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Influence of recombinant human granulocyte colony-stimulating factor (filgrastim) on hematopoietic recovery and outcome following allogeneic bone marrow transplantation (BMT) from volunteer unrelated donors

C Berger[1,2], H Bertz[1], C Schmoor[3], D Behringer[1], K Potthoff[1], R Mertelsmann[1] and J Finke[1]

[1]*Albert Ludwigs University Medical Center, Department of Hematology/Oncology, Freiburg; and* [3]*Albert Ludwigs University Medical Center, Department of Medical Biometry and Statistics, Freiburg, Germany*

Summary:

Effects of recombinant human granulocyte colony-stimulating factor (rhG-CSF, filgrastim) on hematopoietic recovery and clinical outcome in patients undergoing allogeneic bone marrow transplantation (BMT) from volunteer unrelated donors (VUD) were analyzed retrospectively. Additionally, the influence of baseline patient and transplant characteristics on hematopoietic recovery was evaluated. From January 1994 to March 1996, 47 consecutive adult patients received VUD-BMT. GVHD prophylaxis was cyclosporin A/short course methotrexate/prednisolone, and in four patients additional ATG. Post-transplantation, cohorts of patients received rhG-CSF (5 μg/kg/day) ($n = 22$) or no rhG-CSF ($n = 25$) in a non-randomized manner. The patient groups with and without rhG-CSF were rather comparable with respect to baseline patient and transplant characteristics. Median time to neutrophil counts (ANC) >500/μl was 14 days with rhG-CSF *vs* 16 days without rhG-CSF ($P = 0.048$), to ANC >1000/μl was 15 *vs* 18 days ($P = 0.084$). Neutrophil recovery was accelerated in patients receiving more than the median MNC dose of 2.54 × 10^8/kg with a median time to ANC >1000/μl of 13 days *vs* 19 days ($P = 0.017$). RhG-CSF did not influence platelet recovery and incidence of infectious complications. Incidence of acute GVHD II–IV was 50% with rhG-CSF and 28% without rhG-CSF ($P = 0.144$), but death before acute GVHD II–IV occurred in 9% of patients with and 20% of patients without rhG-CSF. The median follow-up time was 38 and 36 months in patients with and without rhG-CSF, respectively. Survival at 2 years post-transplant was 39% (95% confidence interval (18%, 60%)) in patients with rhG-CSF and 24% (95% confidence interval (7%, 41%)) in patients without rhG-CSF. Administration of rhG-CSF after VUD-BMT may lead to more rapid neutrophil recovery, but did not influence the incidence of infectious complications. Patients receiving rhG-CSF showed a slightly higher incidence of acute GVHD II–IV. Higher numbers of MNC in the marrow graft accelerated hematopoietic engraftment.

Keywords: filgrastim; G-CSF; allogeneic; unrelated donor; bone marrow transplantation; GVHD

High-dose chemotherapy followed by allogeneic bone marrow (BMT) or peripheral blood stem cell transplantation (PBSCT) is increasingly used for patients with poor-risk hematological malignancies. The myeloablative therapy is associated with prolonged pancytopenia, which can result in serious morbidity and life-threatening complications due to infections and bleeding.[1,2] Hematopoietic growth factors (HGF) have been investigated following allogeneic BMT and PBSCT as potential means of decreasing the period of aplasia and thus reducing infectious complications, early toxicity and death rate post-transplant.[3–5] Initial concerns over potential aggravation of graft-versus-host disease (GVHD) and increased incidence of relapse in patients treated for myeloid leukemias have not been confirmed.[3–15]

Several trials, analyzing the use of G-CSF following related BMT have shown a significantly accelerated neutrophil recovery in patients receiving G-CSF.[4,5,9–15] However, most trials failed to detect any beneficial effect of G-CSF on platelet recovery, incidence of infectious complications and clinical outcome.[4,11–15]

Data concerning the use of HGFs following volunteer unrelated donor (VUD) BMT are more limited.[4,5,14–18] Whereas the influence of GM-CSF following VUD-BMT on hematopoietic recovery and outcome has been investigated in some trials,[16–18] only one analysis of G-CSF after VUD-BMT in adults has been published until now.[14] However, in this retrospective analysis of G-CSF following related as well as VUD-BMT, results of hematopoietic recovery and outcome have been compared with data of historical controls only in related BM recipients. Thus, no adequate trial concerning the use of G-CSF following VUD-BMT has been performed yet.[4,5]

We now report our experience in 47 consecutive patients treated at a single institution using rhG-CSF (filgrastim) after allogeneic VUD-BMT. Our objectives were to evaluate the efficiency and safety of rhG-CSF regarding hemato-

Correspondence: Dr J Finke, Department of Hematology/Oncology, Freiburg University Medical Center, Hugstetterstr 55, 79106 Freiburg, Germany
[2]Present address: Clinical Research Division, Fred Hutchinson Cancer Research Center, 1124 Columbia Street, Seattle, WA, USA
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poietic recovery and possible effects on GVHD and to analyze clinical outcome post-transplant.

