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Successful transplantation of peripheral blood stem cells mobilized by chemotherapy and a single dose of pegylated G-CSF in patients with multiple myeloma

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Summary:

Following induction therapy and 4 g/m^2 cyclophosphamide, a single dose of 12 mg polyethyleneglycol-conjugated G-CSF (pegfilgrastim; $n = 12$) or daily doses of unconjugated G-CSF (8.5 μg/kg/day) ($n = 12$) were administered to myeloma patients. Pegfilgrastim was associated with an earlier leukocyte recovery (12 *vs* 14 days) and peripheral blood CD34+ cell peak (12 *vs* 15 days). The peripheral blood CD34+ cell peak was lower in the pegfilgrastim group (78 *vs* 111/μl). Following high-dose melphalan (200 mg/m^2) and autografting, leukocyte and platelet reconstitution was similar in both groups and stable blood counts were observed 100 days post transplant. In summary, a single dose of pegfilgrastim after chemotherapy is capable of mobilizing a sufficient number of CD34+ cells for successful autografting with early engraftment and sustained hematological reconstitution in patients with myeloma. These data provide the basis for randomized studies evaluating the optimal dose and time of pegfilgrastim as well as long-term outcome in larger cohorts of patients.

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High-dose therapy and autologous blood stem cell transplantation (ABSCT) are widely used in myeloma.[1–4] Approximately 2.5×10^6 CD34+ cells/kg bodyweight provide rapid and sustained hematopoietic reconstitution after myeloablative therapy.[5] Different methods have been used to mobilize hematopoietic stem and progenitor cells into the peripheral blood such as cytotoxic therapy alone, hematopoietic growth factors, or a combination of both.[6–8]

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Recently, a polyethylene glycol (PEG)-conjugated form of G-CSF has been introduced (pegfilgrastim, Neulasta™).[9] In this formulation, filgrastim is bound covalently to a 20 kDa PEG molecule which increases the serum half-life of G-CSF due to decreased renal elimination.[10] As a result, therapeutic serum levels of G-CSF are maintained over a period of about 2 weeks following the subcutaneous injection of a single dose of pegfilgrastim. In patients with lymphoma, pegfilgrastim is as effective as filgrastim in shortening the time of neutropenia after cytotoxic chemotherapy. The spectrum of side effects is similar to that encountered in patients who have received filgrastim.[11–13] However, systematic data on the ability of pegfilgrastim to mobilize stem cells after chemotherapy as well as on the engraftment potential of pegfilgrastim-mobilized CD34+ cells are lacking.

Patients and methods

After having obtained written informed consent, 24 patients with newly diagnosed myeloma were studied. Treatment protocols were approved by the local ethics committee of the Heinrich Heine University Duesseldorf. Patients were treated with an induction therapy consisting of 1–6 cycles of either 4×10 mg/m^2 idarubicin p.o. in combination with 4×20 mg/m^2 dexamethasone p.o. (ID), or 2 mg vincristine i.v. combined with 36 mg/m^2 doxorubicine i.v. given as a 96 h infusion and 4×40 mg dexamethasone p.o. (VAD). Following induction therapy, all patients received a total dose of 4 g/m^2 cyclophosphamide administered on two consecutive days.

A cohort of 12 consecutive patients who are referred to as the 'pegfilgrastim group' received a single dose of 12 mg pegfilgrastim on day 4 (median, range 3–6) after cyclophosphamide. Mobilization kinetics, apheresis results and transplantation outcomes were retrospectively compared with 12 patients with similar characteristics, who had received a median daily dose of 8.5 μg (range 4.6–11.9 μg)/kg bodyweight of unconjugated G-CSF (11 filgrastim, one lenograstim) subcutaneously starting 5 days (median, range 2–7) after cyclophosphamide. The growth factor was given daily until a sufficient number of CD34+ cells could be

harvested. In the following, these patients will be referred to as the 'G-CSF group'.

Patients of the pegfilgrastim group did not show any significant differences with regard to age, gender, body weight, subtype of the myeloma, stage, or induction therapy, when compared to the G-CSF group. The patients' characteristics are summarized in Table 1.

