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# Harvesting Peripheral Blood Progenitor Cells From Healthy Donors: Retrospective Comparison of Filgrastim and Lenograstim

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Mobilization of $CD34^+$ into peripheral blood is attained by either glycosylated (lenograstim) or non-glycosylated recombinant G-CSF (filgrastim). 101 donors, 57 males, median age 42 years (range 16–63) entered this retrospective study. Group I (55 cases) received filgrastim and group II lenograstim subcutaneously for 5–6 days. The peak number of $CD34^+$ cells/μl blood observed on day 4 and 5 was not significantly different in the two groups. No differences were shown in terms of both circulating CFU-GM at the time of harvesting and $CD34^+$ target of collection. The most frequent side effects were bone pain (18.2% grade I; 36.4% grade II, 7.3% grade III), headache (18.2%), nausea (9.1%), fever (5.5%) and a mild splenomegaly (>2cm) (5.5%) in filgrastim group, and bone pain (37.0% grade I, 26.1% grade II, 2.2% grade III), headache (17.4%), nausea (15.2%), fever (4.4%) and a mild splenomegaly (4.3%) in lenograstim group, respectively. $CD34^+$ collection was associated with thrombocytopenia, which was not significantly different between the two groups. No donor in either group developed long-term adverse effects. We conclude that both G-CSFs are comparable in terms of $CD34^+$ cell collection, safety and tolerability. J. Clin. Apheresis 20:129–136 

**Key words: filgrastim; lenograstim; stem cell mobilization; healthy donors; allogeneic transplantation; G-CSF.**

## INTRODUCTION

Granulocyte colony stimulating factor (G-CSF) is a haemopietic growth factor that stimulates proliferation and differentiation of myeloid precursor cells as well as some of the functional properties of mature granulocytes [1, 2]. Following its molecular identification and subsequent production as recombinant human protein [3, 4], G-CSF has been increasingly used as a therapy for several forms of neutropenia and as a mobilizer of progenitor cells for autologous or allogeneic transplantation [5, 6].

Two forms of G-CSF are available on the market: lenograstim and filgrastim [7, 8]. These 2 G-CSFs differ in chemical structure; in particular lenograstim is glycosylated whereas filgrastim is not [9, 10]. G-CSF glycosylation is advantageous in terms of in vitro stability to temperature, ph and degradation by proteases [11–14]. Moreover, glycosylation optimises the erythropoietin effects on red blood cell proliferation [15] and increases the biological potency of qualitative and quantitative colony formation of human bone marrow cells [16]. Similarly, it has been documented that lenograstim has a greater capacity to stimulate the colony growth of both purified $CD34^+$ and unmanipulated PBSC [17].

G-CSF is used in healthy donors to mobilize allogeneic peripheral blood progenitor cells (PBPC) [18–20]. After initial encouraging results were reported in the literature, the number of allogeneic PBPC transplants increased rapidly. This extraordinary success is most likely due to an accelerated post-transplant recovery of blood counts, in particular of platelets. The incidence and severity of acute GVHD seem comparable to that of marrow transplants but there is also hope of better disease control through an augmentation of the so-called GVL (graft-versus-leukaemia) effect.

A dose-response relationship of G-CSF and progenitor cell mobilization has been shown by several investigators [21–26]. High doses of G-CSF are

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generally well tolerated, but may be accompanied by intense muscleskeletal pain [27]. The key remaining question is: what do the structural differences between the two types of G-CSFs mean for the donor?

The objectives of this study was to retrospectively compare the capability of equivalent dose of lenograstim and filgrastim in mobilizing allogeneic PBPC and to evaluate their safety and tolerability.

## PATIENTS AND METHODS

### Study Design/Data Collection

This was a retrospective study of healthy stem cell donors receiving two different types of recombinant human granulocyte colony-stimulating factor (glycosylated G-CSF, lenograstim or non-glycosylated G-CSF, filgrastim) for mobilizing allogeneic PBPC at the our Bone Marrow Transplant Unit between November 1995 and April 2004. During this evaluative period, both types of G-CSF were available in our institution for physicians to prescribe according to preference.