Patients and methods

Forty-seven consecutive adult patients with hematological malignancies (22 male/25 female) received as their first transplantation allogeneic VUD-BMT from January 1994 to March 1996. Two further patients were excluded because of a different G-CSF schedule. All patients were treated according to standard BMT protocols after oral and written informed consent. Eligibility for BMT included adequate cardiac, pulmonary, hepatic and renal function prior to transplantation. Patients' characteristics are shown in Table 1. The patients' median age was 35 (18–53) years. Patients with acute myeloid leukemia (AML) or acute lymphoblastic leukemia (ALL) in first complete remission (1st CR), and chronic myeloid leukemia (CML) in first chronic phase (1st CP) were classified as early disease. The advanced disease group included patients with AML, ALL and CML beyond 1st CR/CP, myelodysplastic syndrome (MDS) and relapsed or refractory disease status. Thirty-one of 47 patients had advanced disease at the time of BMT. Donors were matched by serotyping for class I and high resolution SSOP DNA testing for class II. Forty-three patient/donor pairs were HLA-identical for HLA A, B, DR and DQ. Two patients had an HLA-A micromismatch, another two had an HLA-DR minor mismatch.

Table 1 Characteristics of patients with allogeneic volunteer unrelated donor BMT

Characteristics of patients	*Without rhG-CSF (n = 25)*	*With rhG-CSF (n = 22)*
Year of BMT		
1994	14	10
1995– March 1996	11	12
Sex (male/female)	9/16	13/9
Median age in years (range)	35 (22–53)	36 (18–49)
Diagnosis		
AML	9	6
CML	10	11
ALL	3	4
MDS	3	1
Remission		
Early disease: CR1/CP1	7	9
Advanced disease:		
CR2/CP2	5	1
AP/BC	3	4
no remission	10	8
CMV status (patient/donor)		
neg/neg	6	10
neg/pos	5	5
pos/neg	9	7
pos/pos	5	—
Sex mismatch		
male recipient/female donor	3	4[a]
Median no. of infused (range)		
Mononuclear cells × 10^8/kg	2.5 (1.2–4.25)	2.75 (1.53–7.5)
CFU-GM × 10^4/kg	10.6 (2.79–23.87)	11.15 (1.5–30.65)
Preparatory regimens		
BU/CY	11	13
TBI/CY	10	4
TBI/VP16/CY	4	5
GVHD prophylaxis		
CSP/MTX/PSE	23	20
CSP/MTX/PSE/ATG	2	2

Patient groups were comparable without significant differences regarding the parameters shown.
[a]Data of one patient were missing.

Conditioning regimens

Twenty-four patients received busulfan (4 mg/kg/day) on days −7 to −4, and cyclophosphamide (60 mg/kg/day) (CY) on days −3 and −2.[19–21] Seizure prophylaxis with phenytoin 4 × 200 mg p.o. was started on day −8 until day −3 and then reduced until day −1. Fourteen patients received fractionated total-body irradiation (TBI) 2 Gy twice a day on days −6 to −4 (12 Gy) and CY (60 mg/kg/day) on days −3 and −2. Nine patients received TBI (days −7 to −5), VP-16 (50 mg/kg; day −4), CY (60 mg/kg; day −2). Cystitis prophylaxis in patients receiving CY consisted of mesna (100 mg/kg/day, c.i.v.) on days −3 until −1 and forced diuresis.

Donors/Transplantation

Bone marrow harvesting was performed under general anesthesia using standard procedures. Immunophenotypic analysis by flow cytometry (FACScan analyser, Becton Dickinson, Heidelberg, Germany)[22] and progenitor cell assays were performed as described previously.[23] Bone marrow was reinfused without freezing on day 0, administered by central venous access. In cases of blood group incompatibility HAES separation was performed.[20]

Administration of granulocyte colony-stimulating factor (rhG-CSF)

During the study period all consecutive patients treated by allogeneic VUD-BMT as their first transplantation were included. Patients received rhG-CSF, filgrastim (Amgen, München, Germany) ($n = 22$), or did not receive rhG-CSF ($n = 25$) in a non-randomized manner, depending on the investigator's choice. RhG-CSF was applied from day +1 until an ANC >3000/μl at 5 μg/kg/day by continuous 24 h infusion. Table 1 summarizes the characteristics of the different patient groups. Patient groups with and without rhG-CSF were rather comparable with respect to baseline patient and transplant characteristics.

