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Haemopoietic growth factors

Recombinant human granulocyte colony-stimulating factor (rh-G-CSF) may accelerate hematopoietic recovery after HLA-identical sibling allogeneic peripheral blood stem cell transplantation

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Summary:

We studied the effects of recombinant human granulocyte colony-stimulating factor (G-CSF) on hematopoietic recovery and clinical outcome in patients undergoing allogeneic peripheral blood stem cell (PBSC) transplantation. Fifty-six patients with hematological malignancies who underwent allogeneic PBSC transplantation between 1995 and 1998 were entered into this study. Twenty-eight patients who received daily G-CSF from day +1 after allogeneic PBSC transplantation until the absolute neutrophil count (ANC) reached $>0.5 \times 10^9$/l for 3 consecutive days were compared with 28 patients (control group) who did not receive G-CSF in a non-randomized manner. The study group and the control group were comparable with respect to baseline patient and transplantation characteristics. Median times to ANC of $>0.5 \times 10^9$/l and 1×10^9/l with or without G-CSF were 12 days (range 8–21), 13 days (10–32) ($P = 0.04$) and 13 days (9–21), 15 days (11–44) ($P = 0.02$), respectively. Median times to reach a platelet count of $>20 \times 10^9$/l with and without G-CSF were 11 days (0–20) and 13 days (9–26), respectively ($P = 0.03$). The incidence of febrile episodes was significantly lower with G-CSF, 75% *vs* 100% ($P = 0.008$). Patients receiving G-CSF had less grade III–IV mucositis than those who did not receive G-CSF ($P = 0.01$). There was also no increase in the incidence and severity of acute GVHD in patients using G-CSF ($P = 0.22$). Although the number of relapsing patients was greater in the G-CSF group (seven *vs* three patients), this was not statistically significant ($P = 0.24$). Disease-free and overall survival rates did not differ between the two groups ($P = 0.58$ and 0.53, respectively). The administration of G-CSF after allogeneic PBSC transplantation provided faster neutrophil and platelet engraftment associated with less severe mucositis and less febrile episodes. *Bone Marrow Transplantation* (2001) **27,** 499–505.

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Recombinant human growth factors such as granulocyte colony-stimulating factor (G-CSF) and GM-CSF have been used successfully to accelerate hematopoietic recovery after both allogeneic and autologous bone marrow transplantation (BMT).[1–11] In the allogeneic setting BMT has been increasingly replaced by peripheral blood stem cell (PBSC) transplantation over a period of 10 years.[12] Allogeneic PBSC transplantation provides faster platelet and neutrophil engraftment than alloBMT, regardless of growth factor administration, and there is no risk of general anesthesia for donors. PBSC transplantation provides early engraftment as PBSC yield includes faster engrafting committed progenitors compared to BMT.[12–19] Therefore, whether growth factors may further accelerate hematopoietic recovery in the allogeneic PBSC transplantation is obscure. On the other hand, initial concerns about administration of growth factors following allogeneic BMT were based on the probability of their potential effect on the incidence of graft-versus-host disease (GVHD)[13] and relapse rates which was not confirmed with recent studies.[14,15,17,20]

Taken together, the efficacy and the safety of growth factor administration after allogeneic PBSC transplantation remains elusive. This study addressed whether G-CSF further accelerates engraftment, decreases infectious complications and increases the risk of GVHD and relapse in patients undergoing allogeneic PBSC transplantation.

Materials and methods

Patient characteristics

Fifty-six patients with hematological malignancies who underwent allogeneic PBSC transplantation between 1995 and 1998 in our department were entered into this study. Twenty-eight patients who received daily G-CSF from day +1 after allogeneic PBSC transplantation until the absolute neutrophil count (ANC) reached $>0.5 \times 10^9$/l for 3 consecutive days were compared with 28 patients (control

group) who did not receive G-CSF in a non-randomized manner. In both groups, the patients were comparable with regards to their age, sex, diagnosis, disease status at transplantation, CMV serology (all were seropositive) and GVHD prophylaxis. Patient characteristics are given in Table 1. All patients received peripheral blood stem cells from their HLA-identical sibling donors. Daily G-CSF was given intravenously from day +1 after allogeneic PBSC transplantation until the ANC reached $>0.5 \times 10^9$/l for 3 consecutive days.