The main endpoints of this study were to compare the efficacy of equivalent doses (μg) of lenograstim and filgrastim in mobilizing allogeneic PBPC (i.e. difference in peak value of $CD34^+$ cells in blood, number of $CD34^+$ cells collected, aphaeresis requirement to reach the target number of CD34+ cells) and to compare the safety and tolerability of the 2 G-CSFs given to healthy subjects.

### Donor Characteristics

One hundred and one consecutive, not selected healthy individuals received G-CSF as PBPC mobilization agent. Fifty seven donors were males and 44 females. Their median age was 42 years (range 16–63), with 1 donor being less than 18 years old (16 years). Their median body weight was 72 Kg (range 47–113 Kg). These donors underwent overall 230 procedures of collection which form the basis of the present analysis. Written informed consent of donors was obtained after a detailed description of the potential side effects and risks of G-CSF mobilization and leukapheresis compared to bone marrow donation with general anaesthesia. Concerning the unknown possible long-term effects, donors were informed about in vitro results and animal experiments, that G-CSF could lead to stimulation of tumour or leukaemia growth [28] but that, to the best of our knowledge, no carcinogenic effect of G-CSF is known up to now.

The donors were seen at our institution two to three weeks before starting mobilization. The workup included a detailed history, clinical examination, abdomen ultrasound and ECG. Laboratory evaluation included blood counts and differentials, clinical chemistry, infectious diseases markers, and β-HCG in female donors to exclude pregnancy. All donors received a form addressed to their general practitioner, explaining the procedure and the use of growth factors in this setting. Both G-CSF and paracetamol were prescribed. All donors were informed that in the case of headaches or bone pain they could take the analgesic at the prescribed dosage. The donors were monitored prospectively at our institution, daily during G-CSF administration and at regular intervals until the normalization of blood count, and, as a follow-up, every three months after the last treatment.

### PBPC Mobilization

Healthy donors received random filgrastim (Group I, n. 55) or lenograstim (Group II, n. 46) as mobilizing agent according with the different schedules reported by the literature [21–26]. The groups were well balanced for age, body-weight and sex (Table I). In all cases the 1st G-CSF administration was performed in the hospital, and continued on an outpatient basis at the donor's home, either as self–administration or injection by the family physician. Filgrastim and lenograstim were administered subcutaneous as twice daily for 4–7 days. All injections were given at the same time in the morning and in the evening following blood sampling. Day 0 was conventionally that of pre-treatment evaluation and day 1 the first day of G-CSF administration.

### Study Medication

Filgrastim was supplied as single-use ampoules containing 480 μg/1.6 ml (i.e. 300 μg/ml) of active substance. Lenograstim was supplied as vials containing 368 μg of sterile lyophilised preparation to be reconstituted with 1.4 ml sterile water at a final concentration of 263 μg/ml. A large international collaborative study, comparing different research-use preparations of G-CSF in a wide range of in vitro bioassays and immunoassays, demonstrated that recombinant products containing the same mass of G-CSF differ significantly in biological activity. Based on this study, an international potency standard was established, assigning a specific activity of 100, 000 IU/μg to filgrastim and 127, 760 IU/μg to lenograstim, respectively [28].

### Aphaeretic Collection

Following administration of the G-CSF, $CD34^+$ cells were monitored at day 4 and daily, until aphaeresis completion. Aphaeretic collections were

**TABLE I. Characteristics of Healthy Donors**

| | Group I: Filgrastim | Group II: Lenograstrim | *P** |
|---|---|---|---|
| No. donors | 55 | 46 | |
| Sex | | | |
| Male | 33 | 24 | *NS*[a] |
| Female | 22 | 22 | |
| Age, median and range, years | 42 (19–63) | 41.5 (16–62) | *NS*[b] |
| Donor weight, median and range, kg | 72 (48–113) | 76 (47–105) | *NS*[b] |
| Recipient weight, median and range, kg | 71 (50–95) | 71.5 (40–103) | *NS*[b] |
| G-CSF μg/kg/day | | | |
| Mean ± SD, range | 9.5 ± 1.7 (5–12.8) | 9.2 ± 1.6 (6.2–14) | *NS*[b] |
| G-CSF MIU/kg/day | | | |
| Mean ± SD (range) | 0.95 ± 0.17 (0.51–1.28) | 1.19 ± 0.22 (0.81–1.79) | *< 0.0001*[b] |