Supportive care

All patients were housed in single rooms conditioned with HEPA-filtered air. All received heparin 200 U/kg/day c.i.v., starting before conditioning until day +30 for prophylaxis of veno-occlusive disease.[20] Standard antibiotic prophylaxis consisted of trimethoprim-sulfamethoxazol (320 mg/1600 mg/day) (TMP-SMZ) until day −1, and fluconazol

(200 mg/day) or itraconazol (400 mg/day) until day +35. Colistin was administered beginning on day −1 until day +35. Acyclovir was given from day +1 to day +14. All patients received total parental nutrition (TPN) as long as clinically indicated. Empiric broad spectrum intravenous antibiotic treatment was started in the event of fever, positive blood cultures, invasive infection in an organ or fluid, or rapid increase of C-reactive protein according to standard procedures.[20] Immunoglobulin (10 g) was administered every 10 days from day −1 to +100. After engraftment, patients received TMP-SMZ (320 mg/1600 mg/day) twice weekly as prophylaxis for *Pneumocystis carinii* infection. In case of intolerance, pentamidine was used (monthly inhalations of 300 mg). All patients were monitored weekly for CMV infection with blood tests for CMV-PCR and CMV antigenemia. Patients with two consecutive positive results of CMV-PCR or detectable CMV antigen received pre-emptive therapy with gancyclovir or foscarnet, and immunoglobulin. Only CMV-negative, leukocyte-depleted and irradiated blood products were used.

GVHD prophylaxis

GVHD prophylaxis consisted of cyclosporine/methotrexate/prednisone (CSP/MTX/PSE) as previously described.[24] Anti T lymphocyte globulin (ATG, Fresenius, Bad Homburg, Germany) was added in four patients with minor HLA differences. In brief, CSP was started on day −3 with 2.5 mg/kg twice a day with a target trough level between 200–350 ng/ml as determined by a fluorescence polymerization immunoassay (Abbot, Wiesbaden, Germany). PSE was begun on day +7 (0.5 mg/kg/day), increased to 1 mg/kg on day +15 until day +29 when patients were gradually tapered off steroids. MTX (15 mg/m^2 i.v.) was given on day +1 and 10 mg/m^2 on days +3 and +6. ATG (30 mg/kg/day) was administered from day −3 to −1 (12-h infusion). Patients were graded for GVHD on a three times weekly basis using established criteria.[25]

Data handling

Granulocyte engraftment was defined as the first of 3 consecutive days with an ANC >500/μl and >1000/μl, respectively. The day of platelet engraftment was the day the platelet count exceeded 20 000/μl without platelet transfusions for at least 3 days thereafter. Platelets were transfused to achieve a platelet count >15 000/μl, packed red blood cells (RBC) were given to keep the hemoglobin >8.0 g/dl. Clinical variables including infections were analyzed during neutropenia, as well as during hospitalization, starting the day after BMT. Fever was classified according to the WHO classification (grade I: ⩽38.0°C, II: 38.1–40°C, III: >40°C for <24 h, IV: >40°C for >24 h duration, axillary). We evaluated the duration of febrile neutropenia, defined as number of days with both fever of ⩾WHO II and ANC <1000/μl, and total febrile days (⩾WHO II). Documented sepsis was defined in febrile patients as the occurrence of a single positive blood culture for a pathogeneic organism.[12] Patients with invasive infection in an organ or fluid were classified as having a clinically and/or microbiologically documented infection. Data were collected in a retrospective manner.

Statistical analysis

Primary endpoints were hematopoietic recovery (defined as time to ANC >500/μl and ANC >1000/μl, time to platelets >20 000/μl, number of platelets- and RBC-transfusions), incidence and severity of acute GVHD, incidence of cGVHD, and survival within 3 years post-transplant. Secondary endpoints were number and type of infectious complications, duration of TPN and hospitalization. Patient groups treated with G-CSF and patients treated without G-CSF were compared. Additionally, comparisons in time to hematopoietic recovery were made with respect to patient characteristics: year of transplantation (1994 *vs* 1995/1996), sex, age (⩽ median 35 years *vs* >median 35 years), disease status (early *vs* advanced), conditioning regimen (TBI *vs* no TBI), number of transplanted MNC (<median 2.54 × 10^8/kg *vs* ⩾median 2.54 × 10^8/kg), and the number of transplanted CFU-GM (<median 10.8 × 10^4/kg *vs* ⩾median 10.8 × 10^4/kg). The distribution of time to hematopoietic recovery, duration of hospitalization, onset of acute GVHD, and onset of chronic GVHD was estimated by cumulative incidence rates.[26] Survival distributions in patients treated with and without G-CSF were estimated by the Kaplan–Meier method and 95% confidence intervals were calculated at 2 years post-transplant. Differences between groups with respect to time-to-event data were tested by the logrank test. Differences between groups with respect to the number of platelets- and RBC-transfusions were tested by means of Wilcoxon rank-sum test. Differences with respect to the incidence of aGVHD ⩾grade II was tested by Fisher's exact test. A two-sided P value of ⩽0.05 was considered to indicate statistical significance. No adjustment for multiple testing was performed.