Conditioning regimens

Patients were conditioned with busulfan 4 mg/kg/day for 4 days starting on day −8 and cyclophosphamide 60 mg/kg/day on days −4 and −3. One patient with lymphoblastic lymphoma (LL) was conditioned with total body irradiation (TBI) only.

Mobilization and blood stem cell collection

G-CSF was used for donor stem cell mobilization at a dose of 10 μg/kg bodyweight subcutaneously for 5 days. On the fifth day of G-CSF, leukapheresis was performed with a continuous flow cell separator (Cobe Spectra; COBE BCT, Lakewood, CO, USA, or Fenwall CS 3000+; Baxter Healthcare Systems, Deerfield, IL, USA) using peripheral veins. A median of 2 (range 1–5) leukaphereses was necessary to reach the target number of $CD34^+$ cells, which was $\geqslant 4 \times 10^6$/kg. After each collection, harvested yields were transfused immediately on the same day. The first infusion day was accepted as day 0.

Prophylaxis and treatment of GVHD

Diagnosis and grading of GVHD were based on the accepted clinical criteria.[21] Patients were evaluated for acute GVHD if they survived 21 days and had evidence of engraftment. Patients who survived for more than 100 days were evaluated for chronic GVHD. Cyclosporin A (CsA) plus short-term MTX were used for GVHD prophylaxis in all patients. CsA was administered on day −1 until day 180 unless there was no sign of GVHD. MTX was initiated on day +1 at a dose of 15 mg/m^2 and given on days +3, +6, +11 at a dose of 10 mg/m^2. When clinical grade II–IV GVHD was diagnosed, methylprednisolone (1–2 mg/kg/day intravenously with gradual tapering) was commenced. In the event of progression ATG or ALG was added to this treatment. Chronic GVHD was treated with CsA plus methylprednisolone.

Supportive care

Platelet suspensions and packed red blood cells (RBC) were transfused when the platelet and hemoglobin levels were less than 20×10^9/l and 8.0 g/dl, respectively. Ciprofloxacin, fluconazole and acyclovir were administered for antimicrobial prophylaxis. A third generation cephalosporin or carbapenem and amikacin were initiated intravenously for neutropenic febrile episodes. Vancomycin on day 5 followed by amphotericin B was added to the treatment in the event of persistent fever. All antibiotics were discontinued after 5 consecutive afebrile days unless there was a clinical or microbiological documented infection or clinical deterioration.

Engraftment

Neutrophil engraftment was defined as of the first day of the 3 consecutive days with ANC of $\geqslant 0.5 \times 10^9$/l. Platelet engraftment was defined as the time to reach a sustained platelet count of $\geqslant 20 \times 10^9$/l for 7 days without platelet transfusion. Engraftment failure was defined if there was no sign of engraftment after day 30. In all patients, sex mismatches, blood group incompatibility and molecular polymorphism were investigated by forward and reverse

Table 1 Characteristics of patients

Characteristics of patients	*With G-CSF (n = 28)*	*Without G-CSF (n = 28)*	*P value*
Sex (male/female)	19/9	17/11	NS
Median age in years (range)	36 (13–44)	38 (17–47)	NS
Diagnosis			NS
CML	15	18	
AML	10	10	
ALL	1	0	
LL	1		
MDS	1		
Remission			
Early disease: CR1/CP1	7/14	7/17	
Advanced remission	4	4	
Sex mismatch			
Male recipient/Female donor	5	8	NS
Median of infused (range)			
MNC $\times 10^8$/kg (median)	7.7 (2.71–29.3)	6.4 (3.1–38.2)	NS
$CD34^+$ cells $\times 10^6$/kg (median)	6.4 (2.99–30.8)	6.5 (2.1–18.9)	NS
Conditioning regimens			
BU/CY	27	28	
TBI	1	0	

ABO blood grouping, conventional cytogenetics, fluorescent *in situ* hybridization (FISH) and variable tandem repeat polymorphism (VNTR) to determine the origin of chimerism.