**NS* = not significant; [a]Fisher's exact test; [b]Kruskal-Wallis test.

**TABLE II. Number of $CD34^+$ Cells/μl Blood in Healthy Donors.**

| | Group I: Filgrastim (mean ± SD) | Group II: Lenograstim (mean ± SD) | *P** |
|---|---|---|---|
| Day 4 | 46.0 ± 33.4 | 39.8 ± 22.1 | *NS* |
| Day 5 | 57.8 ± 36.4 | 48.3 ± 26.5 | *NS* |

**NS* = not significant; Kruskal-Wallis test.

started at our institution starting either on day 4 (66.7% of donors) or on day 5 and they were performed on an outpatient basis using an automated continuous-flow blood cell separator (Fresenius, AS 104 or 204 system) through bilateral peripheral venous access. No donors needed the placement of a catheter. The target $CD34^+$ cell dose to be collected was set at $4 \geq \times 10^6$ per kg of recipient's body weight (BW). The target amount of blood to be processed was approximately 3 times the donor's blood volume for at least the first collection. Donors who, after one or two leukapheresis procedures, had reached at least the minimal number of $CD34^+$ cells considered acceptable for allotransplantation ($< 2 \times 10^6$ $CD34^+$ cells/kg recipient's BW) were allowed to continue PBPC collection at the discretion of the aphaeresis attending physician. Filgrastim or lenograstim were continued until the completion of PBPC collection.

## Measurement of Nucleated Cells and Flow Cytometric Analysis

Cell counts of peripheral blood and the leukapheresis product were performed on an automated hematology analyzer (Coulter Corporation, Miami, FL). Differential counts were done microscopically on a Wright stained smear. The mononuclear cell count was obtained by multiplying the number of leukocytes with the sum of the percentage of lymphocytes and monocytes from the differential count.

The same investigator performed all flow cytometric analyses blindly. The stem cell content was evaluated by flow cytometry. The quantities of $CD34^+$ cells in the peripheral blood and leukapheresis component were determined by flow cytometry using FACScan (Becton Dickinson, Palo Alto, CA, U.S.A.). After lysis of RBCs in an ammonium chloride lysis solution and washing with phosphate-buffered saline with 0.5% human serum albumin, 100 L of cell suspension was stained with fluorescein isothiocyanate (FITC)-conjugated monoclonal anti-CD34 antibody (Becton Dickinson) and with phycoerythrin (PE)-conjugated anti-CD45 antibody (Becton Dickinson) at the concentrations recommended by the manufacturer. An irrelevant monoclonal antibody was used as a negative isotope control. A total of $1.0 \times 10^5$ cells were analyzed by the FACScan research software (Becton Dickinson).

## Progenitor Colony Assay

To determine CFU-GM (colony forming unit of granulocyte/macrophage progenitors) derived colonies, $2 \times 10^5$ nucleated cells were cultured in triplicate in 24-well dishes in the presence of methyl-cellulose in Iscove's MDM, L-glutamine, fetal bovine serum, 2-mercaptoethanol, penicillin (100 U/ml) and streptomycin (100 μg/ml), rhGM-CSF, rhSCF and rhIL-3 (Stem Cell Technologies, Vancouver, BC). The wells were incubated in a humidified incubator containing 5% CO2 and at 37°C. After incubation for 7 days, the colonies containing 50 or more cells were counted under an inverted microscope.

## Adverse-event Assessment

PBPC donors were interviewed daily during collection on adverse events and oral analgesic intake. Their subjective complaints were recorded on an adverse event assessment form. They were asked to rate the G-CSF-related adverse events as mild (grade 1), moderate (grade 2) or severe (grade 3). G-CSF-related adverse events that prompted the discontinuation of the drug were rated as grade 4.

