Single-dose pegfilgrastim for the mobilization of allogeneic CD34+ peripheral blood progenitor cells in healthy family and unrelated donors

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Background and Objectives. Short-term treatment with granulocyte colony-stimulating factor (G-CSF) has been established as the standard regimen for mobilizing allogeneic peripheral blood progenitor cells (PBPC) from healthy donors. The pegylated form of filgrastim (pegfilgrastim) has a longer elimination half-life because of decreased serum clearance and might be a convenient alternative for stem cell mobilization.

Design and Methods. Twenty-five family (n=15) or unrelated (n=10) healthy donors received a single-dose of 12 mg pegfilgrastim for mobilization of allogeneic PBPC. Donors with inadequate mobilization (blood CD34+ cells ≤5/μL on day 3 or ≤20/μL on day 4) were given additional daily doses of 10 μg/kg conventional filgrastim. Leukapheresis was planned to start on day 5.

Results. All harvests were completed successfully. In 20 out of 25 donors (80 %) only a single apheresis was necessary. Additional non-pegylated filgrastim had to be given to only one 74-year old family donor. The maximum concentration of circulating CD34+ cells occurred on day 5 (median 67/μL, range 10-385/μL). The median yield of CD34+ cells was 9.3 (range 3.2-39.1)×10^6/kg of the recipient´s body weight. The median number of T cells in the apheresis products was 3.9 (range 2.7-10.8)×10^8/kg. Bone pain, headaches and transient elevations of alkaline phosphatase and lactate dehydrogenase were the main adverse events.

Interpretation and Conclusions. The study shows that collection of allogeneic PBPC after administration of a single dose of pegfilgrastim is feasible. The toxicity profile, graft composition and impact on the recipients´ outcome need further investigation.

Kwy words: pegfilgrastim, stem cell mobilization, allogeneic donors.

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Short-term treatment with recombinant human granulocyte colony-stimulating factor (rhG-CSF) followed by leukapheresis is the common procedure used to obtain CD34+ peripheral blood progenitor cells (PBPC) from allogeneic donors. In some studies addressing the optimal dosage and schedule of rhG-CSF, a higher stem cell yield was found after twice daily administration than after single-dose treatment during steady-state hematopoiesis in patients as well as in allogeneic donors.[1-4] An intra-individual crossover study in four healthy volunteers showed higher minimum G-CSF serum levels after twice daily doses than after once daily doses and an increased CD34+ cell count in the peripheral blood on day 4, which is indicative of an association between G-CSF trough blood levels and mobilization efficacy.[5] This hypothesis has also been supported by the observation of a sufficient and rapid increase of CD34+ cells during continuous subcutaneous infusion of G-CSF (72 μg/day) for 5 days in a small group of healthy volunteers.[6] Pegfilgrastim (Neulasta™, Amgen Inc., Thousand Oaks, USA) is a covalently bound conjugate of filgrastim and monomethoxypolyethylene glycol. It has a longer elimination half-life than the unconjugated filgastrim because of decreased serum clearance.[7] After chemotherapy a single injection of pegfilgrastim is equivalent to daily filgrastim in enhancing neutrophil recovery.[8,9] Pegfilgrastim has also been shown to induce CD34+ progenitor cell mobilization in animal models,[10] preliminary human studies,[11,12] as well as in patients with lymphoproliferative malignancies, who received a single pegfilgrastim injection as an adjunct to chemotherapy.[13-15]

Given the possible schedule-dependent mobilization efficacy of non-pegylated rhG-CSF, pegfilgrastim could be an interesting choice for mobilizing PBPC since its specific pharmacokinetic properties could make it superior to conventional, non-

pegylated G-CSF in terms of mobilizing stem cells into the peripheral blood. Furthermore, the more convenient once-per-mobilization dose is an attractive manner of administration, particularly for allogeneic PBPC donors.

Design and Methods

Growth factor treatment, leukaphereses and donor monitoring

Between July 2003 and November 2004 15 family and 10 unrelated volunteer donors were treated with a single dose of pegfilgrastim (Neulasta™; Amgen, Thousand Oaks, CA, USA) before leukapheresis for collection of PBPC for allogeneic transplantation. There were 9 female and 16 male donors and their median age was 43 years (range 28-74). All donors underwent a pre-donation health check-up including medical history, physical examination, abdominal ultrasound, electrocardiography, full blood count, blood chemistry analysis and a search for infectious disease markers.