Relapse

Patients who had hematological evidence of primary disease were accepted as having hematological relapse. In patients with CML, the analysis of Philadelphia chromosome starting from +12 months by conventional cytogenetic and FISH or PCR were performed to evaluate the cytogenetic or molecular remission.

Statistics

The differences between the two groups (recipient age, mononuclear and $CD34^+$ cell count, febrile neutropenic days, duration of antibiotic therapy, transfusion requirements and duration of mucositis) were compared by Wilcoxon rank sum (Mann–Whitney) test. Chi-square test was used to compare differences between the two groups for recipient and donor sex and ABO mismatch, diagnosis, aGVHD grade II–IV, cGVHD (limited and extensive), transplant-related mortality and causes of death. The probabilities of neutrophil and platelet recovery, and estimation of survival were compared using the method of Kaplan–Meier with log rank analysis.

Results

Engraftment

The median numbers of infused MNC in the G-CSF and control group were 7.7×10^8/kg (2.71–29.3) and 6.4×10^8/kg (3.1–38.2), respectively ($P = 0.13$) (Table 1). The median numbers of infused amount of $CD34^+$ cells in the G-CSF and control group were 6.4×10^6/kg (2.99–30.8) and 6.5×10^6/kg (2.1–18.9), respectively ($P = 0.36$).

Engraftment was significantly faster in the G-CSF group than in the control group. Median time to reach an ANC of $\geq 0.5 \times 10^9$/l and $\geq 1 \times 10^9$/l with and without G-CSF were 12 days (8–21), 13 (10–32) ($P = 0.04$) and 13 days (9–21), 15 (11–44) ($P = 0.02$), respectively (Figure 1, Table 2). Median time to reach a platelet count of $\geq 20 \times 10^9$/l with and without G-CSF was 11 days (0–20) and 13 (9–26), respectively ($P = 0.03$) (Figure 2). Transfusion requirements were similar in both groups ($P = 0.14$) (Table 2).

Transplant-related complications

Infectious complications: Consistent with early engraftment, the percentage of patients developing febrile episodes was markedly less in the G-CSF group than in the control group (75% *vs* 100%) ($P = 0.008$). Five patients in the G-CSF group and four patients in the control group had documented infection ($P = 0.81$). There was no significant difference between the two groups in terms of duration of febrile days, antibiotic and antifungal administration (Table 3).

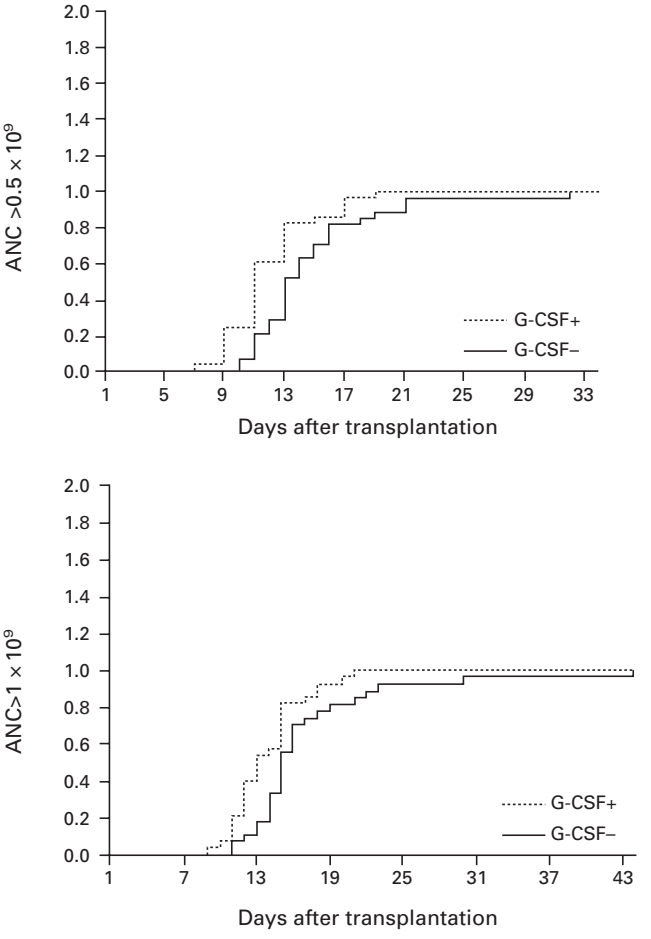


Figure 1 Probability of reaching ANC of $>0.5 \times 10^9$/l and $>1 \times 10^9$/l with and without G-CSF.

