

# Clinical implications, safety, efficacy of recombinant human Granulocyte Colony-Stimulating Factors and pegylated equivalent

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## ABSTRACT

A wide use of recombinant human granulocyte colony-stimulating factors (G-CSFs) and their pegylated equivalent is a significant step forward in the treatment of both solid tumors and hematological malignancies. Evidence-based use of these molecules resulted in more intensive treatments, safely extended to frail and elderly patients, and development of response- and comorbidity-tailored approaches. The available G-CSFs are filgrastim, and the long-acting pegfilgrastim, which are produced in *E. Coli* cells, and are chemically different from native human G-CSF, and lenograstim, a molecule produced in mammalian cells, with a chemical structure identical to native human G-CSF. These chemical differences produce a diverse interaction with receptors and stimulated neutrophils. For instance, lenograstim binds to receptors in the same way of endogenous ligand, and neutrophils obtained from stimulation with this G-CSF have a physiological activity profile similar to neutrophils normally generated in humans. Conversely, the different interaction between filgrastim and G-CSF receptor is more frequently associated with morphological abnormalities, reduced motility and chemotaxis and a reduced response to bacterial stimuli in induced neutrophils.

On this background, we reviewed available evidence in order to analyze the impact of these chemical and pharmacodynamic differences among G-CSF molecules on safety, particularly in healthy peripheral-blood stem-cells donors, functional qualities of induced neutrophils, and mobilization of hematopoietic stem cells.

*Key words:* Granulocytes-colony stimulating factors; Lenograstim; Filgrastim; Pegfilgrastim; Neutrophils; Stem cells mobilization; Autologous stem cells transplantation

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## INTRODUCTION

The diffuse use of granulocyte-colony stimulating factors (G-CSFs) has changed several therapeutic approaches in oncology and hematology, leading to a safe exploitation of more intensive chemotherapy; especially, among drug regimens exhibiting hematological toxicity as the main, if not exclusive, dose-limiting toxicity. The use of rHuG-CSF reduces the risk of febrile neutropenia and documented infections when administered prophylactically to patients receiving systemic chemotherapy [1]. G-CSF use is associated with a significant decrease in the need to reduce the dose of, or otherwise delay, chemotherapy, with a trend toward a reduction in infection-related mortality [1]. These cost-effective molecules are currently used as part of supportive care, febrile neutropenia prophylaxis and as mobilizing agent to collect hematopoietic stem cells both from patients themselves and healthy donors. In this way, G-CSFs allowed a worldwide and safe use of both autologous and allogeneic transplantation as part of the therapeutic armamentarium in onco-hematology, with dramatic improvement in overall outcome of some malignant disorders.

Neutropenia is a quite common complication during anticancer chemotherapy; it is diagnosed in 20-40% and in 50-70% of patients with solid tumors and hematological malignancies, respectively. Both neutropenia severity and duration are strongly related with the incidence of infective episodes, with an increase in infections incidence from 5% up to over 40% when absolute neutrophil count (ANC) declines from more than 1 000/ $\mu$ l to <100/ $\mu$ l. Febrile neutropenia is seen most often during the first courses of chemotherapy, and prophylactic administration of G-CSF ensures protection for high-risk patients, reducing hospital admissions, antibiotic prescriptions and other related costs. There is consensus across international guidelines that a primary prophylaxis with G-CSF must be used if expected risk of febrile neutropenia is  $\geq$ 20%, while it should be considered in chemotherapies with intermediate risk of febrile neutropenia (10-20%) in elderly patients as well as in patients with disseminated disease, poor performance status, and/or treated with extended-field irradiation [2].

Several commercial products are available as recombinant human G-CSF. Filgrastim has

been the first developed G-CSF. It has been synthesized by amplifying recombinant human DNA in *E. Coli* bacterial cells. Subsequently, a similar technique has been used to introduce recombinant DNA inside mammalian cells, which has resulted in the synthesis of a glycosylated molecule, lenograstim, which has a chemical structure that is undistinguishable from native human endogenous G-CSF. More recently, filgrastim was included in a polyethyleneglycol (PEG) polymer to obtain a pegylated form of G-CSF, named pegfilgrastim, with a permanence in blood circulation for up to 16 days after a single dose administration [2]. As above described, lenograstim is a glycosylated molecule, chemically undistinguishable from native human endogenous G-CSF, with the same sequence and number of aminoacids ( $n=174$ ), whereas filgrastim, its biosimilars and pegylated form are non-glycosylated G-CSF. Glycosylation confers certain pharmacodynamic advantages, making lenograstim more stable to pH and temperature variations, and less susceptible to proteolysis. Lenograstim can be stored and delivered in a wide range of temperature (up to 30 °C), while filgrastim and its biosimilars need to be maintained in the cold chain (2-8 °C). These chemical and pharmacodynamic differences among available G-CSFs lead to relevant peculiarities in induced neutrophil functionality and stem cells mobilization efficacy as well as to variations in safety and cost-effective profiles.