Results

Transplantation

In patients receiving rhG-CSF post-transplantation (n = 22), the median transplanted nucleated cell (MNC) count was 2.75 × 10^8/kg (1.53–7.45), with a median of 11.15 × 10^4/kg (1.5–30.65) infused CFU-GM. A median of 1.85 × 10^6/kg CD34$^+$ cells (0.04–6.4, n = 12) was infused. Bone marrow cultures showed a median growth of 85 (8–268) CFU-GM/2 × 10^5 cells after 14 days. Patients without rhG-CSF (n = 25) received a median of 2.5 × 10^8/kg MNC (1.2–4.25), 10.6 × 10^4/kg CFU-GM (2.79–23.87), and 1.3 × 10^6/kg CD34$^+$ cells (0.14–6.8, n = 8). Bone marrow cultures showed a median growth of 86 (22–214) CFU-GM/2 × 10^5 cells after 14 days. The difference was not significant.

Hematological recovery

The results of hematopoietic recovery are shown in Table 2. Forty-four of 47 patients (94%) achieved complete neu-

Table 2 Hematopoietic recovery following volunteer unrelated donor BMT

Variables	*Without rhG-CSF (n = 25)*	*With rhG-CSF (n = 22)*	*P value*
Median days to (range)			
ANC $>500/\mu$l	16 (11–30)	14 (10–22)	0.048
ANC $>1000/\mu$l	18 (13–32)	15 (12–29)	0.084
Plts $>20\,000/\mu$l	24 (10–40)	25 (13–150)	0.62
Median no. units (range)			
Plts transfusions	69 (15–242)	102 (33–300)	0.12
RBC transfusions	10 (0–73)	13 (1–56)	0.18

ANC = absolute neutrophil count; plts = platelets; RBC = red blood cells; DFS = disease-free survival.

trophil engraftment. Primary engraftment failure occurred in two patients receiving HLA-matched BM in blast crisis of CML (G-CSF: $n = 1$, no G-CSF: $n = 1$). In both cases, pretransplant bone marrow biopsy revealed extensive marrow fibrosis. In one of them, a second bone marrow infusion from the same donor without further conditioning was given, but again no engraftment was achieved. The patient died due to invasive aspergillosis (day +65). The other patient received rhG-CSF-mobilized PBSC after leukapheresis twice from her haplo-identical son, but did not engraft and died due to pneumonia (day +122). Another patient, suffering from MDS (RA), developed secondary graft failure after VUD-BMT (with G-CSF), which did not resolve despite intensive treatment with hematopoietic growth factors (G-CSF, GM-CSF, IL-3). He died due to invasive aspergillosis (day +57).

Neutrophil recovery was slightly accelerated in patients receiving rhG-CSF (Table 2, Figure 1). Median time to ANC $>500/\mu$l was 14 days (10–22) with rhG-CSF *vs* 16 days (11–30) without rhG-CSF ($P = 0.048$). The median time to ANC $>1000/\mu$l was 15 days (12–29) *vs* 18 days (13–32), but the difference was not statistically significant ($P = 0.084$). The recovery of platelets, as well as the median number of platelets- and RBC-transfusions were not significantly influenced by the administration of rhG-CSF.

Additionally, we compared patient groups defined by characteristics detailed in Table 1 with respect to the speed of hematopoietic recovery. The only relevant difference was with respect to the number of MNC infused. Time to ANC $>1000/\mu$l was significantly shorter in patients receiving a MNC dose of $\geqslant 2.54 \times 10^8$/kg with a median time of 13 days compared with 19 days in patients receiving less than the median MNC dose of 2.54×10^8/kg ($P = 0.017$) (Figure 2). Moreover, platelet recovery to $>20\,000/\mu$l tended to be shorter in patients receiving $\geqslant 2.54 \times 10^8$/kg MNC with a median time of 21 days *vs* 27 days ($P = 0.055$) (Figure 2). The question arises whether this observation was caused by imbalances of other factors in patients receiving more than and less than the median MNC dose. But the groups were rather balanced with respect to the other factors listed in Table 1. In particular, there was also no large imbalance with respect to the administration of G-CSF (50% in patients with $\geqslant 2.54 \times 10^8$/kg MNC and 43% in partients receiving $<2.54 \times 10^8$/kg MNC).