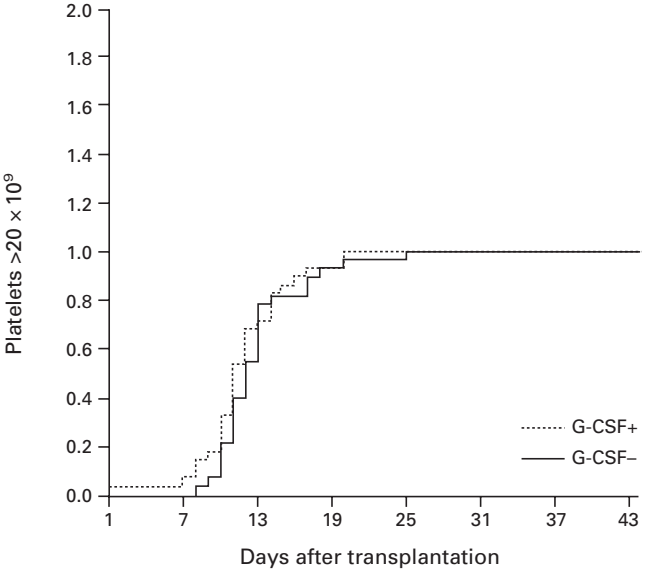


Figure 2 Probability of reaching platelets $>20 \times 10^9$/l with and without G-CSF.

Table 2 Hematopoietic recovery following allogeneic PBSC transplantation

Variables	*With G-CSF (n = 28)*	*Without G-CSF (n = 28)*	*P value*
Median days to (range)			
ANC $>0.5 \times 10^9$/l	12 (8–21)	13 (10–32)	0.04
ANC $>1 \times 10^9$/l	13 (9–21)	15 (11–44)	0.02
Platelets $>20 \times 10^9$/l	11 (0–20)	13 (9–26)	0.03
Platelets $>50 \times 10^9$/l	14 (10–54)	15 (11–35)	NS
Median No. units (range)			
Platelet transfusions	4 (1–10)	3 (0–26)	NS
RBC transfusions	1 (0–7)	(0–12)	NS

ANC = absolute neutrophil count; RBC = red blood cells.

Table 3 Early complications following allogeneic PBSC transplantation

Variables	*With rh G-CSF (n = 28)*	*Without G-CSF (n = 28)*	*P value*
No. febrile neutropenic patients	21	28	0.008
Median days of (range)			
Neutropenic fever	5 (1–10)	3 (1–23)	NS
Parenteral antibiotics	5 (3–23)	9 (5–28)	NS
Parenteral amphotericin B	8 (4–17)	11 (7–20)	NS
Death due to			NS
Aplasia	0	0	
Infection	0	0	
AGVHD	2	3	
Other	1	1	

Mucositis: Although the overall incidence of mucositis was not different between the groups, the frequency of severe mucositis (grade III–IV) was significantly less in patients receiving G-CSF than those not receiving G-CSF (11/21 *vs* 19/21, respectively, $P = 0.01$). There was no significant difference between the two groups regarding the duration of mucositis. Median number of days of mucositis in the G-CSF and control group were 6 (1–16) and 8 (3–20), respectively ($P = 0.23$) (Table 4). Duration of total parenteral nutrition (TPN) was not also different between the two groups ($P = 0.25$).