In the present review, we analyze available evidence on the above-mentioned differences among G-CSFs and explore their impact on clinical practice. In fact, a better knowledge of these differences may result in a more personalized use of G-CSFs according to patient's age and characteristics, comorbidity, social well-being, illness condition, and treatment goal.

## CHEMICAL AND PHARMACODYNAMIC DIFFERENCES

The main characteristic of G-CSFs synthesized to be used in human beings is the homology with endogenous human growth factor, which is a 174 amino-acid glycoprotein with a glycosylation in position 133. Filgrastim was the first synthesized G-CSF; its production requires the use of *E. Coli* bacterial cells.

The product of bacterial cell transfected with recombinant DNA which codifies for the G-CSF is slightly different from native human G-CSF. In fact, filgrastim has a different primary structure (amino-acid sequence with a methionine added in N-terminal) and does not have a glycosylation in position 133 [3]. Lenograstim, which is synthesized by transfection of a fragment of recombinant DNA inside a mammalian cell (Chinese hamster ovary – CHO), has a chemical structure that is undistinguishable from native human endogenous G-CSF [4, 5] (Figure 1). These biochemical differences not only are relevant from a chemical-physical perspective, but they also result in different *in vitro* and clinical performances of these G-CSFs.

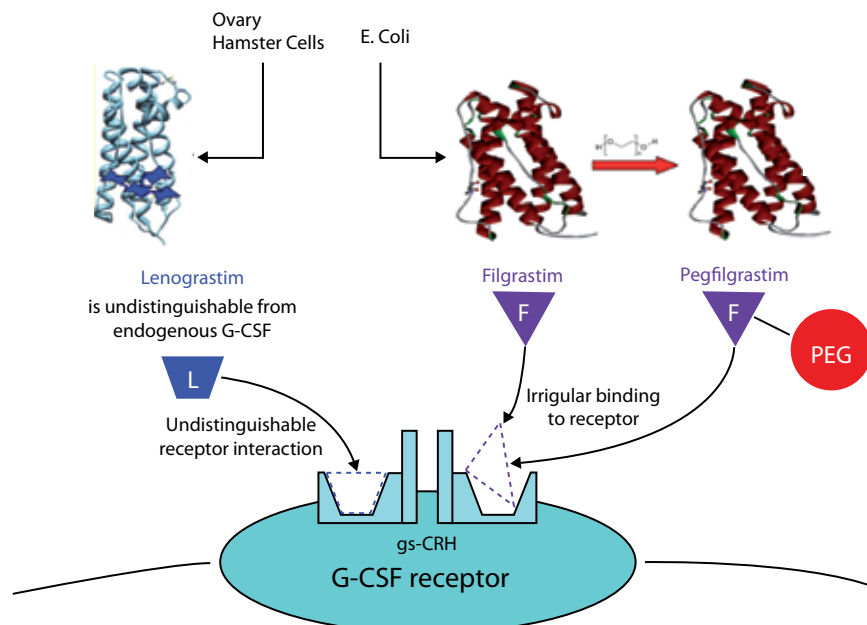
Pegfilgrastim undergoes the same production process as filgrastim, i.e. in *E. Coli* cells, with the addition of a PEG polymer in the final phase of production to prolong drug bioavailability in peripheral blood [2]. In fact, thanks to a mediated neutrophil mechanism, pegfilgrastim is capable of staying in the bloodstream for up to 16 days after a single dose administration: 2

days to reach peak neutrophil counts [6] and at least 14 days from this peak to be eliminated by the same neutrophils [2].

Chemical differences have a relevant impact on drug stability other than efficacy (see below). In fact, both filgrastim and pegfilgrastim are unstable at room temperature and at physiological pH levels; these are the main reasons why these G-CSFs must be stored at a temperature of between 2-8 °C and at an acidic pH level, whereas, similarly to those happens with endogenous human G-CSF, lenograstim is stable at both room temperature and physiological pH [7]. Moreover, glycosylation confers to G-CSF a higher resistance human serum proteins. In fact, *in vitro* studies have demonstrated that glycosylated G-CSF obtained from a CHO expression system is resistant to serum inactivation unlike non-glycosylated molecule obtained from an *E. Coli* expression system. A number of mechanisms have been proposed to explain how serum inactivates non-glycosylated G-CSF including binding of

FIGURA 1

## INTERACTION BETWEEN DIFFERENT G-CSFS AND HUMAN G-CSF RECEPTOR



Lenograstim synthesized in mammalian cells is undistinguishable from endogenous human G-CSF in terms of amino acid sequence (in terms of number), composition of the O-linked sugar chains (glycosylation in position 133), position of sulfur bridges, tertiary structure, and biological activity measured in formation colony *in vitro* tests. Filgrastim is different from endogenous human G-CSF in primary, secondary and tertiary structures. Pegfilgrastim is the pegylated long-acting equivalent of filgrastim.

