatitis B surface antigen, and hepatitis C virus antibody, and their complete blood counts were measured. A full-time member of the nursing staff performed cytokine injection, blood drawing, and donor monitoring in the Dept of Oncology at Kyungpook National University Hospital (Taegu, South Korea). Symptoms and side-effects were elicited and recorded daily by attending doctors. All recipients received G-CSF starting on post-transplant day 1 for 8 days, then received GM-CSF until the WBC count reached $>2.0 \times 10^3/\mu\text{l}$ for 2 consecutive days. Various parameters including CD34⁺ cell, MNC and CD3⁺ cell counts on the harvested products, and engraftment data were statistically analyzed to compare the mobilizing efficacy in the three groups. Daily cytokine injection was performed at 6:00 am and apheresis was started at 10:00 am on day 5. All patients and donors gave informed consent for mobilization with GM-CSF-containing regimens. Boosting with bone marrow cells from the same donors was performed on patients who manifested engraftment failure.

PBSC harvest

Forty-eight healthy donors were asked and agreed to undergo a large volume (15 liters) leukapheresis that was started on the 5th day after cytokine administration. Leukapheresis was performed with Fenwal CS 3000+ blood cell separator (Baxter, Healthcare, Deerfield, IL, USA) using acid-citrate-dextrose as anticoagulant. Venous access was obtained by venipuncture of the femoral vein. The minimum target number of MNCs was $>3 \times 10^8/\text{kg}$ recipient body weight and of CD34⁺ cells was $>3 \times 10^6/\text{kg}$ for successful transplantation. For patients with hematological malignancies with high-risk of relapse, PBSC were additionally harvested for reserving extra PBSC on successive days for the prophylactic use as donor lymphocyte infusion (DLI) as previously reported.⁷

Laboratory analysis

Peripheral blood counts were performed before the mobilization treatment and on the day of stem cell harvesting, and 24 h following apheresis in donors. Peripheral blood CD34⁺ cell counts were measured just before apheresis. Total mononuclear cell and CD34⁺ cell counts were measured on the apheresis component. Aliquots of the blood samples were incubated with phycoerythrin-conjugated monoclonal anti-CD34 (HPCA2; Becton Dickinson, San Jose, CA, USA) for 20 min at room temperature, then lysed with a lyse-no-wash standard assay and finally incubated for 10 min at 4°C. Cells were processed with FACSsort analyzer (Becton Dickinson). Data acquisition was performed with Cellquest software (Becton Dickinson)

GVHD prophylaxis and supportive care of recipients

Prophylaxis against acute graft-versus-host disease (GVHD) consisted of methotrexate (MTX) and cyclosporin A (CsA). All patients were scheduled to receive MTX 15 mg/m² on day 1 and 10 mg/m² on days 3, 6, and 11. CsA was administered intravenously at a dose of 2.5–5.0 mg/m²/day starting on the day before PBSCT. Oral CsA, 6 mg/kg/day was substituted for intravenous administration when tolerated. Acute GVHD was diagnosed and graded using established criteria.⁸ All hemoderived transfusion products were irradiated prior to use.

Data analysis

Data are reported as the mean \pm s.d. unless otherwise noted. Mean values were compared with Student's *t*-test or ANOVA test and percentages with the chi-square test. *P* value of <0.05 was considered significant. All statistics were conducted with SPSS 10.0 for Windows. The main end points of this study were an apheresis yield and engraftment data.

Definitions

Mobilization failure was defined as the failure to harvest more than $3 \times 10^6/\text{kg}$ CD34⁺ cells. Pre-collection WBC count was defined as donor's WBC count checked just before apheresis. WBC engraftment was defined as the first of 3 consecutive days with an absolute neutrophil count $>0.2 \times 10^3/\mu\text{l}$. Platelet engraftment was defined as the first of 3 days with platelet counts of $20 \times 10^3/\mu\text{l}$ or above without requiring transfusion. Engraftment failure was defined as an absolute neutrophil count less than $0.2 \times 10^3/\mu\text{l}$ on day 28.