Table 4 Mucositis following allogeneic PBSC transplantation

Variable	*With G-CSF (n = 28)*	*Without G-CSF (n = 28)*	*P value*
No. of patients with mucositis			
Grade 0	7	7	NS
Grade 1–2	10	2	0.01
Grade 3–4	11	19	
Duration of mucositis median days (range)	6 (1–16)	8 (3–20)	NS
Duration of TPN median days (range)	9 (3–18)	13 (2–34)	NS

GVHD: There was no significant difference in the incidence of aGVHD between two groups. Ten out of 28 patients in the G-CSF group and nine out of the 28 patients in the control group developed severe acute GVHD (grade II–IV) ($P = 0.22$). Chronic GVHD occurred in 50% (14/28) of patients in the G-CSF group and in 67% (19/28) of the patients in the control group ($P = 0.22$). The extensive cGVHD rate was not higher in the G-CSF group than in the control group (50% *vs* 57%) ($P = 0.36$).

Outcome and relapse

Seven of 28 patients (25%) in the G-CSF group and three of 28 patients (10.7%) in the control group relapsed ($P = 0.24$). The seven patients relapsing in the G-CSF group, three with AML and four with CML, relapsed 3–20 months after transplantation. One patient died 3 months after donor lymphocyte infusion (DLI), and 12 months after his first transplantation. The three patients relapsing in the control group, one with AML and two with CML, relapsed at the 5th, 8th and 10th months. All died due to disease progression.

There was no difference between the two groups regarding causes of death. One patient in the G-CSF group committed suicide in the 3rd month and one patient in the control group was admitted dead to the hospital on post-transplant day 60. Three patients in the G-CSF group and four patients in the control group died in the first 100 days (Table 3). Two out of three in the study group and three out of four in the control group died of aGVHD. As of February 2000, patients in the G-CSF group had 22 months of median follow-up (range 2–52) with expected disease-free survival of 75% for 12 months and 60% for 18 months. The control group according to Kaplan–Meier survival estimations did not reach a median survival (range 1–47). Their expected disease-free survivals for 12 and 18 months were 64% for each, and there was no difference between the two groups ($P = 0.58$) (Figure 3). The probability of overall

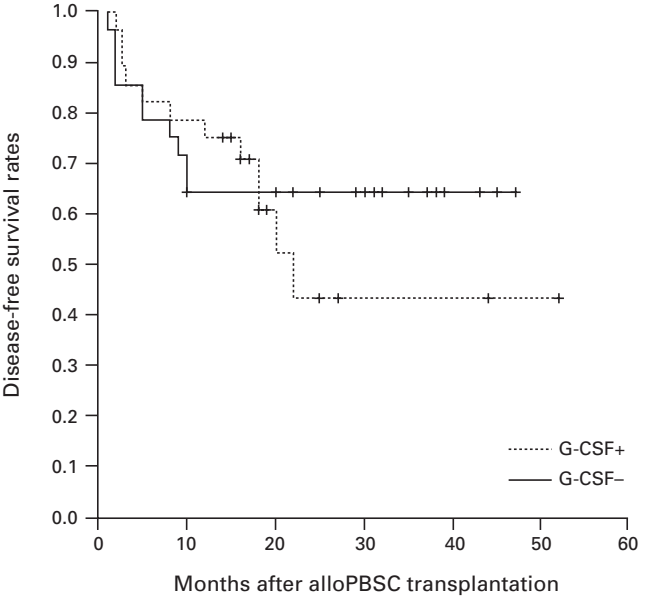


Figure 3 Probability of disease-free survival of patients with and without G-CSF.

survival for patients receiving G-CSF was 75% and 65% at 12 and 18 months, respectively. For the control group, these were 64% at 12 and 18 months, which was not statistically significant ($P = 0.53$) (Figure 4).

Discussion

To achieve early engraftment and thus to encounter less infectious complications and mortality, growth factors have been administered after alloBMT. Most of the studies have shown that G-CSF hastens granulocyte engraftment in patients receiving bone marrow from either HLA-full matched siblings or volunteer unrelated donors.[1–11,22]