the cytokine to  $\alpha$ 2-macroglobulin [8] and destruction of the cytokine by serum proteases [9]. The human serine protease enzyme elastase (derived from neutrophils) is able to degrade G-CSF leading to decreased biological activity [10], with glycosylated G-CSF being relatively resistant to elastase degradation. More recent studies suggested that elastase is not responsible for the degradation of G-CSF in serum although this did not exclude the involvement of other serum derived proteases. *In vitro* removal of carbohydrate residues from glycosylated G-CSF by using an enzymatic approach has demonstrated that glycosylation is important in protecting against serum inactivation [11]. This was also confirmed in a mutated G-CSF lacking glycosylation sites. Overall, these observations suggest that human serum reduces the biological activity of non-glycosylated G-CSF in a dose, and temperature dependent manner and deduces that the mechanism of inhibition is dependent upon  $\alpha$ 2-macroglobulin bound serum protease enzymes [11].

The above-mentioned structural differences contribute to influence pharmacodynamic of G-CSFs, in particular, the interaction with receptors. Lenograstim and endogenous G-CSF have almost identical affinity for G-CSF receptors [12, 13]. On the contrary, filgrastim has shown an irregular binding to receptor [12, 13] (Figure 1); the resulting receptor stimulation by filgrastim and lenograstim is different [12, 13]. The G-CSF receptor is present in various steps of the hematopoietic process, from neutrophil proliferation to neutrophil differentiation. In particular, G-CSF is the responsible for the maturation phase of the neutrophil cytoskeleton [14]. Neutrophils induced by filgrastim exhibit an immature immune phenotype, which suggests that non-physiological interaction between this drug and receptors present along the different steps of the maturation of neutrophil cytoskeleton might lead, ultimately, to the formation of defective PMNs [15]. Decreased inflammatory responses, measured with the skin chamber technique, has been observed in filgrastim-induced neutrophils collected from healthy young and elderly volunteers [15]. This effect has been attributed to the relative immaturity of the circulating neutrophils or to alterations in neutrophil phenotype induced by filgrastim [15].

## DIFFERENCES IN INDUCED NEUTROPHIL FUNCTIONS IN VITRO AND IN ANIMAL MODELS

Treatment with rHuG-CSF causes enhancement of functions such as phagocytosis, superoxide anion generation, chemiluminescence, bacterial killing, and antibody-dependent complement-mediated cytotoxicity. Neutrophil phenotype changes after rHuG-CSF administration, with an immediate direct activation of circulating neutrophils and delayed increased expression of important effector molecules directly involved in neutrophil functions, such as CD14, CD32, CD64. These effects may have useful clinical consequence in patients with increased risk of infections, such as cancer patients, subjects with hematologic diseases (myelodysplasia, aplastic anemia), congenital neutropenia, and AIDS. Other changes which characterize neutrophils after rHuG-CSF administration are represented by significant impairment of CD16 expression, chemotaxis, and reduced *in vivo* migration of neutrophils to inflammatory sites. These effects may be explained by bone marrow modifications induced by rHuG-CSF themselves. In fact, treatment with rHuG-CSF cause a significant acceleration of transit time of cells belonging to the myeloid lineage, along with amplification of the mitotic pool and a relative decrease of elements of the post-mitotic pool. It is possible that rHuG-CSF cause a relative immaturity of circulating neutrophils due to the accelerated bone marrow transit time of myeloid cells. It is known that both CD16 expression and chemotaxis properties are acquired by neutrophils in the late stages of maturation, but the time necessary to acquire full functional maturity seems to be shortened by rHuG-CSF administration, and this kinetic aspect may play a non-negligible role in the modification of neutrophil behavior. The impact on neutrophils functions varies among the different available G-CSFs, which should be taken into account with caution at the time to indicate these molecules in high-risk patients.

Therapeutic efficacy of G-CSFs results from a quantitative effect, that is the amplification of the number of neutrophils in the peripheral blood, but also from a qualitative effect, which regards the antimicrobial efficacy of released PMN. The latter effect has been investigated in *in vitro* studies that have demonstrated that