Infectious complications

As summarized in Table 3, the application of rhG-CSF did not influence the duration of febrile episodes, number of patients with at least 1 day of febrile neutropenia and use of parenteral antibiotics during hospitalization. Moreover, no difference was found in the incidence of FUO, documented infection and septicemia, as well as fatal infectious complications with respect to the rhG-CSF application. Five patients with rhG-CSF died during hospitalization due to infectious complications: two patients with BM failure due to invasive aspergillosis (day +57) or pneumonia (day +122), and another three patients after engraftment due to aspergillosis (day +44, +109) and pneumonia (day +182). In two patients without rhG-CSF, aspergillosis was fatal during neutropenia (day +17, +65). After engraftment one patient with TMP/SMZ intolerance receiving pentamidine died due to toxoplasmosis (day +51), another patient died due to CMV infection (day +123).

Graft-versus-host disease (GVHD)

Table 4 summarizes incidence and onset of acute GVHD in patients with and without rhG-CSF. The patient groups were comparable with respect to distribution of age, disease status, frequency of 'male recepient/female donor'-sex mismatch, and GVHD prophylaxis (Table 1). HLA minor differences were observed in two patients each of the G-CSF group (HLA-A micromismatch), and the group without G-CSF (HLA-DR minor mismatch).

Comparing patients with and without rhG-CSF, we observed similar overall incidences of acute GVHD I–IV, but a non-significant increase of clinical relevant acute GVHD II–IV in the G-CSF group. The overall incidence of acute GVHD I–IV was 77% in patients with rhG-CSF (17 of 22 patients) and 60% in the group without rh G-CSF (15 of 25 patients). In contrast, acute GVHD II–IV occurred in 11 of 22 patients (50%) with rhG-CSF, and in seven of 25 patients (28%) without rhG-CSF, but due to the small number of patients the difference was not statistically significant ($P = 0.14$). The event competing to the event acute GVHD II–IV is death before its occurrence within the first 100 days. This was observed in 9% (two of 22 patients) treated with rhG-CSF and in 20% (five of 25 patients) not treated with rhG-CSF. Mortality from acute

Table 3 Infectious complications following volunteer unrelated donor BMT

Variables	*Without rhG-CSF (n = 25)*[a]	*With rhG-CSF (n = 22)*
Median days of (range)		
Neutropenic fever	3 (0–8)	3 (0–13)
Fever (total)	3 (0–9)	5 (0–16)
Parenteral antibiotics	25 (6–62)	19 (10–124)
Parenteral Amphotericin-B	4 (0–22)	8 (0–141)
Parenteral nutrition	31 (17–65)	30 (17–137)
In hospital[b]	56 (17–124)	52 (33–138)
No. of patients with fever (WHO)[c]		
Grade I	3	4
Grade II	21	18
Grade III	0	0
Grade IV	0	0
Infectious events during neutropenia (after engraftment until discharge)		
No. of patients with FUO	9 (2)	4 (3)
No. of septicemic episodes[d]	4 (2) 13 (11)	5 (2) 20 (13)
No. of documented infections[e]		
Urinary tract infection	9 (4)	9 (6)
Infection of central catheter	1 (2) 0 (0)	1 (1) 1 (1)
Herpes simplex virus	1 (2)	3 (2)
Respiratory tract infections		
GI tract infections	2 (3)	6 (3)
No. of fatal infectious complications	2[f](2)[g]	2[h](3)[i]

FUO = fever of unknown origin.
[a]Data concerning infectious complications were available in 24 out of 25 patients only.
[b]Median was estimated from cumulative incidence rates.
[c]Fever was classified according to the WHO classification (grade I: ≤38.0°C, II: 38.1–40°C, III: >40°C for <24 h, IV: >40°C for >24 h duration, axillary).
[d]Documented sepsis: occurrence of a single positive blood culture for a pathogeneic organism in a febrile patient (>38°C).
[e]Clinically and/or microbiologically documented infection: invasive infection in an organ, an otherwise sterile specimen of tissue or fluid, cellulitis, and catheter site infections (exit site or within the tunnel track).
[f]Aspergillosis ($n = 2$).
[g]Aspergillosis ($n = 1$), pneumonia ($n = 1$).
[h]Toxoplasmosis, CMV infection.
[i]Aspergillosis ($n = 2$), pneumonia ($n = 1$).