Results

Donor characteristics

Table 1 summarized the donor characteristics. Forty-eight normal healthy donors were included. Median age was 34 years (range: 21–65 years). Male to female ratio was 1:1. Mean body weight was 62.4 kg. Hematological values in 48 donors just before apheresis were (mean \pm s.d.) WBC, $20.8 \pm 11.1 \times 10^3/\mu\text{l}$; Hb, 13.6 ± 1.7 g/dl; platelets,

Table 1 Donor characteristics

	No. of Donors	Median age (range, years)	Gender (M/F)	Weight (kg)
Total	48	34 (21–65)	24/24	62.4 \pm 9.9
Group I (GM-CSF)	9	35 (21–51)	6/3	64.3 \pm 7.2
Group II (GM/G-CSF ^a)	20	34 (21–65)	10/10	61.6 \pm 10.5
Group III (GM→G-CSF ^b)	19	30 (23–52)	8/11	63.7 \pm 11.2

^aConcurrent combination regimen.

^bSequential combination regimen.

Table 2 The mobilization and transplantation results according to GM-CSF based cytokine regimens in normal healthy donors

Cytokine Regimen	Patient No.	Pre-collection WBC ($\times 10^3/\mu\text{l}$)	MNC ($\times 10^8/\text{kg}$)	CD3 ⁺ cell ($\times 10^8/\text{kg}$)	CD34 ⁺ cell ($\times 10^6/\text{kg}$)	Acute GVHD (Gr 2–4)	Engraftment days WBC	PLT
Group I (GM)	9	14.60 \pm 7.26	2.64 \pm 1.55 ^b	1.65 \pm 0.95 ^b	3.28 \pm 2.46	42.9%	17	19 ^b
Group II (GM/G)	20	21.55 \pm 12.77	4.77 \pm 2.53	3.14 \pm 1.62	9.03 \pm 9.44	44.4%	14	14
Group III (GM→G)	19	23.54 \pm 6.45 ^a	5.56 \pm 1.94	3.21 \pm 1.08	15.66 \pm 11.11 ^b	73.3%	14	15

^a*P* < 0.05 vs regimen GM-CSF.

^b*P* < 0.05 vs other two regimens.

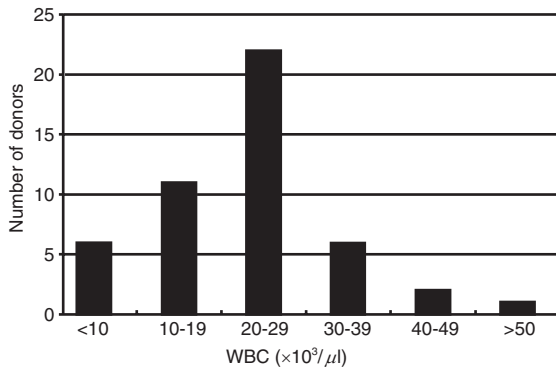


Figure 1 Histogram of pre-collection WBC counts in normal healthy donors.

189.7 \pm 53.1 $\times 10^3/\mu\text{l}$. Pre-collection WBC counts of three groups were as follows: 14.6 \pm 7.3 $\times 10^3/\mu\text{l}$ for group 1; 21.6 \pm 12.8 $\times 10^3/\mu\text{l}$ for group 2; 23.9 \pm 6.4 $\times 10^3/\mu\text{l}$ for group 3-1; 22.7 \pm 7.0 $\times 10^3/\mu\text{l}$ for group 3-2. There were no differences of age, body weight, and gender ratio among the three donor groups. As shown in Figure 1, 45.8% of donors showed pre-collection WBC counts in range of 20–29 $\times 10^3/\mu\text{l}$, 22.9% in range of 10–19 $\times 10^3/\mu\text{l}$, and 12.5% in range of 30–39 $\times 10^3/\mu\text{l}$ (Figure 1).