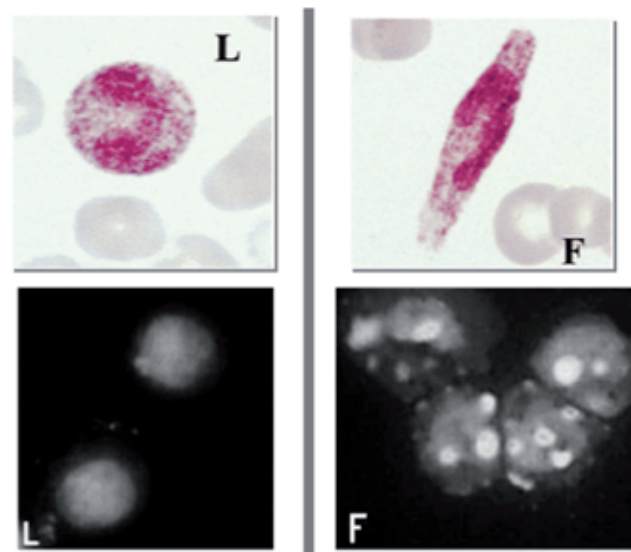
stimulation with lenograstim induces a neutrophil production that is similar to physiological neutrophils, whereas filgrastim produced morphologically anomalous neutrophils [16] (Figure 2). Structural abnormalities in the neutrophils of donors stimulated with filgrastim and, particularly, structural abnormalities of actin and cytoplasmic extrusions, called “blebs” have been demonstrated after phalloidin-FITC staining using fluorescent immunohistochemistry techniques [17, 18]. On the contrary, treatment with lenograstim was associated with F-actin content, distribution, and polymerization kinetics indistinguishable from those displayed by control neutrophils collected from blood donors. These morphological peculiarities result from an anomalous kinetic polymerization of the actin induced by filgrastim, whereas stimulation with lenograstim is followed by a more physiologic process [16, 18] (Figure 3-C).

Cellular motility is based principally on the polymer properties of actin. Thus, neutrophils stimulated by filgrastim show anomalies in movement respect to those released after lenograstim stimulation (Figure 3-B). The effects are visible on the capability of the neutrophils to migrate, to do diapedesis and to form

phagosomes, such the vacuole that engulfs the pathogen in the process of phagocytosis [17] (Figure 3-A). In fact, lenograstim-induced neutrophils display normal chemotaxis values ( $133.5 \pm 10.3$  mm) as compared with normal range (120-160 mm) without differences with basal values ( $137.9 \pm 11.1$  mm), with overall normal kinetics. Filgrastim-induced neutrophils display a defective chemotaxis ( $107.5 \pm 16.3$  mm) as compared with values before administration ( $131.6 \pm 17.7$ mm). Moreover, the typical chemotactic “peak” is replaced by a Gaussian pattern, just as under random conditions. These different effects can be explained by overexpression of  $\beta 2$  integrin or by imperfect cytoskeleton in filgrastim-induced neutrophils, with a consequent reduced chemotactic response. Moreover, membrane deformability strictly depends on a well assembled cytoskeleton and correlates with neutrophil maturation. Recently, a marked contrast between blood ANC and skin localization in filgrastim-induced neutrophils was attributed to the structural immaturity of the circulating neutrophils caused by the accelerated entry into the blood [18]. Major cell morphological modifications, which reflect

FIGURA 2

NEUTROPHILS MORPHOLOGY AFTER G-CSF STIMULATION ASSESSED BY FLUORESCENT IMMUNOHISTOCHEMISTRY TECHNIQUES AND WITH FITC-PHALLOIDIN [16, 17]



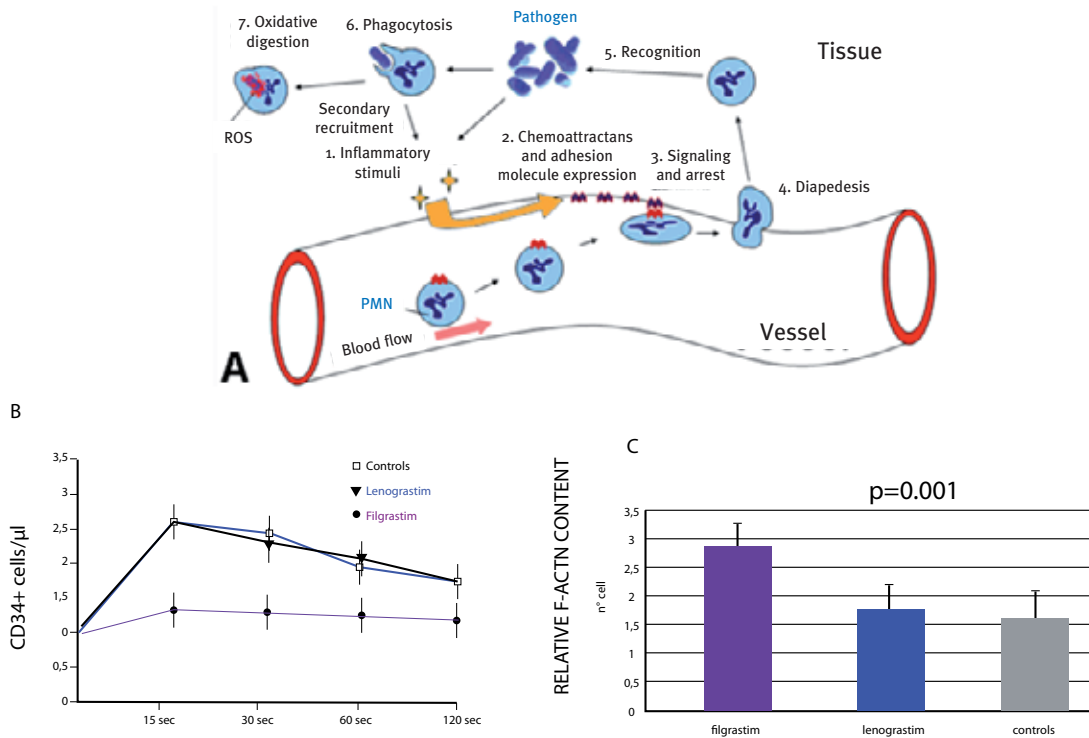
Lenograstim (L) determines a neutrophil production similar to physiological neutrophils. Conversely, stimulation with filgrastim produces morphologically anomalous neutrophils with cytoplasmic extrusions called “blebs” (F).