GVHD was not increased in patients receiving rhG-CSF. Whereas four of 22 patients (18%) with rhG-CSF died due to refractory acute GVHD (day +85, +132, +182, +262), acute GVHD was fatal in three of 25 patients (13%) without rhG-CSF (day +44, +57, +80).

The rate of chronic GVHD within 3 years in patients surviving the first 100 days post-transplant was 71% in 18 patients with rhG-CSF, and 50% in 16 patients without rhG-CSF. The rate of the competing event death before occurrence of cGVHD was 17% in patients with G-CSF and 44% in patients without rhG-CSF.

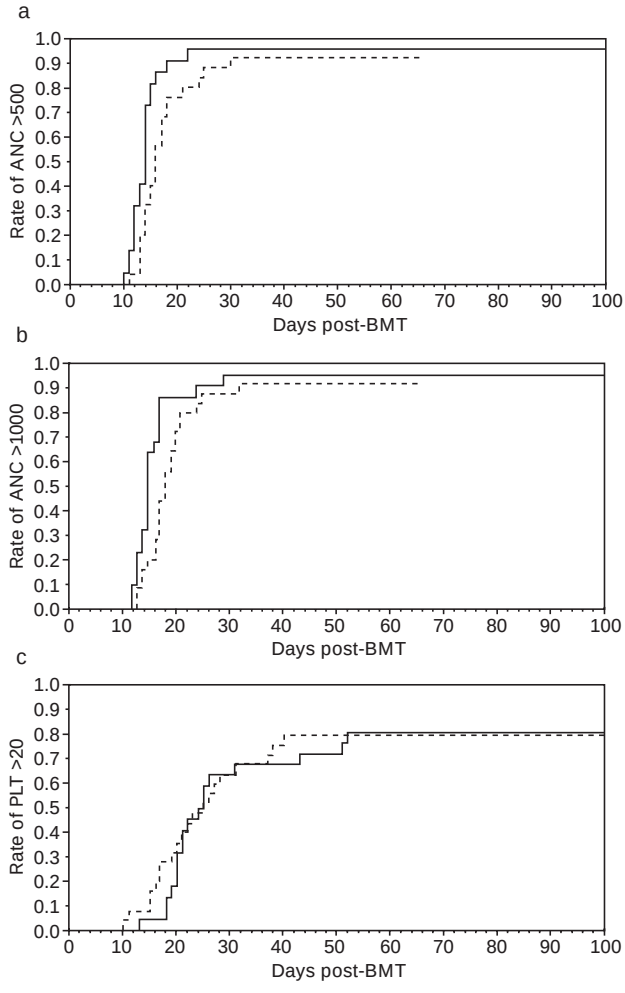

Figure 1 Probability of reaching (**a**) ANC $>0.5 \times 10^9$/l ($P = 0.048$); (**b**) 1.0×10^9/l ($P = 0.084$); (**c**) platelet count $>20 \times 10^9$/l ($P = 0.62$) in patients receiving (——) or not receiving (---) rhG-CSF.

Patients' outcome/Survival

The median follow-up time was 38 months for the rhG-CSF group and 36 months for the group without rhG-CSF. Minimum follow-up of patients alive was 2 years, except for one patient in the G-CSF group who was lost to follow-up at 5 months post-transplant. Figure 3 shows the survival rate of patients treated with G-CSF and patients treated without G-CSF. The 2-year survival rate was 39% (95% confidence interval (18%,60%)) in the G-CSF group and 24% in the group without G-CSF (95% confidence interval (7%,41%)). The observed difference in survival is not significant and has to be interpreted with caution because treatment was assigned in a non-randomized manner and patient numbers are small.

Four of 22 patients (18%) receiving rhG-CSF died up to day +100 due to aspergillosis ($n = 2$), GVHD ($n = 1$), or graft failure ($n = 1$). In the group without G-CSF, nine of 25 patients (36%) died up to day +100: causes of death were ARDS ($n = 2$), GVHD ($n = 3$), and in each one graft failure, aspergillosis, toxoplasmosis, and relapse. In the G-CSF group, nine patients died beyond day +100 due to relapse ($n = 3$), infection ($n = 2$), GVHD ($n = 3$) or graft