**TABLE III. Aphaeretic Collection**

| | Group I: Filgrastim | Group II: Lenograstim | *P** |
|---|---|---|---|
| Day of aphaeresis start | | | |
| Day 4 | 37 (67.3%) | 29 (63%) | *NS*[a] |
| Day 5 | 16 (29.1%) | 16 (34.8%) | *NS*[a] |
| Day 6 | 2 (3.6%) | 1 (2.2%) | *NS*[a] |
| No. Aphaeresis, median, range | 2 (1–4) | 2 (0–4) | *NS*[a] |
| 1 | 4 (7.3%) | 4 (8.7%) | *NS*[a] |
| 2 | 35 (63.6%) | 29 (63%) | *NS*[a] |
| 3 | 11 (20%) | 7 (15.2%) | *NS*[a] |
| 4 | 5 (9.1%) | 5 (10.9%) | *NS*[a] |
| $CD34^+$ cell dose collected $\times 10^6$, day 4 | 389 ± 271 | 434 ± 251 | *NS*[b] |
| $CD34^+$ cell dose collected $\times 10^6$/kg donor, day 4 | 5.4 ± 3.9 | 5.9 ± 3.2 | *NS*[b] |
| CFU-GM/μl 1st aphaeresis | 30.0 ± 19.3 | 24.9 ± 17.8 | *NS*[b] |
| Blood Volume processed on day 4, mean ± SD (range) | 14 ± 2.6 (9–20) | 15.8 ± 2.9 (10.2–21.1) | *0.001*[a] |
| $CD34^+$ cell dose collected $\times 10^6$, day 5 | 370 ± 212 | 441 ± 241 | *NS*[b] |
| $CD34^+$ cell dose collected $\times 10^6$/kg donor, day 5 | 5.0 ± 2.6 | 5.9 ± 2.9 | *NS*[b] |
| CFU-GM/μl 2nd aphaeresis | 31.7 ± 18.7 | 29.0 ± 22.6 | *NS*[b] |
| Blood Volume processed on day 5, mean ± SD (range) | 13.7 ± 3.2 (6–20) | 15.4 ± 3.2 (8–21) | *0.020*[b] |
| Total dose $CD34^+$ cells collected $\times 10^6$ | 835 ± 423 | 867 ±413 | *NS* |
| Total dose $CD34^+$ cells collected $\times 10^6$/kg donor | 10.4 ± 4.0 | 10.2 ± 2.9 | *NS* |
| No. of donors who collected a $CD34^+$ cell dose $>4 \times 10^6$/kg with the 1st aphaeresis | 28 (50.9%) | 25 (55.5%) | *NS*[a] |
| No. donors failed mobilization | 0 | 1 | *NS*[a] |

**NS* = not significant; [a]Fisher's exact test; [b]Kruskal-Wallis test.

### Statistical Analysis

All statistical calculations were performed using the statistical package SPSS for Windows, release 11.5, 2002 software (SPSS UK, Working, Surrey, United Kingdom). Data are presented by using values expressed as mean ± SD or median (range) or percentage. Statistical comparisons for binary variables were performed using two-way tables for the Fisher's exact test and multiway tables for the Pearson's Chi-square test. The non-parametric Kruskal-Wallis was employed to test the differences between two groups. A value of $P \leq 0.05$ was considered significant for all statistical calculations.

## RESULTS

### Donor Demographics and G-CSF Administration

Main donor characteristics are shown in Table 1. Filgrastim (Group I) and lenograstim (Group II) were administered in 55 and 46 donors, respectively. The two groups were well balanced in terms of gender, age and body-weight. Moreover, the median G-CSF dose calculated as μg/kg/day was equally distributed between the two groups, while a statistically significant difference in the number of administered units was demonstrated (0.95 ± 0.17 MIU/kg in filgrastim group, vs. 1.19 ± 0.22 MIU/kg in lenograstim group, $P = 0.0001$). Finally, median duration of G-CSF administration was 5 days (range 4–7) in both groups.

### Cell Harvest

The kinetics of $CD34^+$ cell mobilization into peripheral blood and aphaeretic product of healthy donors is shown in Table 2. $CD34^+$ cells blood peak value was slightly higher in filgrastim group on both day 4 and 5 (mean values: 46.0 *vs.* 39.8 and 57.8 *vs.* 48.3, respectively); however differences were not statistically significant.