Large volume leukapheresis (LVL)

Tables 2 and 3 summarized the data on the harvest and LVL. A total of 83 aphereses was performed. Harvested MNC, CD3⁺ cell and CD34⁺ cell counts per apheresis were 4.64 \pm 2.57 $\times 10^8/\text{kg}$, 2.80 \pm 1.54 $\times 10^8/\text{kg}$, and

10.41 \pm 11.61 $\times 10^6/\text{kg}$, respectively. Thirty-one donors (64.6%) received two or three aphereses on successive days to harvest the target dose of stem cells for transplantation or reserve additional PBSCs for use as prophylactic DLI to enhance the graft-versus-tumor (GVT) effects.

The mobilization effects of GM-CSF based cytokine treatments (Table 2)

Two donors (4.2%) showed the mobilization failure. One was in group 1 and the other was in group 2. The mean counts of harvested MNC, CD3⁺ cells, CD34⁺ cells in group 1 was 2.64 \pm 1.55 $\times 10^8/\text{kg}$, 1.65 \pm 0.95 $\times 10^8/\text{kg}$, 3.28 \pm 2.46 $\times 10^6/\text{kg}$, respectively. Two (25%) out of eight evaluable patients who received an allogeneic PBSCT from group 1 showed engraftment failure. Their transplanted CD34⁺ cell dose was 2.8 $\times 10^6/\text{kg}$ and 1.0 $\times 10^6/\text{kg}$, respectively. The mean counts of harvested MNC, CD3⁺ cells, CD34⁺ cells in group 2 was 4.77 \pm 2.53 $\times 10^8/\text{kg}$, 3.14 \pm 1.62 $\times 10^8/\text{kg}$, 9.03 \pm 9.44 $\times 10^6/\text{kg}$, respectively. The mean counts of harvested MNC, CD3⁺ cells, CD34⁺ cells in group 3 was 5.56 \pm 1.94 $\times 10^8/\text{kg}$, 3.21 \pm 1.08 $\times 10^8/\text{kg}$, 15.66 \pm 11.11 $\times 10^6/\text{kg}$, respectively. Figure 2 depicts the differences of harvested MNC, CD3⁺ cell and CD34⁺ cell counts among three groups. The harvested CD34⁺ cell count (*P* < 0.05) was statistically higher in group 3 than in groups 1 or 2. Pre-collection WBC count in donors (*P* < 0.05), harvested MNC (*P* < 0.05) and CD3⁺ cell count (*P* < 0.05) of group 2 or 3 were significantly higher than those of group 1 (Figure 2). Recipients who received stem cells mobilized with combinations of GM-CSF and G-CSF showed an earlier recovery of WBC and platelet counts than those with GM-CSF alone.

Table 3 The side-effects of mobilization in normal healthy donors

Side-effects ^a	Group 1 (n = 9)	Group 2 (n = 20)	Group 3 (n = 19)
Headache	6 (67%)	6 (32%)	8 (42%)
Myalgia	6 (67%)	17 (89%)	12 (63%)
Fever	2 (22%)	9 (47%)	7 (37%)
Skin rash	2 (22%)	3 (16%)	3 (16%)

^aIncluded all cases of complaints of symptoms over grade 1 on WHO scale.

The comparison of mobilizing effects between lenograstim and filgrastim

There were no significant differences of pre-collection WBC (23.9 \pm 6.4 vs 22.7 \pm 7.1 $\times 10^3/\mu\text{l}$), harvested MNC (5.5 \pm 2.0 vs 5.8 \pm 1.8 $\times 10^8/\text{kg}$), CD3⁺ cell (3.0 \pm 1.2 vs 3.6 \pm 0.7 $\times 10^8/\text{kg}$), and CD34⁺ cell (17.0 \pm 12.7 vs 12.2 \pm 3.8 $\times 10^6/\text{kg}$) counts between group 3-1 (*n* = 13) and group 3-2 (*n* = 6).