For mobilization the donors received a single subcutaneous injection of 12 mg pegfilgrastim in the morning of day 1. According to the study protocol, if four consecutive donors failed to produce a sufficient yield of $CD34^+$ progenitor cells the dose of growth factor was to be increased to 18 mg pegfilgrastim for the subsequent participants. If the peripheral blood $CD34^+$ cell concentration was found to be ≤5/μL on day 3 or ≤20/μL on day 4, the donors were to be treated with additional non-pegylated filgrastim at a dose of 2 × 5 μg/kg (Neupogen™; Amgen, Thousand Oaks, CA, USA).

Leukapheresis was planned to start on day 5 of the mobilization regimen, although apheresis could be performed optionally on day 4 in donors with white cell counts exceeding 70×10^9/L to prevent complications of hyperviscosity. The harvests were performed as large-volume, continuous-flow collections using a Cobe Spectra blood cell separator (software version 7.0, Gambro BCT Inc., USA) through bilateral peripheral venous accesses. During the first apheresis about four times the donor´s blood volume was processed at a rate of 50 to 120 mL/min. Anticoagulation was achieved with ACD-A (ratio 1:12-20) and heparin (5,000 IU). A second collection on day 6 was optional and depended on the yield of $CD34^+$ progenitor cells obtained during the first procedure. The duration of the second leukapheresis was limited to 3 hours. The target PBPC dose to be collected was decided by the individual transplant centers on the basis of the recipient's body weight.

From day 1 to day 6 the donors were seen daily by a physician and blood samples were taken so that the full blood cell count could be monitored (Sysmex XE-2100, Sysmex Corporation Ltd., Kobe, Japan). The number of peripheral blood $CD34^+$ cells was determined by flow cytometry on days 3 to 6. Blood chemistry analyses were repeated on days 5 and 30. At the first leukapheresis, donors were asked to describe the side effects of growth factor treatment especially the severity of bone pain and headache and the need for analgesic medication using a standardized questionnaire. According to the WHO toxicity criteria the severity of the maximum pain was classified by the donors on a scale from 0 to 4 (in which 0 denotes no pain, 1-mild pain, 2-moderate pain, 3-severe pain, 4-very severe pain). After completion of the leukaphereses the donors were monitored, depending on their willingness, with at least one clinical and blood count examination per week until day 30.

The protocols for pegfilgrastim mobilization and leukapheresis were approved by the Institutional Review Board of the University Hospital Dresden, Germany.

Harvest cell counts and flow cytometric evaluations

All leukapheresis products were analyzed for total nuclear and mononuclear cell counts (Sysmex XE-2100, Sysmex, Norderstedt, Germany). $CD34^+$ cells in peripheral blood and apheresis samples were quantified using a dual platform assay combining flow cytometry (FACS Calibur, BD Sciences, Heidelberg, Germany) and leukocyte counting. Two-color immunophenotyping was performed with the CD34PE/CD45FITC reagent (8G12/2D1, BD Sciences, Heidelberg, Germany). The samples were incubated with the antibody combination mentioned above for 15 min at room temperature and then the red cell were lysed for 10 min at room temperature with FACS lysing solution (BD Sciences, Heidelberg, Germany). After a washing step cells were resuspended in 500 μL phosphate-buffered saline (Seromed, Berlin, Germany). The measurement and analysis procedures were carried out according to previously described guidelines.[16] The laboratory has successfully participated in annual interlaboratory quality control tests.

Statistical analysis

The data were managed using Microsoft Excel and SPSS for Windows software. Descriptive quantitative parameters of clinical characteristics and results are presented as medians with ranges. The relationship between peripheral blood $CD34^+$ cell count and PBPC yield was analyzed by linear regression and correlation analysis.