Most of cases initiated aphaeresis in both groups on day 4 (67.3% in group I *versus* 63% in group II), while the remaining cases started the procedure on day 5; a small number of donors (3.6% group I and 2.2% group II) underwent aphaeretic procedure on day 6 (Table 3). At first leukapheresis, the number of $CD34^+$ cells collected both at day 4 and 5 was higher, although not statistically different, in lenograstim group, possibly due to the significantly more elevated blood volume processed (Table 3). Overall, no significant difference was demonstrated between the two groups in terms of either the absolute number of stem cell collected or the number of $CD34^+$ cells calculated pro kg of donor weight. A $CD34^+$ cell dose $> 4 \times 10^6$/kg, assumed as a reasonable progenitor number for allogeneic transplantation, was reached at the 1st aphaeresis in a fairly matching percentage of donors (50.9% in filgrastim *versus* 55.5% in lenograstim groups). The blood number of CFU-GM, as measured on the day of the 1st and 2nd aphaeresis, was similar in the two groups. Finally, only 1 donor (lenograstim Group) failed mobilization (Table 3).

**TABLE IV. Effects of G-CSF on Haematological Parameters†**

| | Group I: Filgrastim | Group II: Lenograstim | *P** |
|---|---|---|---|
| Leukocytes ($\times 10^9$/L), baseline | 6.9 ± 1.6 | 7.2 ± 1.8 | *NS* |
| Leukocytes ($\times 10^9$/L), on day 4 | 38.3 ± 8.6 | 41.6 ± 12.7 | *NS* |
| Leukocytes ($\times 10^9$/L), on day 5 | 41.6 ± 9.6 | 42.9 ± 12.0 | *NS* |
| Leukocytes ($\times 10^9$/L), peak | 47.2 ± 10.3 | 49.0 ± 12.0 | *NS* |
| Leukocytes: time to baseline value, days | 7.1 ± 5.8 | 8.6 ± 7.4 | *NS* |
| Platelets ($\times 10^9$/L), baseline | 229 ± 52 | 230 ± 51 | *NS* |
| Platelets ($\times 10^9$/L), nadir | 93 ± 31 | 103 ± 38 | *NS* |
| Platelets: time to baseline value, days | 7.1 ± 3.6 | 8.3 ± 5.6 | *NS* |

†Values are expressed as mean ± SD.
*NS = not significant; Kruskal-Wallis test.

**TABLE V. Incidence and Grading of Side Effects Reported During G-CSF Administration**

| | Group I: Filgrastim (% donors) | Group II: Lenograstim (% donors) | *P** |
|---|---|---|---|
| Bone pain | | | |
| Absent | 38.2 | 34.8 | |
| Mild | 18.2 | 37.0 | *NS*[a] |
| Moderate | 36.4 | 26.1 | |
| Severe | 7.3 | 2.2 | |
| Headache | 18.2 | 17.4 | *NS*[b] |
| Splenomegaly | 5.5 | 4.3 | *NS*[b] |
| Fever | | | |
| No. | 94.5 | 95.7 | |
| Yes WHO = 1 | 5.5 | 2.2 | *NS*[a] |
| Yes WHO = 2 | 0 | 2.2 | |
| Nausea | 9.1 | 15.2 | *NS*[b] |
| Insomnia | 7.3 | 15.2 | *NS*[b] |

NS = not significant. [a]Pearson's Chi-square test. [b]Fisher's exact test.

Table 4 shows the effects of G-CSF on hematological parameters. Leukocyte and platelet kinetics were substantially equivalent after both filgrastim and lenograstim administration. In particular, leukocyte peak values were $47 \times 10^9$/L and $49 \times 10^9$/L in the filgrastim group and in the lenograstim group, respectively ($P$ = ns). On the other hand, the nadir of platelet counts reached mean values of $93 \times 10^9$/L and $103 \times 10^9$/L in the I and II groups, respectively; however, such a decrease was not complicated by bleeding manifestations. Moreover, a comparative analysis performed before and immediately after aphaeresis procedures showed an expected drop off in platelet counts, which reached a value below $80 \times 10^9$/L in 5 cases in the 1st group and in 3 cases in the 2nd group; in these cases, autologous platelets were retransfused. To note, the haemoglobin concentration decreased slightly but not significantly in both groups (data not shown). Finally, leukocyte and platelet counts returned to normal values in about one week in both groups. Notably, hemoglobin concentration, leukocyte and platelet counts, evaluated after one moth, showed no significant differences as compared to baseline values in both groups.