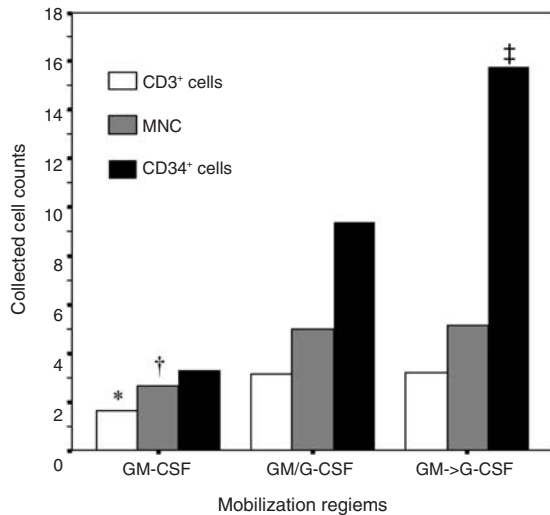


Figure 2 The comparison of collected cell counts among 3 mobilization regimens. * $P < 0.05$ vs the other two regimens; † $P < 0.05$ vs the other two regimens; ‡ $P \leq 0.05$ vs the other two regimens (CD3⁺, $\times 10^8/\text{kg}$; MNC, $\times 10^8/\text{kg}$; CD34⁺, $\times 10^6/\text{kg}$).

Side-effects of LVL and mobilization

Post-apheresis hematological values were WBCs, $20.6 \pm 10.7 \times 10^3/\mu\text{l}$; Hb, $13.1 \pm 1.6\text{g/dl}$; platelet, $78.1 \pm 24.9 \times 10^3/\mu\text{l}$. Peripheral blood platelet count dropped significantly in most of the donors after apheresis ($P = 0.001$), but clinically significant thrombocytopenia was not observed. The side-effects of mobilization treatments were mild and limited to fever, myalgia and bone pain that were relieved by acetaminophen, and did not interfere with daily activities. There were no differences in side-effects among the three groups (Table 3).

The difference of cell dose and engraftment data between group with GVHD (+) and with GVHD (-)

Twenty-two (55%) out of evaluable 40 patients manifested an acute GVHD. There was no significant difference of the incidence of acute GVHD grade 2–4 among the three recipient groups. The comparisons between recipient groups with and without GVHD showed no differences in the harvested MNC (5.08 ± 2.64 vs $5.64 \pm 2.95 \times 10^8/\text{kg}$, $P = 0.266$), CD3⁺ cell (2.99 ± 1.75 vs $3.32 \pm 1.81 \times 10^8/\text{kg}$, $P = 0.681$), CD34⁺ cell counts (12.15 ± 15.42 vs $13.30 \pm 13.19 \times 10^6/\text{kg}$, $P = 0.637$) and the engraftment day (WBC, 15 vs 15 days, $P = 0.347$; platelets, 17 vs 26 days, $P = 0.041$).

Factors to predict the amounts of harvested stem cells

Using linear regression analysis, a highly significant correlation was found between the collected MNC counts and the pre-collection WBC counts in donors ($r = 0.388$, $P = 0.001$), between the collected CD34⁺ cell counts and the pre-collection WBC counts in donors ($r = 0.281$, $P = 0.013$), and between the collected CD34⁺ cell counts and pre-collection peripheral blood CD34⁺ cell counts ($r = 0.483$, $P = 0.001$).

Discussion

Even though there is conflicting evidence^{9,10} about the impact of CD34⁺ cell dose on survival or chronic GVHD, it is reasonable to accept the fact that CD34⁺ cell dose has a dramatic positive influence on survival in allogeneic settings. The higher CD34⁺ cell doses may improve outcome in engrafting patients by leading to earlier hematopoietic recovery. Also CD34⁺ cell dose has an independent effect on relapse in patients with diverse relapse probabilities.¹¹ Previous studies^{12,13} concluded that relapse probability was significantly lower for recipients of the higher dose of CD34⁺ cells in T cell-depleted transplantation settings. Especially in hematological malignancies with a high risk of relapse, attempts to improve the outcome and relapse rate following allogeneic PBSCT should include strategies to transplant sufficient stem cells and enhance the GVT effects. Therefore, appropriate cytokine treatment is essential for the harvest of a sufficient number of stem cells in allogeneic PBSCT.