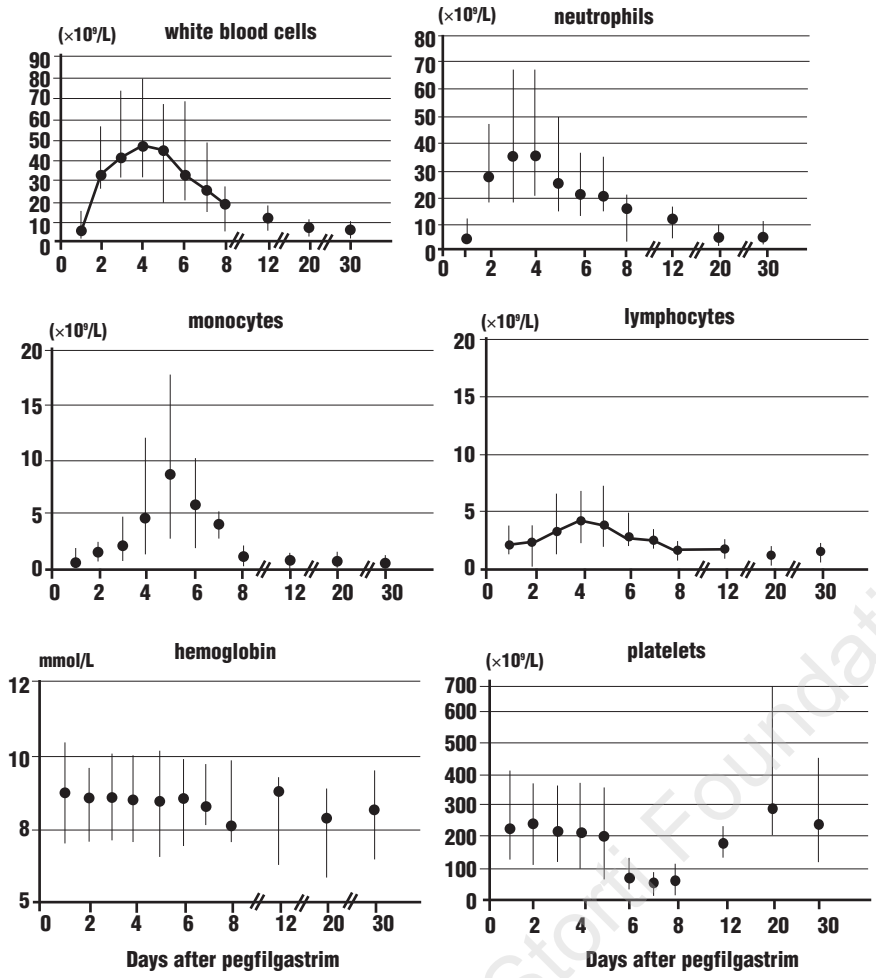

Figure 1. Course of peripheral blood leukocytes after subcutaneous administration of pegfilgrastim (median dose 161 μg/kg, range 126-203) to healthy allogeneic stem cell donors in steady state hematopoiesis. Leukapheresis was performed on day 5.

Results

Blood cell counts and safety

The median dose of pegfilgrastim administered was 161 μg/ kg (range 126-203 μg/ kg). The total dose of growth factor was reduced to 9 mg for two female donors because they weighed less than 60 kg, and increased to 15 mg in one male donor who weighed more than 100 kg. Within 24 hours after pegfilgrastim administration an increase in all peripheral leukocyte subsets was observed (Figure 1). The maximum white cell counts were a median of 7-fold (range 4 to 14-fold) higher than the baseline values and occurred in 13 donors (52%) on day 4. The maximum numbers of neutrophils, monocytes and lymphocytes were increased a median 10-fold, 21-fold and 2.5-fold, respectively. The highest leukocyte count observed in our series of donors was 82×10^9/L and occurred in a 40-year old unrelated female donor (body weight 72 kg, pegfilgrastim dose 167 μg/kg) on day 4. Leukapheresis was not started prematurely but as scheduled on day 5 because she was completely well and free from symptoms of hyperleukocytosis. After completion of stem cell collections the number of leukocytes declined rapidly. White cell counts at the upper normal limit and neutrophilia were found in all examined donors on day 12 (n=5), whereas completely normalized blood cell counts were found on days 20 (n=6) and 34 (n=13).

Toxicity of cytokine treatment

Bone pain and headaches were the main adverse effects of growth factor administration. These events occurred in 76% and 44% of donors, respectively. Clinical toxicities are summarized in Table 1. Twenty-two out of 25 (88%) donors required pain medication with oral paracetamol at a median daily dose of 1 g (range 0-4 g/day). None of the donors required opioid analgesics. Pegfilgrastim caused mean 4-fold increases of both alkaline phosphatase and lactate dehydrogenase and increases were observed in all donors. A mean 2-fold increase of aspartate aminotransferase occurred in five donors (24%), whereas a mean 3-fold elevation of alanine aminotransferase was seen in six cases (29%). All elevated parameters had returned to baseline values at the re-evaluations 4 weeks after collection.

Table 1. Clinical toxicities after administration of a single-dose of pegfilgrastim (median dose 161 μg/kg, range 126-203) in 25 allogeneic stem cell donors.