## G-CSF related adverse events

The main adverse events reported by the donors were bone pain (18.2% grade 1; 36.4% grade 2; 7.3% grade 3), headache (18.2%), nausea (9.1%), fever (5.5%) and a mild splenomegaly (> 2cm exceeding the marginal cost at physical examination) (5.5%) in Group I, and bone pain (37.0% grade I, 26.1% grade II, 2.2% grade III), headache (17.4%), nausea (15.2%), fever (4.4%) and a mild splenomegaly (4.3%) in group II, respectively (Table 5). Prophylaxis with paracetamol was done in all donors. All donors experienced side effect resolution within 2-4 days of G-CSF cessation. None of the side effects caused discontinuation of G-CSF administration.

At a median of 28.7 months (range 0.3–102.8) from mobilization all of donors reported no complaints and no long-term effects.

## DISCUSSION

There is increasing interest in the use of filgrastim or lenograstim-mobilized PBPC for allogeneic transplantation. This donation modality appears to have several appealing features, such as: the avoidance of general anaesthesia, surgery (marrow harvesting), the risk of autologous or allogeneic blood transfusions, as well as the potential to make the donation process more donor-friendly and convenient. At the present time, it is important to ascertain how this donation approach compares to the time-honoured and established marrow harvesting, a procedure with a remarkable safety record over the past 30 years[30].

Lenograstim and Filgrastim are produced by recombinant DNA technology [7, 8]. The first is produced in culture from mammalian cells. It is glycosylated, making it a true glycoprotein identical to the endogenous human molecule. The second, on the other hand, is produced in culture from bacterial cells, and this difference in the production processes accounts for the differences in the amino-acid sequences and glycosylation between the two molecules.

Published reports indicate that lenograstim is more active in vitro than filgrastim on a weight-by-weight basis. Nissen [31] compared mass equivalent doses of lenograstim, filgrastim and research-use deglycosylated G-CSF by neutrophil colony assays in normal human bone marrows. Lenograstim stimulated neutrophil colony formulation at doses 16 times lower than that of filgrastim (and deglycosylated G-CSF) and was twice as potent at maximal colony stimulation. Pedrazzoli [17] demonstrated the superior potency of lenograstim as compared to filgrastim, in stimulating the growth of CFU-GM from whole blood and purified $CD34^+$ cells. A large international collaborative study, comparing different research-use preparations of G-CSF in a wide range of in vitro bioassays and immunoassays, demonstrated that recombinant products containing the same mass of G-CSF differ significantly in biological activity. Based on this study, an international potency standard was established, assigning a specific activity of 100 000 IU/μg to filgrastim and 127 760 IU/μg to lenograstim, respectively [24].

In human normal donors, two randomised trials indicated the superior specific activity of lenograstim. Simulating the allogeneic donor setting, Hoglund and his colleagues [32] randomised 32 male volunteers to receive either 10 μg/kg/day of Lenograstim followed by 10 μg/kg/day of Filgrastim or vice versa. Experimentally, this was a randomised; single blind, crossover study where each volunteer acted as his or her own control. Each volunteer was administered lenograstim or filgrastim as subcutaneous injections once daily for 5 days followed by a wash-out period of 4 weeks then a further 5 days of the alternative G-CSF product. There was a significant difference in favour of lenograstim for mobilisation of both $CD34^+$ cells and CFU-GM. In a similar crossover study by Watts [33], filgrastim and lenograstim were administered at a dose of 5 μg/kg/day subcutaneously for 6 days. The blood peek levels of WBC and CFU-GM were significantly higher with lenograstim as compared with filgrastim.