G-CSF has been widely employed for the purpose of mobilizing stem cells for allogeneic PBSCT.^{1–3} There is limited experience in the mobilization of PBSC with other cytokines or combination regimen in normal healthy donors. *In vitro* studies have demonstrated synergistic effects on highly enriched human marrow progenitor cells between GM-CSF and G-CSF.¹⁴ Lane *et al*¹⁵ concluded that subjects treated with the combination of G- and GM-CSF showed an equivalent mobilization of CD34⁺ cells and CFU-GM, as G-CSF alone. However, whether a new mobilization regimen will lead to an improvement in the outcome of transplantation or a better result in the mobilization failure rate than G-CSF alone needs to be evaluated. In the autologous setting, $250 \mu\text{g}/\text{m}^2$ GM-CSF alone was inferior to $250 \mu\text{g}/\text{m}^2$ G-CSF alone for the mobilization of CD34⁺ cells.¹⁶ Madero *et al*¹⁷ concluded that concurrent mobilization with G-CSF and GM-CSF in children did not enhance hematological recovery in comparison with mobilization using G-CSF alone in autologous setting. Mobilization with GM-CSF alone has been very rarely used in allogeneic settings.¹⁸ Lane *et al*⁵ concluded that GM-CSF alone proved to be the poorest at mobilizing CD34⁺ cells in a quantity comparison study of the efficacy of various mobilization regimens in normal healthy donors. However, they suggested that the concurrent GM-CSF and G-CSF regimen was associated with yields of CD34⁺ cells equal to those seen with G-CSF alone and with greater yields of primitive CD34⁺ cells (CD34⁺/CD38⁻/HLA-DR⁺ subset). Ho *et al*¹⁹ illustrated that the cloning efficiency of CD34⁺ cells primed with combination of GM-CSF and G-CSF was twice than that of G-CSF mobilized CD34⁺ cells. They also concluded that different growth factors and regimens can preferentially mobilize different CD34⁺ subsets from normal donors and that the combination of G-CSF and GM-CSF might be an optimal regimen. We therefore hypothesized that combination regimen with GM-CSF and G-CSF might induce a better result for transplantation or, at least, a similar result compared to G-CSF alone. Sargramostim (Leucogen[®]) used in this trial was a glycosylated form of GM-CSF derived from yeast and marketed in Korea.

In a randomized trial¹⁶ of G-CSF (filgrastim), GM-CSF

(sargramostim), or sequential GM-CSF and G-CSF for mobilization in autologous setting, G-CSF alone or sequential GM-CSF and G-CSF were superior to GM-CSF alone. This observation is consistent with those presented here. In our trial, GM-CSF alone was inferior to a concurrent combination or a sequential combination regimen in the yields of harvested MNC or CD34⁺ cell counts. Among three different mobilization groups, group 1 manifested the most delayed recovery time of platelet count after an allogeneic PBSCT. We decided to stop enrolling further cases for mobilization with GM-CSF alone because of the poor yield of stem cells and late recovery of blood cell counts after transplantation in nine patients who received an allogeneic PBSCT primed with GM-CSF alone. Even though we did not compare GM-CSF alone and G-CSF alone in our study, it is clear that the mobilization results of GM-CSF alone were inferior to those that have been expected from G-CSF alone. Lane *et al*'s comparison studies^{5,15} between G-CSF alone and GM-CSF alone showed that mobilization with G-CSF alone resulted in a higher CD34⁺ cell dose than GM-CSF alone. Fischmeister *et al*²⁰ observed that CD34⁺ cell counts in PB start to increase above baseline levels of less than 1/ μ l on the 3rd day with 5 μ g/kg/day of G-CSF or GM-CSF. The highest values of 29.5/ μ l (\pm 18.6, G-CSF) and of 8.0/ μ l (\pm 4.8, GM-CSF) were reached on day 5 after 4 days of stimulation. This shows G-CSF to be more than three times as effective as GM-CSF in increasing PBSCs. Holm²¹ suggested that mobilization with G-CSF alone would not be optimal for all individuals. Corringham *et al*⁶ previously showed that mobilization with a combination of GM-CSF and G-CSF was successful in several patients who manifested a mobilization failure with G-CSF alone. In our trial, poor mobilization was observed only in one (2.5%) out of 39 patients who received allogeneic PBSCT with stem cells primed with concurrent or sequential combination regimens and observed side-effects were not a major concern. The sequential addition of GM-CSF to G-CSF might be adopted as a salvage regimen in donors who failed to mobilize the target dose of stem cells with G-CSF alone.