All patients were followed with routine physical and blood examinations in our outpatient clinic on a regular basis depending on their general condition. When the patients had gone through the nadir of the white blood cell count (WBC), the CD34+ cells in the peripheral blood were measured as early as the WBC rose above 1×10^9/l. The concentration of CD34+ cells was determined according to the International Society of Hematotherapy and Graft Engineering (ISHAGE) guidelines.[14] A leukapheresis was performed 1 day after CD34+ cells exceeded a level of 10 μl in the peripheral blood, and continued until at least 4×10^6 CD34+ cells per kilogram bodyweight were collected. After successful mobilization and leukapheresis, peripheral blood stem cells were cryopreserved in liquid nitrogen and checked for viability and clonogenic growth before transplantation.

Patients were re-evaluated and received a high-dose therapy with a total dose of 200 mg/m^2 melphalan administered on two consecutive days, followed by autologous transplantation of at least 2×10^6 CD34+ cells per kilogram bodyweight. All patients received a standard supportive treatment including antibiotic, antimycotic and antiviral therapy. No growth factors were given after transplantation.

Student's *t*-test was used to compare the pegfilgrastim group with the G-CSF group regarding mobilization, leukapheresis and transplantation characteristics. *P*-values smaller than 0.05 are indicated (Tables 1–4).

Results

Mobilization

Following the administration of cyclophosphamide recovery of leukocytes to counts of more than 1×10^9/l was observed after 12 days (median, range 7–14 days) in patients of the pegfilgrastim group and after 14 days (median, range 11–15) in patients of the G-CSF group ($P = 0.05$). There were no differences with regard to the time needed for platelet recovery to counts of greater than 20 000 or 50 000/μl respectively (Table 2). The maximum number of CD34+ cells in the peripheral blood was noted on day 12 (median, range 11–16) in patients of the pegfilgrastim group. This was significantly earlier in comparison to patients of the G-CSF group in whom the median peak was observed on day 15 (range 12–18). On the other hand, the median maximum CD34+ cell count in the peripheral blood was 78/μl (range 20–1055/μl) in the pegfilgrastim group and 111/μl (range 28–760) in the G-CSF group. This difference was not statistically significant ($P = 0.81$).

Leukapheresis

In line with the earlier appearance of the peripheral CD34+ cell peak, the first apheresis in the pegfilgrastim group was performed 2 days earlier in comparison to the G-CSF group (median day 13 *vs* 15, range 11–15 *vs* 12–18, $P = 0.01$, Table 3). Within the G-CSF group, one apheresis procedure was sufficient to obtain at least 4×10^6 CD34+ cells/ kg bodyweight in all patients. The same was true for 11 out of 12 patients in the pegfilgrastim group, whereas one patient needed a second apheresis to obtain an amount of CD34+ cells above this threshold. The total number of collected CD34+ cells/kg bodyweight was 7.4×10^6 (median, range 4.9–38.0 $\times 10^6$) in the pegfilgrastim group and 10.8×10^6 (median, range 5.0–87 $\times 10^6$) in the G-CSF

Table 1 Patient characteristics

	Pegfilgrastim group	*G-CSF group*
Number of patients	12	12
Age	51 years (41–65)	51 years (32–62)
Gender (m/f)	9/3	7/5
Bodyweight	78 kg (66–107)	77 kg (51–97)
IgG, A, D	7, 3, 0	10, 1, 1
LC	2	0
Stage II, III	4, 8	4, 8
Stage A, B	8, 4	10, 2
Induction therapy (cycles)	4 (1–6) ID/VAD	3 (1–5) ID/VAD
Radiotherapy (total)	4 (33%)	6 (50%)
Extensive radiotherapy[a]	1 (8%)	3 (25%)

[a]Patients with radiotherapy of seven or more vertebra and/or pelvis.