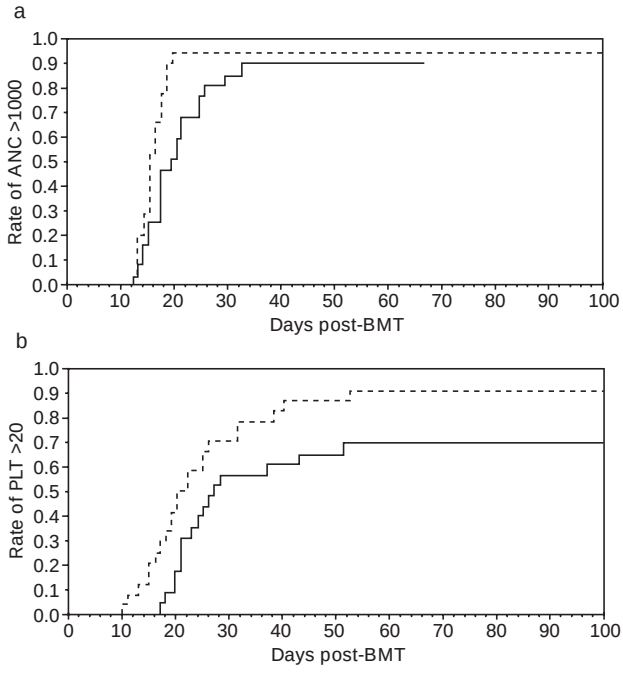

Figure 2 Probability of reaching (**a**) ANC $>1.0 \times 10^9$/l ($P = 0.017$) (**b**) platelet count $>20 \times 10^9$/l ($P = 0.055$) in patients receiving less (—) or more (---) than the median MNC dose of 2.54×10^8/kg.

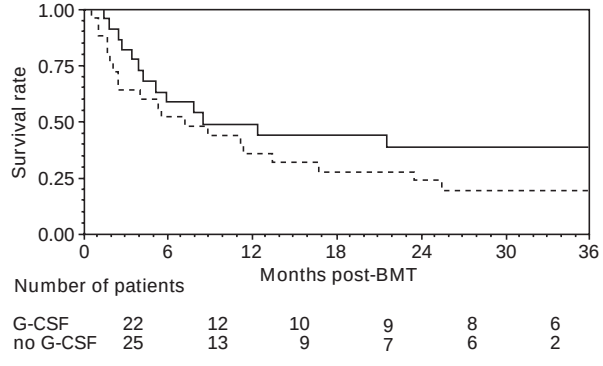

Figure 3 Probability of survival of patients undergoing volunteer unrelated donor BMT with (—) or without (---) rhG-CSF ($P = 0.20$).

failure ($n = 1$) and in the group without G-CSF 12 patients died beyond day +100 because of relapse ($n = 6$), infection ($n = 3$) and one each ARDS, hemolytic uremic syndrome (HUS) and unclear sudden death at home 1245 days after bone marrow transplantation.

Discussion

Hematopoietic growth factors have been increasingly used following allogeneic BMT or PBSCT in an attempt to accelerate myeloid recovery and reduce the length of the high-risk period of bone marrow aplasia.[4–18]

We observed an accelerated neutrophil recovery in patients receiving rhG-CSF as compared to patients without rhG-CSF. Administration of rhG-CSF did not influence platelet recovery. Several former non-randomized and randomized trials in matched related BMT demonstrated, that G-CSF significantly shortens neutropenia, but does not affect platelet recovery.[4,9–14] Until now, few data analyzing the use of hematopoietic growth factors following VUD-BMT have been published.[4,5,14–18] Several trials using GM-CSF in unrelated BM recipients have shown a significantly accelerated neutrophil recovery in patients treated with GM-CSF.[4,5,16–18] In contrast, only one retrospective analysis concerning the use of G-CSF following VUD-BMT in adults has been performed until now.[14] In that trial, the influence of G-CSF on hematopoietic recovery and clinical outcome in 30 patients undergoing related BMT and 20 patients undergoing VUD-BMT have been evaluated. However, in contrast to the data of related BM recipients, the results of hematopoietic recovery and outcome following VUD-BMT have not been compared with historical controls. Therefore, the less pronounced effect of G-CSF on neutrophil recovery after VUD-BMT, observed in our analysis, has to be proved in further randomized trials.