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FIGURA 3

NEUTROPHIL CAPABILITY OF RECOGNIZING THE PATHOGEN (A) [19]



The kinetic distribution of polymerization processes of the PMN for inflammatory stimuli, confirms that lenograstim leaves the potential reactivity of the PMN intact, guaranteeing polymerization processes, and, thus, motility, similar to controls (B). The anomalous neutrophil obtained with filgrastim show a “motor” anomaly after chemo-attractants stimulation that is reflected both in its migration and phagocytosis capabilities.

The comparison of the polymerization process of actin filaments among filgrastim, lenograstim and controls (C) shows the high rates of polymerization induced by filgrastim. reduce the motility of PMNs. Vice versa, lenograstim does not alter the structure of actin filaments (as in controls), guaranteeing motility of PMN for inflammatory stimuli.

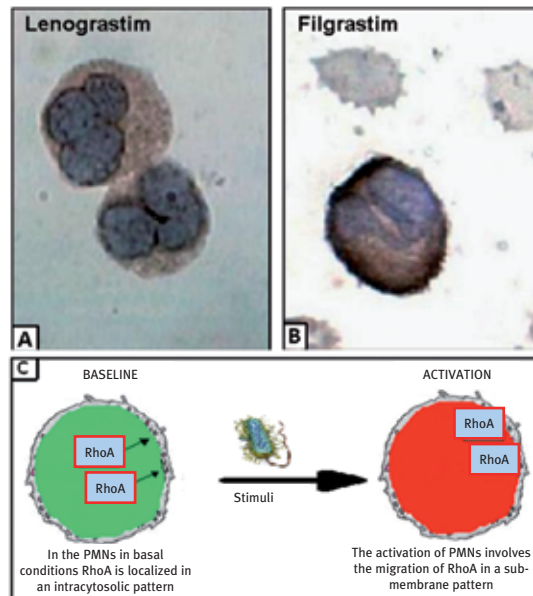
structural defects responsible for the motility disorders of induced neutrophils, are more common after filgrastim than lenograstim administration. These figures suggest that lenograstim seems to respect neutrophil motility more than filgrastim, which, theoretically, may be due to glycosylation.

Filgrastim and lenograstim stimulation results in varied expression of recognition receptors in released neutrophils. The anomalies of the receptors involved in the process of interaction between neutrophils and the surrounding environment causes anomalies in the recognition of the circulating pathogens, as well as diapedesis (migration across the endothelium) (Figure 3). For instance, the expression recognition of receptors CD16 and CD10 is reduced after stimulation with

filgrastim or pegfilgrastim in comparison with neutrophils stimulated by lenograstim [19]. Likewise, the expressions of CD49, an antigen involved in adhesion mechanisms, and of CD14, an activation marker involved in the defense against Gram negative pathogens, show a significantly reduced expression after stimulation with filgrastim respect to lenograstim [19, 20] (Figure 3-A). Conversely to those happened with lenograstim, stimulation with filgrastim is followed by an anomalous expression of the MAC1 complex, which is an important factor involved in neutrophil-endothelial interactions [19] (Figure 3-A). In addition to being involved in recognition and adhesion mechanisms, the above-mentioned markers increase the production of reactive oxygen substances (ROS) during oxidative

FIGURA 4

## NEUTROPHIL STATUS AFTER G-CSF STIMULATION [17]



(A) Lenograstim-mobilized PMNs under the same experimental conditions are not activated: RhoA is expressed in the cytosol. (B) Immunocytochemical brown staining of RhoA shows that the protein is associated with the cell membrane in filgrastim-mobilized cells. The anomalous neutrophil resulting, shows activation abnormalities following a bacterial stimulation and more likely head towards a “functional blockage”. (C) In physiological PMN exposed to bacterial stimulus, the RhoA protein migrates to a sub-membrane position causing the neutrophil to pass from a “basal” state to an “activated” state.

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damage [19] (Figure 3-A). This may suggest that oxidative digestive process is also anomalous in neutrophils that have been induced by filgrastim or pegfilgrastim stimulation [19].