Using a different approach de Arriba [34] compared bio equivalent doses of glycosylated and non-glycosylated G-CSF for mobilization of PBPC. 31 patients with stage II-IV breast cancer were randomised to receive either lenograstim 0.82 MU/kg/day or filgrastim 0.84 MU/kg/day for 4 days or until completion of leukaphereses. Mobilization of $CD34^+$ cells, whether measured in blood or leukapheresis products, did not differ between the two growth factors. However, the dose of approximately 0.80 MU/kg was contained in 8.4 mg/kg of filgrastim and in only 6.4 mg/kg of lenograstim, and authors concluded that, for an identical result, 31% more filgrastim is needed as opposed to lenograstim.

The simplest technique to compare filgrastim and lenograstim is to measure the ability to increase the number of hematopoietic stem cells in an organism (measured in particular in circulating blood). These stem cells will be the origin of mature blood cells. Increasing the number of hematopoietic stem cells is actually what is expected from a G-CSF whether it be in hematology (mobilization of stem cells) or in the reduction of chemotherapy-induced neutropenia. The stem cell count ($CD34^+$ cells) is an objective and an especially quantitative measure on the effect of G-CSF on bone marrow. From a scientific point of view, we can use two techniques: a) identify the dosage which would give exactly the same number of circulating $CD34^+$ cells; b) administer the same dosage of the two products to two groups of donors or patients and measure the number of circulating $CD34^+$ cells.

In our series we have demonstrated that using a comparable dose of the two products, the number of $CD34^+$ cells, mobilized in a cohort of healthy donors, is similar. This result is not in line with literature data [32], possibly due to a larger number of subjects analysed in our study which, in turn, should counterbalance the bias of the large inter-individual variation in stem cell mobilisation in response to G-CSF. Furthermore, we utilized a twice-daily dosing regimen which could be considered as an extra cause to justify the conflicting results.

The number of progenitor cells required for rapid and sustained allogeneic engraftment has not been established. A target harvest yield of $\geq 4 \times 10^6$ $CD34^+$ cells kg recipient body weight, corresponding to a total of $250–400 \times 10^6$ $CD34^+$ cells to an adult 60–80 kg recipient, has been proposed by several investigators [34]. 50.9% donors in group I and 55.5% in group II mobilizes a $CD34^+$ cell dose $> 4 \times 10^6$/kg with the 1st aphaeresis (no statistical difference) and only a poor responders (peak $CD34^+$ cells $< 26/\mu l$) were observed (in lenograstim group). Interestingly, the target of collection is reached in lenograstim group with a larger blood volume processed.

In clinical practice, G-CSF is usually dosed in μg/kg. However, previous studies indicate that filgrastim and lenograstim differ in specific activity in vitro. Thus lenograstim has a specific activity of 127 760 IU/μg, whereas filgrastim has a specific activity of 100 000 IU/μg. The present study doesn't confirm that a corresponding difference in potency exists *in vivo*, showing a significant statistical difference in terms of international biological unit administered to healthy donors (1.19 MIU/kg/day in lenograstim group vs 0.95 MIU/kg/day in filgrastim group, $P = 0.001$) to obtain the same results in terms of mobilization of progenitor cells.

At doses ≤10 μg/kg/day G-CSF is generally well tolerated with low incidence of clinically relevant side effects. The most common reported adverse event, which is partly, dose related, is bone pain [36–40]. In our study lenograstim and filgrastim have a similar profile and frequency of side effects. Both G-CSFs were well tolerated, although essentially most of the donors experienced some degree of bone pain. However, G-CSF-related adverse events were never so severe to cause drug discontinuation, and did respond well to paracetamol and not lasting beyond day 5–6. The rapid and pronounced increase in leukocytes is of some concern, although the duration of leukocytosis is short and no donors experienced any symptom of hyperviscosity. A significant drop in platelet counts occurred in almost all subjects (no difference between the drugs) and the days to return to baseline value were similar between the two groups.

In conclusion, we suggest the that the two G-CSFs are similar in terms of efficacy. However, only a larger sample size can definitely answer which is the best type of G-CSF to be used.

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