Adverse event	*Intensity*	*N (%)*
Bone pain	Total	19 (76)
	Mild	1 (4)
	Moderate	8 (32)
	Severe	10 (40)
Headache	Total	11 (44)
	Mild	2 (8)
	Moderate	5 (20)
	Severe	4 (16)
Local reaction at the injection site	Total	7 (28)
	Mild	5 (20)
	Moderate	2 (8)
Other complaints	Total	13 (52)
	Insomnia	5 (20)
	Tiredness	5 (20)
	Sweating	2 (8)
	Hypotension	1 (4)

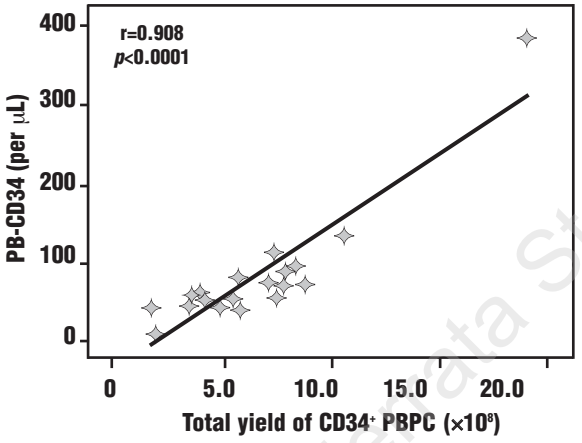

Figure 3. Correlation between blood CD34⁺ (PB-CD34) cell count and yield of CD34⁺ PBPC in donors mobilized with single-dose pegfilgrastim.

Mobilization efficacy and stem cell yield

The stem cell collections were successfully completed in all donors. In 20 out of 25 donors (80 %) sufficient PBPC yields were achieved by a single apheresis. The maximum number of circulating CD34⁺ cells was found on day 5 (median 67/μL, range 10-385/μL). CD34⁺ cell counts on day 4 (median 61/ μL, range 4-135/ μL) ranged below the values on day 5 but were higher than those on day 6 (median 30/μL, range 9-180/ μL) (Figure 2). Additional doses of non-pegylated filgrastim had to be given to only one 74-year old family donor, who had a peripheral blood CD34⁺ cell count of 4/μL on day 3.

The characteristics of leukapheresis products are presented in detail in Table 2. There was a significant correlation between the concentration of blood CD34⁺ cells in the morning before leukapheresis and the number of stem cells harvested (Figure 3). Given the substantial increase of monocytes in the periph-

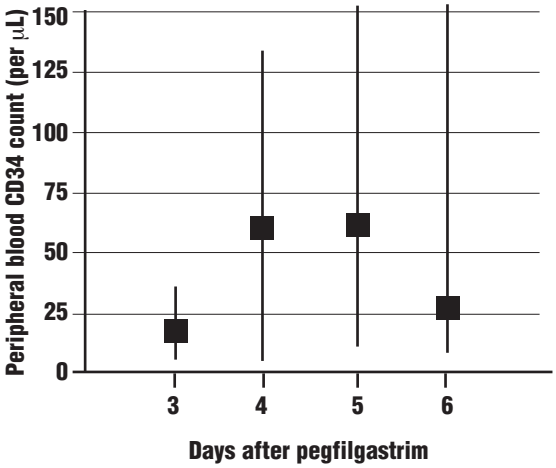

Figure 2. Concentration of CD34⁺ cells in the peripheral blood after a single dose of pegfilgrastim (median dose 161 μg/kg, range 126-203) in allogeneic stem cell donors.

Table 2. Characteristics of the leukapheresis products harvested after administration of a single-dose of pegfilgrastim (median dose 161 μg/kg, range 126-203) to 25 allogeneic stem cell donors. Leukaphereses were performed on day 5 (1st leukapheresis) and 6 (2nd leukapheresis).