The RhoA protein has been identified as the principal regulator of the activation of neutrophils in response to an exogenous stimulus [21, 17]. In baseline scenarios, the RhoA protein is dispersed uniformly inside the cytosol of the neutrophil (intra-cytosolic pattern), and when an exogenous activating stimulus is encountered (for example, a bacterial stimulus), the RhoA protein migrates from the intra-cytosolic position towards a sub-membrane position (Figure 4-C). Discrepancies in localization of the RhoA protein towards the membrane have been evaluated in neutrophils collected from cancer patients undergoing standard chemotherapy and registered in a randomized trial that compared the effects lenograstim and filgrastim stimulation [17]. Exposure of neutrophils to filgrastim resulted in an increased localization of the RhoA protein towards the membrane respect to that

seen in patients treated with lenograstim. This finding suggests that neutrophils stimulated by filgrastim were constitutively activated in an anomalous way (hyperactive), even at the basal level (Figure 4-B). On the contrary, the neutrophils of patients treated with lenograstim showed more physiological levels of activation at the basal state (Figure 4-A). As a consequence, neutrophils stimulated by lenograstim have preserved their capability to respond to endotoxic bacteria when they have been activated by an exogenous stimulus. Conversely, neutrophils exposed to filgrastim head towards a state of “functional blockage” [17]. In other words, baseline hyper-activated state induced by filgrastim more likely result in a failed response to an exogenous pathogen stimulus [17]. Some authors have also reported similar cytoskeletal, migration and activation anomalies in neutrophils collected from patients treated with pegfilgrastim [22, 23]. Such results are easily foreseeable given that pegfilgrastim, in essence, is a long-acting form of filgrastim [7, 24, 25].

Animal models support a major efficacy of lenograstim than filgrastim in treating chemotherapy-induced neutropenia. For instance, the use of these G-CSFs in normal CD rats and in a rat model of iatrogenic neutropenia has clearly demonstrated that neutrophil recovery after chemotherapy was significantly superior after lenograstim than after equal doses of filgrastim [26].

### DIFFERENCES IN INDUCED NEUTROPHIL FUNCTIONS IN CLINICAL OBSERVATIONS

The above-mentioned chemical differences among different G-CSFs suggest that the process of hematopoiesis should resemble physiological patterns in patients treated with lenograstim, thus ensuring a better quality of neutrophil respect to filgrastim and pegfilgrastim [16, 17, 22, 23]. In other words, neutrophils exposed to lenograstim may be more efficient in the prevention of infective complications, often seen in patients with iatrogenic neutropenia, with respect to those stimulated with filgrastim [5]. The clinical impact of the above-mentioned biochemical differences and their impact on neutrophils capabilities demonstrated by *in vitro* studies should be confirmed in clinical trials comparing the efficacy of filgrastim and lenograstim by a randomized design. This kind of trial should demonstrate actual qualitative clinical differences (neutrophil functionality) in terms of reduction of infectious events and not only quantitative differences in absolute neutrophil count (ANC) or duration of neutropenia.

Efficacy of lenograstim and filgrastim has been assessed in a multi-centric, randomized trial where cancer patients managed with myelotoxic chemotherapy were randomly allocated to receive filgrastim or lenograstim to prevent infectious events [27]. The main goal of the trial has been to compare clinical performance of neutrophils exposed to these G-CSFs though the reduction of febrile episodes recorded in the two treatment groups only after an ANC > 500 has been reached. Theoretically, patients in both groups should not have different incidences of febrile episodes under these ANC conditions. Nevertheless, febrile episodes have been significantly more common among patients treated with filgrastim with respect to patients treated with lenograstim (9% vs. 1%, respectively) [23]. In this trial,

the prevailing infectious agent has not been identified, but all documented cases have been caused by bacterial infections (Figure 5). This trial offers firsthand clinical evidence that even though both G-CSFs are capable of increasing the ANCs, only lenograstim seems to guarantee more normally functioning neutrophils, capable of protecting patients from febrile episodes.

In a small prospective trial assessing the role of rHuG-CSF in preventing complications of iatrogenic neutropenia in children and young subjects affected by solid tumors and managed with myelotoxic chemotherapy, both lenograstim and filgrastim administered at a standard paediatric dose of  $250 \mu\text{g}/\text{m}^2 \pm 5\%$  have been associated with similar results in terms of duration of very severe and severe leukopenia, infections, infection-related hospital stay, and antibiotic treatment [28].