The dosing schedule of G-CSF and GM-CSF is important in mobilization protocols. According to Winter *et al*'s study,²² the administration of G-CSF in patients already receiving GM-CSF was more effective in mobilizing stem cells than the addition of GM-CSF to G-CSF. This finding suggests that GM-CSF stimulates more primitive CD34⁺ cells and seems to precondition stem cells and improve G-CSF-induced PBSC mobilization and harvest results. Also in our trial, 3-day priming with 10 μ g/kg/day GM-CSF followed by sequential 10 μ g/kg/day G-CSF seemed to be a more effective strategy to mobilize CD34⁺ cells than a concurrent administration of 5 μ g/kg/day GM-CSF and 5 μ g/kg/day G-CSF in normal healthy donors. The practical significance of this observation for allogeneic PBSCT is unknown. However, the sequential regimen might be a preferred option in special circumstances, such as a high body weight discrepancy between donor and recipient, or in a situation that needs mega-dose transplantation, or where there is a possibility of losing significant numbers of cells during the process of CD34⁺ cell selection. We previously presented that allogeneic PBSCT followed

by prophylactic growth factor-primed DLI using surplus cells additionally reserved at the time of harvest after mobilization with combination of GM-CSF and G-CSF would be one of the enhancing strategies to reduce the relapse risk in hematological patients with a high risk of relapse.^{7,23} Conclusively, through our trial and published reports, the mobilization strategy with sequential combination for a higher yield of stem cells may offer the additional advantage of being able to collect enough cells for potential use as DLI at harvest, which is easy, convenient to donors and cost-effective.

Two forms of G-CSF are available for clinical use. A comparative study²⁴ on the mobilization efficacy between glycosylated form (lenograstim) of G-CSF and non-glycosylated form (filgrastim) of G-CSF reported that lenograstim produced a highly significant 25% greater mobilization of CD34⁺ cells in healthy donors than did the same dosage of filgrastim. This observation suggests that the influence of glycosylation could be an important factor. However, there was no difference of various parameters on the mobilization and harvest between group 3-1 (GM-CSF followed by sequential lenograstim) and 3-2 (GM-CSF followed by sequential filgrastim) in our trial. Even though both G-CSFs proved to produce a similar mobilization result at a dose of 10 μ g/kg/day in sequential use followed by GM-CSF in our trial, we could not exclude that there might be the statistical likelihood that there was no difference due to a relatively small number of patients

Vij *et al*²⁵ suggested that the combined use of G-CSF and GM-CSF for mobilization could reduce the number of T cells infused into the recipients, which might confer a decreased risk for the development of GVHD compared with the single use of G-CSF. However, we failed to confirm this observation in our trial. The harvested CD3⁺ cell dose of groups 2 or 3 in our trial was similar with that of mobilization groups with G-CSF alone reported in the literature.²⁵⁻²⁷ There was no significant difference of the incidence of acute GVHD among three groups of recipients. The incidence of acute GVHD was 55% in our trial and seems to be similar with that of other studies on allogeneic PBSCT from donors primed with G-CSF alone.

In conclusion, GM-CSF-based mobilization was well tolerated in normal healthy donors. A sequential combination regimen with GM-CSF and G-CSF appears to be an excellent mobilization strategy and might be preferred in some clinical situations that need a higher number of stem cells.

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