	First leukapheresis (n=25)	*Second leukapheresis (n=5)*	*Total*
Total number of nuclear cells median (range) [×10⁸]	1033.0 (630.4-1811.7)	562.3 (443.3-1083.5)	1115.0 (630.4-2068.5)
Total number of mononuclear cells median (range) [×10⁸]	822.8 (530.5-1427.6)	430.7 (363.4-631.7)	913.4 (530.5-1650.5)
Total number of CD34+ cells median (range) [×10⁸]	5.47 (1.10-19.06)	1.48 (0.80-2.40)	5.82 (1.72-19.06)
Yield of CD34+ cells/kg body weight of the donor median (range) [×10⁶/kg]	7.28 (1.37-29.32)	2.05 (1.00-3.69)	7.50 (2.37-29.32)
Yield of CD34⁺ cells/kg body weight of the recipient median (range) [×10⁶/kg]	9.30 (1.86-39.71)	2.11 (1.24-4.62)	9.30 (3.22-39.71)
Proportion of CD3⁺ cells median (range) [%]	24.9 (12.2-39.7)	19.2 (10.5-20.6)	
Number of CD3⁺ cells/kg body weight of the recipient median (range) [×10⁸/kg]	3.7 (1.6-10.8)	1.9 (1.1-2.2)	3.9 (2.7-10.8)

LPH: leukapheresis.

eral blood after pegfilgrastim administration we also analyzed the monocyte content of the leukapheresis products. The median proportions of monocytes in the first and second harvests were 41.2% (range 26.8–53.5%) and 37.2% (range 31.8–44.2%), respectively. Converted to absolute numbers the leukapheresis products contained a median of 488×10^8 monocytes (range 175-817×10^8).

Engraftment and graft-versus-host disease

Engraftment data are available for 23 patients. Stable engraftment of neutrophils occurred in 20 patients, whereas platelet reconstitution and independency from transfusions was achieved in 19 cases. The median time intervals to reach absolute neutrophil counts $>0.5\times10^9$/L for three consecutive days and unsupported platelet counts $>50\times10^9$/L were 17 days (range 9–24 days) and 18 days (range 11–21 days), respectively. The patients who failed to achieve hematopoietic reconstitution suffered from advanced malignancies (accelerated chronic myeloid leukemia, n=1; second relapse of acute myeloid leukemia, n=1; early relapse of acute myeloid leukemia with mutated *FLT3-ITD*, n=1) and/or were transplanted from HLA-haploidentical related donors (n=2). Eight out of the 16 patients (50%) who are evaluable for graft-versus-host disease (GVHD) have symptoms of acute GVHD.

Discussion

The study was designed to evaluate the appropriate dose, safety and feasibility of stem cell mobilization with pegfilgrastim administered in a single dose to allogeneic donors. A dose of 6 mg pegfilgrastim has been shown to be equivalent to daily injections of 5 μg/kg filgrastim in chemotherapy-induced neutropenia.[8,9] The efficacy of 6 mg[13,14] or 12 mg[15] pegfilgrastim in patients who had received chemotherapy was comparable to that of conventional, non-pegylated G-CSF in mobilizing autologous PBPC.

Allogeneic donors are usually treated with 10-20 μg/kg of non-pegylated filgrastim for 5 days. Based on such experience we decided to evaluate a single dose of 12 mg pegfilgrastim in allogeneic PBPC mobilization, resulting in body weight-related doses between 200 μg/kg (for a donor of 60 kg body weight) and 120 μg/kg (for a donor of 100 kg body weight). In healthy volunteers the administration of 100 or 300 μg/kg pegfilgrastim has been shown to induce a sufficient increase of $CD34^+$ cells in the peripheral blood[12] with kinetics similar to that of conventional G-CSF. In our series only one 74-year old family donor had to receive additional growth-factor treatment because of small numbers of circulating $CD34^+$ cells, whereas single-dose pegfilgrastim was able to induce an adequate stem cell mobilization for harvesting sufficient numbers of PBPC in the remaining 24 donors. The peak values of blood $CD34^+$ cells and yields of $CD34^+$ cells were not different from those obtained after daily administration of conventional G-CSF,[3,17,18,19] so the results are not indicative of a superior mobilization efficacy of pegfilgrastim in this setting. A direct comparison of the doses of pegylated and non-pegylated filgrastim might be inappropriate, because a continuously raised G-CSF level is suspected to exert a greater biological activity than pulsatile elevations after intermittent injections of unconjugated filgrastim. However, the total cytokine dose in our series was 12,000 μg, which is much higher than the total filgrastim dose in a donor weighing 100 kg who is treated with 10 μg/kg daily for five days.

The different kinetics of circulating $CD34^+$ cells observed in our study could have consequences for the scheduling of leukapheresis procedures. Whereas with conventional G-SCF the peak numbers of peripheral blood $CD34^+$ cells occur in the sequence day 5, day 6, day 4, the maximum concentration after pegfilgrastim administration was also found on day 5, but then decreased rapidly to values lower than on day 4. This observation might indicate that pegfilgastrim mobilizes hematopoietic cells into the peripheral blood more quickly and this hypothesis is is also supported by the finding that the maximum leukocyte count was already found on day 4 in the majority of pegfilgastrim-stimulated donors.