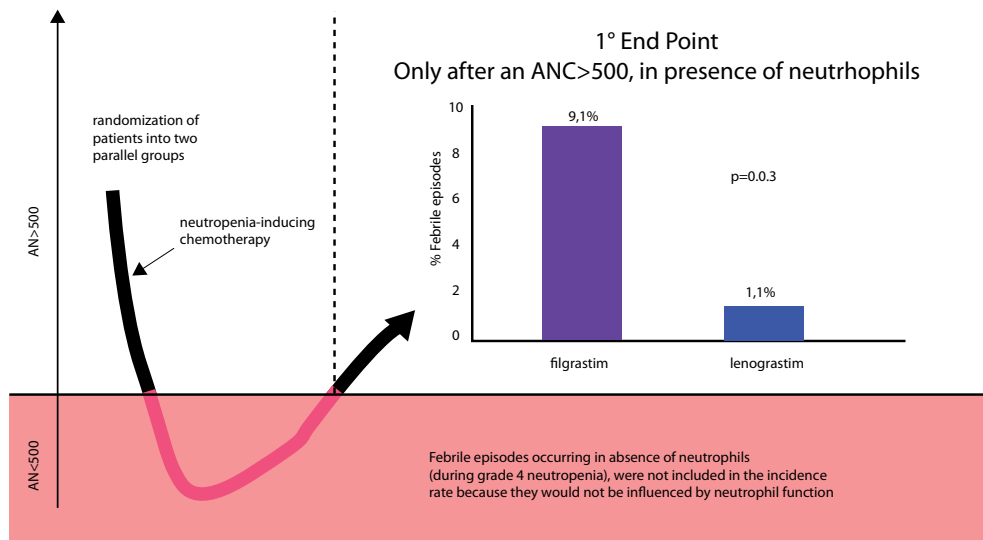
### DIFFERENCES IN MOBILIZATION OF HEMATOPOIETIC STEM CELLS

An important use of G-CSFs regards their capability to stimulate and mobilize hematopoietic stem cells, which can be collected from peripheral blood of both cancer patients and healthy donors. This important attainment has been followed by a worldwide and safe use of both autologous and allogeneic transplantation as part of the therapeutic armamentarium in onco-hematology, with dramatic improvement in overall outcome of some malignant disorders. An important question regards the different effect of glycosylated and non-glycosylated G-CSFs on stem cells mobilization and engraftment rates in patients treated with autologous or allogeneic transplantation.

Different studies compared the mobilizing efficacy of glycosylated and non-glycosylated G-CSFs in cancer patients eligible for high-dose chemotherapy supported by autologous stem cell transplant. Mobilization efficacy of lenograstim has been significantly higher with respect to filgrastim in patients referred to stimulation with high doses of cyclophosphamide [29]. Lenograstim has induced a higher absolute number of collected CD34+ cells, and target of  $3 \times 10^6$  CD34+ cells/kg has been obtained with only two aphaeresis in 75% of patients treated with lenograstim and in 48% of patients treated with filgrastim [29] (Figure 6). Mobilization

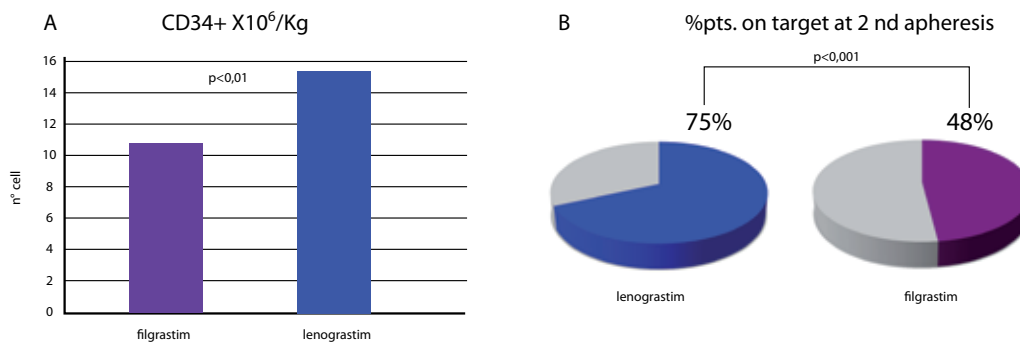


FIGURA 5

**RANDOMIZED, MULTI-CENTRIC, DIRECTLY COMPARATIVE CLINICAL STUDY BETWEEN LENOGRASTIM AND FILGRASTIM ON THE INCIDENCE OF FEBRILE EPISODES IN CANCER PATIENTS WITH AN ANC>500/ $\mu$ L**


The prevailing etiological agent was not identified. However, all documented cases were caused by bacterial infections. This suggests that a functional blockage of neutrophils may have been responsible for the poorer clinical performance seen in filgrastim treated patients.