Although the effect of growth factors following alloBMT has been investigated in many studies, there are very limited data demonstrating the efficacy and safety of G-CSF administration after allogeneic PBSC transplantation. This study was performed in a single institution and showed that neutrophil engraftment was significantly faster in patients treated with G-CSF in comparison with a control group who did not receive G-CSF. The number of infused stem cells, which may have an influence on neutrophil engraftment, was not different between the two groups. In addition, the effect of G-CSF has previously been found less pronounced in those who were given MTX as a component of their GVHD prophylaxis.[7] In the current study all of our patients in both groups received MTX, which might mask the benefit of G-CSF. Przepiorka *et al*[23] randomized 42 patients undergoing allogeneic PBSC transplantation from HLA-matched related donors into two groups of whether to receive G-CSF or not. The group receiving G-CSF (filgrastim) had a shorter time to reach ANC $>0.5 \times 10^9$/l (day 11 *vs* 15, $P = 0.002$). In a retrospective and multicenter study, Urbano-Ispizua *et al*[24] showed that the speed of neutrophil recovery to $>1 \times 10^9$/l was influenced by post-transplant G-CSF administration ($P = 0.02$).[24] However, that study included only a small number of patients, and MTX was not given to all patients for GVHD prophylaxis. G-CSF has also been shown to hasten neutrophil recovery after PBSC transplantation from unrelated donors.[25] Although it has been demonstrated that G-CSF had an inverse impact on platelet engraftment after ABMT[26,27] and its administration was associated with a longer median time to reach 20×10^9/l for platelets (11 *vs* 14 days) after allogeneic PBSC transplantation,[24] we showed that platelet engraftment was significantly earlier in the G-CSF group than in the control group as well. While all patients in the control group received four doses of MTX (days 1, 3, 6 and 11), 25 out of 28 patients in the G-CSF group received the same MTX regimen and the remaining three patients received three instead of four doses (days 1, 3 and 6). The difference in platelet recovery times cannot be attributed to MTX. The platelet recovery times of these three patients who received MTX for 3 days did not further shorten the mean platelet recovery time (their recovery times were 10, 20 and 14 days, respectively). The longer platelet recovery period in the control group might be explained by the higher incidence of febrile neutropenia (100% *vs* 75%) in this group.

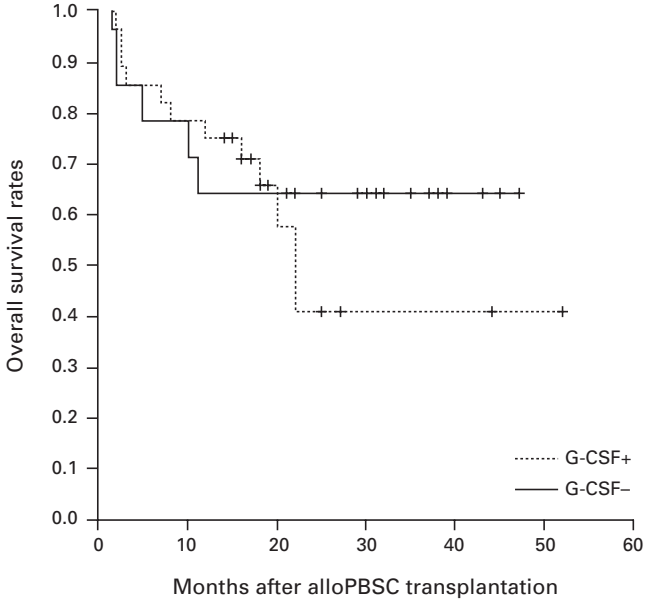


Figure 4 Probability of overall survival of patients with and without G-CSF.