The influence of the transplanted progenitor cell count on hematopoietic recovery has been examined in detail, but the results remain controversial.[27–31] We observed a significantly accelerated neutrophil recovery in patients receiving more than the median MNC dose of 2.54×10^8/kg, as well as a nonsignificant trend to faster platelet engraftment. Most previous trials concerning matched related BM recipients, failed to detect any association between the number of infused MNCs, CFU-GM, or $CD34^+$ cells and

Table 4 Acute GVHD following volunteer unrelated donor BMT

Variables	*Without rhG-CSF (n = 25)*	*With rhG-CSF (n = 22)*	*P value*
Acute GVHD No. of pts			
Grade 0	10	5	0.14[a]
Grade I	8	6	
Grade II	2	3	
Grade III	2	6	
Grade IV	3	2	
Median onset of acute GVHD			
Grade I–IV days (range)	35 (10–100)	35 (10–100)	0.55

[a]Test comparing grade <II *vs* ⩾II.

engraftment.[12,31] However, although our findings could also be explained as random occurrence, patients receiving VUD-BMT using methotrexate-based regimens may require higher doses of MNC for optimal neutrophil engraftment.[31]

We did not observe any differences between the treatment groups with respect to the incidence of infectious episodes, use of intravenous antibiotic therapy and time spent in hospital. Moreover, the use of G-CSF did not reduce the incidence and severity of clinical relevant infections during neutropenia or hospitalization. Although several trials have been performed analyzing the influence of hematopoietic growth factors on the incidence and severity of infectious complications following BMT, the results remained controversial.[4,5,9–15] For example, in one recently published randomized, placebo-controlled study analyzing the use of G-CSF following autologous and allogeneic sibling donor BMT, a significant reduction in days of infection, antibiotic application, or hospital stay was detected.[12] However, those effects were not accompanied by a decreased number of patients with at least 1 febrile day, number of clinically relevant infections or an increased survival. Despite the beneficial effects of G-CSF in reducing the duration of neutropenia, it is unlikely until now, that administration of G-CSF efficiently decreases infectious complications following allogeneic BMT.

Our results showed a non-significantly increased incidence of clinically relevant acute GVHD in patients receiving rhG-CSF compared with patients not receiving rhG-CSF (50% *vs* 28%). However, there was no difference in overall incidence of acute GVHD I–IV or mortality due to acute GVHD. In several trials published previously, the use of G-CSF did not affect incidence and severity of GVHD.[4,5,9–15] The increase of clinically relevant acute GVHD observed in our analysis is of concern, but due to the relatively small number of patients in the different risk groups and the retrospective design, it is unlikely that rhG-CSF contributes to the nonsignificant increase of acute GVHD. No significant differences were observed in the incidence and severity of chronic GVHD between the two groups.

Another major concern in the use of hematopoietic growth factors was a possible increase in the incidence of leukemia relapse, particularly in patients treated for myeloid neoplasms, where G-CSF may increase the proliferation of leukemic cells that express G-CSF receptors.[32–34] None of the trials published up to now, have shown an increased relapse rate in patients treated with G-CSF post-transplantation.[4,9–15,33,34] This is in line with our data and we observed relapse in three of 22 patients receiving rhG-CSF and in seven of 25 patients without rhG-CSF.

In two recently published non-randomized trials, a higher than expected early mortality rate in patients receiving hematopoietic growth factors after VUD-BMT was observed.[14,18] We have not been able to confirm this observation. In contrast, we found slightly higher survival rates in patients undergoing VUD-BMT receiving rhG-CSF. Similar results have been observed in previous trials using GM-CSF after VUD-BMT, demonstrating no increase in mortality or relapse rates in patients receiving GM-CSF compared with the controls.[4,16,17]

Our analysis was designed to examine the role of rhG-CSF on hematopoietic recovery following VUD-BMT and to discern whether rhG-CSF could have a significant impact on complications associated with the marrow transplant procedure during the critical first 3 months. Our data indicate that rhG-CSF may be useful to accelerate neutrophil recovery following VUD-BMT. No negative side effects were observed, above all no delayed platelet engraftment, and no increased incidence of relapse. Although we observed a nonsignificant trend to clinically relevant acute GVHD II–IV, overall incidence of acute GVHD I–IV, and mortality from acute GVHD were not increased. On the other hand, with the limited number of patients in each group we could not detect any obvious clinical benefit, particularly concerning infectious complications and early morbidity post-transplantation. However, a future prospective trial appears to be worthwhile to evaluate a possible positive benefit on long-term survival of rhG-CSF post-VUD transplantation. The individual patient may benefit from more rapid hematopoietic recovery without suffering from negative side-effects. The effects of G-CSF on T lymphocyte alloreactivity has become of interest recently in the context of allogeneic peripheral blood progenitor cell transplantation. A possible influence of rhG-CSF on acute and chronic GVHD or relapsing disease can only be detected with statistical certainty in a large randomized trial including homogenous patient groups with regard to disease and remission pre-BMT.

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