Side effects such as bone pain and headache are the main toxicity of G-CSF-stimulated stem cell collection and occur in about 80% of the donors, whereas fatigue, gastrointestinal or cardiovascular symptoms are rare.[18,20,21] Ten donors (40%) in our series suffered from severe bone pain, but the symptoms could be controlled by oral paracetamol in all cases. Although pain intensity needs to be evaluated in further trials, the frequency and intensity of adverse events in our pegfilgrastim-mobilized donors seem not to have been substantially different from those after conventional G-CSF.

The long half-life of pegfilgrastim could induce concerns of a prolonged or excessive leukocytosis if this drug is administered to healthy donors in steady state hematopoiesis. In 150 adult family donors treated with 1×10 μg/kg or 2×8 μg/kg filgrastim for 5 days the white cell counts increased a median of 5.7-fold and 7.6-fold, respectively.[20] Another study of 40 normal subjects who received filgrastim 2×6 μg/kg daily for 4 to 6 days showed an average 8-fold increase in the numbers of neutrophils and monocytes and a 2-fold increase in the number of lymphocytes.[22] The white cell count returned to baseline values usually

on day 12 or 13.[20,23] Maximum numbers of white blood cells exceeding 80×10⁹/L have been reported in about 5% of G-CSF-mobilized stem cell donors;[17] in our database of more than 2,500 donors treated with short-course non-pegylated G-CSF this proportion is <1% (*data not shown*). In addition to hyperviscosity, excessive rises in white cell count might be associated with splenic enlargement[24] and the potential risk of splenic rupture. Therefore further investigations of pegfilgrastim in healthy donors should include systematic evaluation of spleen size changes.

An interesting finding of our study is the remarkable increase of blood monocytes after pegfilgrastim administration. Conventional G-CSF given for allogeneic stem cell mobilization induces only a 4 to 8-fold increase in the number of monocytes.[20,22] In the light of this observation we analyzed the proportion of monocytes in the leukapheresis products, but the percentages found were not higher than those reported for harvests collected after unconjugated G-CSF administration.[25]

GVHD is still one of the major causes of morbidity and mortality in allograft recipients. In animals the rate of GVHD in donors pretreated with pegylated G-CSF was significantly lower than that in animals receiving the same dose of standard G-CSF. This has been hypothesized to be related to an increased generation of interleukin-10-producing regulatory T cells.[26] Prospective clinical trials are needed to evaluate these effects in the human system. No conclusions on GVHD or survival can be drawn from our small feasibility study. G-CSF-mobilized allogeneic PBPC grafts were reported to contain from 2.8 to 4.4×10⁸ CD3⁺ cells/kg,[20,27,28,29] corresponding to the results in our series. However one of these trials, which compared two different schedules of filgrastim-mobilization (2×8 μg/ kg versus 1×10 μg/kg for five days), found that the twice daily regimen was associated with a significantly higher number of T cells.[20] Although this finding might have been due to the higher G-CSF dose in that arm of the trial, the graft composition after mobilization with pegfilgrastim should be evaluated in further studies.

The results of our study are preliminary because of the small number of donors, the short follow-up and the limited information about the recipients´ outcome. However, we were able demonstrate the feasibility of mobilizing and harvesting CD34⁺ allogeneic PBPC after a single injection of pegfilgrastim. Although the mobilization efficacy seems to be comparable to that of conventional G-CSF, more comfort and improved quality assurance at growth factor administration as well as a lower incidence of GVHD might represent arguments for the use of the more expensive pegfilgrastim. The course of peripheral blood CD34⁺ cells might be indicative of faster mobilization kinetics after pegfilgrastim. Further studies should evaluate efficacy and toxicity of different doses and schedules of pegfilgrastim as well as the impact on the recipients´ outcome.

FK, KH, CR, GE: study design, writing the protocol, IRB discussion; FRK, KH, KP-T, KZ, RO, MB, GR: examination and screening of donors, informed consent, growth factor administration, performing aphereses, clinical visits during mobilization and follow-up; laboratory tests, flow cytometry, stem cell laboratory; UO, MB; FK, UO, MB, LL: writing manuscript including preparing tables and figures, statistics; all named authors have read again and carefully checked the re-submitted version of the manuscript. We confirm that we have no financial conflict of interests that may influence any judgement on the paper.

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