It is not clear if administration of G-CSF provides less infectious complications in patients undergoing allogeneic hematopoietic stem cell transplantation due to faster hematopoietic recovery. In our study, the percentage of patients developing febrile episodes were markedly less in the G-CSF group than in the control group, in part due to the faster neutrophil engraftment. However, we did not observe significant differences between the two groups in terms of antibiotic administration, days on amphotericin B and duration of febrile days. No death related to infection occurred in either group. Therefore, G-CSF administration seems to protect some patients from infection but, once infection occurred, G-CSF may not help to resolve the infection. Despite faster hematopoietic recovery with G-CSF after related or unrelated alloBMT in some studies, this benefit did not translate into less infectious complications.[2,28] However, in another study, G-CSF or GM-CSF administration after alloBMT shortened the time to neutrophil recovery, which resulted in shorter duration of infection and antibiotic administration.[29,30] Lenograstim administration after autologous and allogeneic transplantation hastened neutrophil recovery above 0.5×10^9/l (20 *vs* 14 days, $P < 0.01$) and was associated with fewer days of infection and antibiotic administration.[27] Martin-Algarra *et al*[30] reported that the duration of neutropenia was significantly decreased in the group of patients treated with G-CSF or GM-CSF (median 17 *vs* 20 days; $P < 0.001$), and was accompanied by fewer days of intravenous antibiotic therapy (median 20 *vs* 34 days; $P < 0.001$) and by fewer positive blood and tissue cultures (median 2 *vs* 12 and 13 *vs* 28; $P = 0.02$ and $P = 0.05$, respectively).[30] In line with some literature data, our study also demonstrated the beneficial influence of G-CSF administration against infectious complications.

Clinical studies using subcutaneous G-CSF or GM-CSF have shown a beneficial effect on oral mucositis which is an important non-hematological dose-limiting toxicity after high-dose chemotherapy.[26] In our study, the incidence of

grade III–IV mucositis was significantly reduced in the G-CSF-treated group ($P = 0.01$), which may contribute to the lower occurrence of febrile episodes in the G-CSF group. Even if we exclude the three patients who received less MTX in the G-CSF group, the difference in mucositis still remained significant ($P = 0.03$) in favor of the group receiving G-CSF. These data confirm previous studies. Nemunaitis *et al*[31] reported that the incidence of severe mucositis was less in patients receiving GM-CSF ($P = 0.005$) following alloBMT. Gordon *et al*[32] also demonstrated that the duration of mucositis was significantly shorter in children who received GM-CSF than in those who did not after allogeneic hematopoietic stem cell transplantation.[32] Although it was not statistically significant, the median duration of mucositis and TPN was also shorter in G-CSF-treated patients in our study. G-CSF or GM-CSF may exert their effects by activating neutrophil migration, chemotaxis, phagocytosis and cytotoxicity as well as increasing neutrophil count and by enhancing of proliferation and migration of non-hematopoietic cells, including endothelial cells and keratinocytes.[26]

Although it has been suggested that the administration of G or GM-CSF increased the risk of GVHD development,[7,16] the relationship between growth factor administration and GVHD is controversial following alloBMT.[12,33–35] In addition, there are some reports indicating an increased incidence of GVHD after PBSC transplantation.[14,25] In our study the administration of G-CSF following PBSC transplantation did not increase the incidence and severity of acute or chronic GVHD significantly. This was in line with Przepiorka's study in which neither grade II–IV nor grade III–IV GVHD incidence differed significantly between patients who received G-CSF and those who did not.[23] It has been reported that pretreatment of donor mice with G-CSF polarizes donor T cells toward the production of anti-inflammatory cytokines (IL-4, IL-10), which is associated with reduced severity of acute GVHD. G-CSF also down-regulates allogeneic immune responses by inhibition of TNF-α.[36] It is also possible that exposure to G-CSF switch T4 cells to Th2 rather than Th1 that has established a role in the development of GVHD.

Despite early neutrophil engraftment, transplant-related mortality (TRM) was not significantly different in patients who were given G-CSF following allogeneic sibling BMT in most studies.[2,7] In the allogeneic PBSC transplantation setting, we found similar results to Przepiorka *et al* such as faster neutrophil recovery without significantly decreased TRM. Our study demonstrated similar probabilities of DFS and OS in both groups at 12 and 18 months. These data are consistent with the data obtained after alloBMT studies.[3,16,29,32,34,37]

In conclusion, G-CSF provided faster neutrophil and platelet engraftment associated with less severe mucositis and less febrile episodes after allogeneic PBSC transplantation. The incidence of GVHD was not affected by G-CSF administration. The probabilities of DFS and OS were similar in both groups of patients. The data obtained from this study warrant that further randomized studies containing a large number of patients to evaluate the impact of G-CSF after allogeneic PBSC transplantation are needed. Currently, a study to investigate the effects of delayed administration of G-CSF after allogeneic PBSC transplantation in our institution is being initiated.

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