Table 2 Mobilization data following 4 g/m^2 cyclophosphamide

	Pegfilgrastim group	*G-CSF group*
Cytokine dose (total)*	12 mg	7.5 mg (5.4–10.2)
Cytokine dose (calculated)		8.5 μg/kg/day (7.0–13.3)
Number of applications*	1	11 (8–15)
First day of cytokine application	4 (3–6)	5 (2–7)
Leukocyte reconstitution to $>1 \times 10^9$/l#	12 days (7–14)	14 days (11–15)
Platelet reconstitution to $>20 \times 10^9$/l	11 days (10–15)	12 days (10–18)
Platelet reconstitution to $>50 \times 10^9$/l	13 days (10–17)	14 days (10–23)
Day of peripheral blood CD34+ count maximum*	12 (11–16)	15 (12–18)
Maximal peripheral blood CD34+ cell count	78/μl (20–1055)	111/μl (28–760)

*$P < 0.05$.
#$P = 0.05$.

Table 3 Leukapheresis data

	Pegfilgrastim group	*G-CSF group*
Day of 1st apheresis*	13 (11–15)	15 (12–18)
Number of aphereses	1 (1–2)	1
Processed volume	12.21 (5.6–37.1)	14.91 (8.8–27.1)
CD34+ cells/kg bodyweight	7.4×10^6/kg (4.9–38.0)	10.8×10^6/kg (5.0–87.0)
CD34+ cells/kg bodyweight and volume	558/kg ml (132–4488)	868/kg ml (199–9622)
Mobilization failures	0	0

*$P < 0.05$.

Table 4 Transplantation data following high-dose therapy with melphalan (200 mg/m^2) and autografting

	Pegfilgrastim group	*G-CSF group*
Transplanted patients	8	12
Transplanted CD34+ cells	3.4×10^6/kg (2.5–19.0)	7.5×10^6/kg (2.1–29.0)
Leukocyte reconstitution to $>1 \times 10^9$/l	16 days (13–24)	15 days (12–22)
Platelet reconstitution to $>20 \times 10^9$/l	12 days (10–26)	12 days (10–24)
Platelet reconstitution to $>50 \times 10^9$/l	13 days (10–39)	13 days (11–26)
Leukocyte count on day 100 (10^9/l)	4.4 (3.0–8.2)	4.3 (2.3–8.3)
Hemoglobin level on day 100 (g/dl)	12.6 (10.6–14.0)	12.3 (10.6–14.9)
Platelet count on day 100 (10^9/l)	155 (72–258)	147 (64–291)

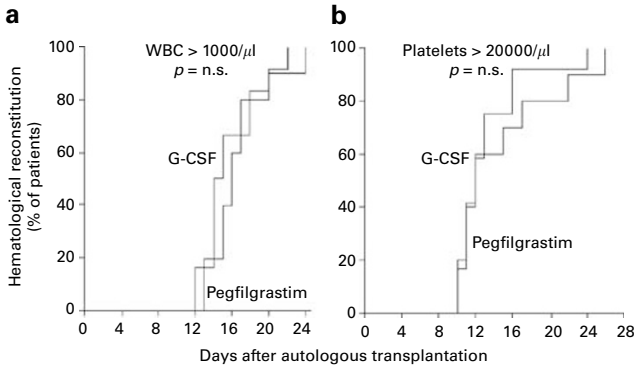

Figure 1 Hematological reconstitution following high-dose therapy with 200 mg/m^2 melphalan and autologous transplantation of blood stem cells mobilized by chemotherapy plus single-dose pegfilgrastim or chemotherapy plus daily unconjugated G-CSF. (**a**) Leukocyte reconstitution ($>1000/\mu$l). (**b**) Platelet reconstitution ($>20\,000/\mu$l).

group (not significant, $P = 0.26$). When we calculated the collected number of CD34+ cells/kg bodyweight/l processed volume, the difference was less significant ($P = 0.60$).

High-dose therapy and autografting

At the time of writing, each of the 12 patients from the G-CSF group and eight patients of the pegfilgrastim group had a minimal follow-up of 100 days after high-dose cytotoxic therapy, which consisted of 200 mg/m^2 of melphalan. The autografts used for transplantation contained a minimum of 2×10^6 CD34+ cells. Patients of the pegfilgrastim group received a median of 3.4×10^6 CD34+ cells per kg bodyweight (range 2.5–19.0 $\times 10^6$) in comparison to 7.5×10^6 (range 2.1–29.0 $\times 10^6$) CD34+ cells/kg bodyweight in patients of the G-CSF group (NS, $P = 0.14$). Despite the lower number of CD34+ cells transplanted, there were no significant differences with regard to the time needed for hematological reconstitution. Leukocyte recovery was achieved after 16 days (median, range 13–24 days) in the pegfilgrastim group and after 15 days (median, range 12–22 days) in the G-CSF group (NS, $P = 0.51$) (Figure 1a). Independence from platelet transfusions (platelets $>20\,000/\mu$l) was observed after 12 days in both the pegfilgrastim and the G-CSF group (Figure 1b). Similarly, reconstitution of platelets to counts of more than $50\,000/\mu$l was achieved after a median of 13 days in both groups. All patients had stable blood counts 100 days after autologous transplantation (Table 4).

Discussion

Our data show that a single dose of pegfilgrastim following cytotoxic chemotherapy is capable of mobilizing sufficient numbers of peripheral blood stem cells for successful autografting after high-dose therapy in patients with myeloma. We chose a relatively high dose of 12 mg, which is twice the recommended dose to shorten neutropenia after conventional chemotherapy. A single dose of pegylated G-CSF increases convenience and improves compliance when compared to the daily application of G-CSF from a practical point of view.[15]

The use of pegfilgrastim was associated with a reduction of 2 days for the time needed to reach a leukocyte count of more than 1×10^9/l and the peripheral blood CD34+ peak in comparison to daily G-CSF. As a result, the first leukapheresis was performed 2 days earlier compared with the patients who had received unconjugated G-CSF. The reason for this phenomenon might be related to the continuously high serum level of G-CSF maintained by pegfilgrastim, possibly providing a more efficient stimulus for the CD34+ hematopoietic stem and progenitor cells

than the pulsatile way of action of daily G-CSF injections. However, these findings may also be related to the small patient number in this feasibility study and larger randomized trials are required to confirm this preliminary observation.

On the other hand, peak levels of CD34+ cells were lower in the pegfilgrastim group than in the G-CSF group, even though the difference was not significant. This was associated with a smaller number of CD34+ cells harvested/kg bodyweight.

The pharmacokinetics of pegfilgrastim could explain the early appearance of the CD34+ cell peak and the lower number of CD34+ cells in the peripheral blood. As shown by pharmacokinetic studies in neutropenic patients, plasma G-CSF levels rise quickly after subcutaneous administration of pegfilgrastim and remain more or less stable due to impaired renal elimination.[10,16–18] When the number of granulocytes increases, pegfilgrastim is eliminated by cellular uptake via the G-CSF receptor and intracellular degradation, as well as by cleavage through neutrophil elastase.[19,20] Thus, the rise of leukocytes leads to a rapid clearance of G-CSF in the pegfilgrastim group, which then may result in less effective mobilization of CD34+ cells. The self-limiting effect of pegfilgrastim may not be relevant in shortening the duration of neutropenia, but may be so for effective stem cell mobilization. In order to utilize the pharmacokinetic advantages of pegylated G-CSF as well as those of unconjugated G-CSF for effective stem cell mobilization, a combination might be worthwhile.

Following high-dose therapy with 200 mg/m^2 melphalan and autografting the time needed for leukocyte and platelet recovery was similar in both patient groups. There were no graft failures and adequate blood counts were achieved on day 100 post transplantation. Pegfilgrastim following chemotherapy apparently mobilizes CD34+ cells, which provide early engraftment and sustained hematological reconstitution.

In conclusion, mobilization, harvesting and autografting of pegfilgrastim-mobilized peripheral blood stem cells could be successfully achieved in patients with myeloma. Further studies are needed to address optimal dose and schedule of pegfilgrastim administration.

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