FIGURA 6

**MOBILIZATION OF CD34+ CELLS WITH LENOGRASTIM VS. FILGRASTIM**


Absolute mobilization (A) and percentage of dose target of  $3 \times 10^6$  CD34+cells/kg obtained with only two aphaeresis procedures (B).

seems to be earlier when patients receive lenograstim, but improvement regards a one-day early availability of stem cells [27]. A prospective randomized study comparing the efficacy of bioequivalent doses of glycosylated and non-glycosylated G-CSFs for mobilizing peripheral blood progenitor cells in breast cancer patients undergoing FEC chemotherapy (5Fluorouracil, Epirubicin and Cyclophosphamide) has showed that both lenograstim and filgrastim produce a comparable number of mobilized CD34+

cells, but mobilizing dose of lenograstim was almost a quarter than that of filgrastim [30]. In line with this study, another randomized trial has demonstrated that a 25% dose reduction of lenograstim is as efficacious as full dose of filgrastim both in terms of mobilization efficacy and number of aphaeresis procedures [31]. However, the effects of bioequivalent doses of G-CSFs should be interpreted cautiously since the large individual variability in response to G-CSF in cancer patients [32]. Moreover,

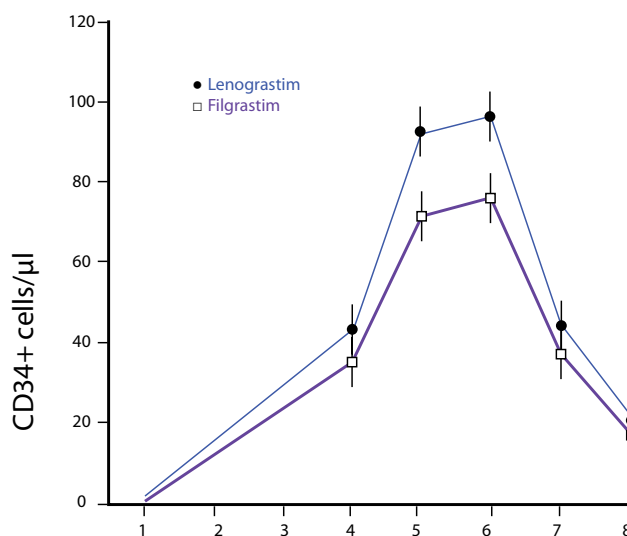
increasing the dose of filgrastim may compensate for this activity difference, probably without a deleterious effect on the patient.

The mobilizing capability of G-CSF in healthy donors is an important issue considering the growing role of allogeneic transplant both in onco-hematology and other medical fields. Accordingly, different aspects of mobilizing efficacy of lenograstim and filgrastim have been compared in randomized trials. A randomized clinical study comparing CD34+ cells mobilizing rates after filgrastim or lenograstim in healthy volunteers has demonstrated that lenograstim exhibits a higher mobilization efficacy, with a significant increment of the area under curve in days 4-8 [33] (Figure 7). A small randomized trial assessing hematological effects of lenograstim and filgrastim administered at a dose of 5 µg/kg/day, for 6 days, administered following a crossover principle after a 4-week washout period has demonstrated that lenograstim administration is followed by a significantly higher peak of WBC, and a higher GM-CFC count [34]. Importantly, serum concentrations of G-CSF molecules have been significantly higher with filgrastim, which excludes that the difference in bio-efficacy could be due to a difference in G-CSF stability. A large study

focused on the mobilizing efficacy of G-CSFs administered at a dose of 10 µg/kg/day for 4 consecutive days and an aphaeresis procedure on the fifth day has been performed in 400 healthy donors with the aim to collect between 4 and 10 x 10<sup>6</sup> CD34+ cells/kg [35]. The target number of CD34+ cells has been obtained with a single aphaeresis procedure in 63% of donors and with two procedures in 81%. Only 2% of donors have mobilized a number of cells inferior to the target level (defined as “poor mobilizers”). The average of collected CD34+ cells has been significantly higher in the group of donors treated with lenograstim with respect to the filgrastim group (5.1 x 10<sup>6</sup> and 4 x 10<sup>6</sup> CD34+/kg, respectively), with a ~40% increase [35] (Figure 8). An additional randomized study has demonstrated that the positive effect of lenograstim on CD34+ cells mobilization in healthy unrelated donors is more evident among males than females [36]. Five hundred and one donors have been randomly allocated to receive lenograstim or filgrastim at 10 µg/kg BW per day, with aphaeresis on day 5. The number of CD34+ cells collected has been 11.5% higher in the lenograstim group (7.19 x 10<sup>6</sup> vs. 6.44 x 10<sup>6</sup>/kg BW donor; p<0.03), with a significantly higher amount of harvested

FIGURA 7

NUMBER OF CD34+ CELL/µL OF BLOOD IN 30 HEALTHY VOLUNTEERS RECEIVING G-CSFS



Both lenograstim and filgrastim have been administered at a dose of 10 µg/kg s.c. once daily for 5 consecutive days.

progenitors cells among males ( $7.73 \times 10^6$  vs.  $6.88 \times 10^6$ ;  $p < 0.01$ ) [36].

All together, this cumulative evidence, mostly coming from randomized trials, strongly suggest that lenograstim produce a better CD34+ cells mobilization both in cancer patients and healthy donors. The superiority of lenograstim has been attributed to its physiological properties which, thanks to its greater affinity to receptors, cause a more powerful peripheral mobilization in stem cells. However, the clinical impact of this advantage, measured in terms of better engraftment and lower septic complications, remains to be confirmed by future trials.

## DIFFERENCES IN SAFETY DATA

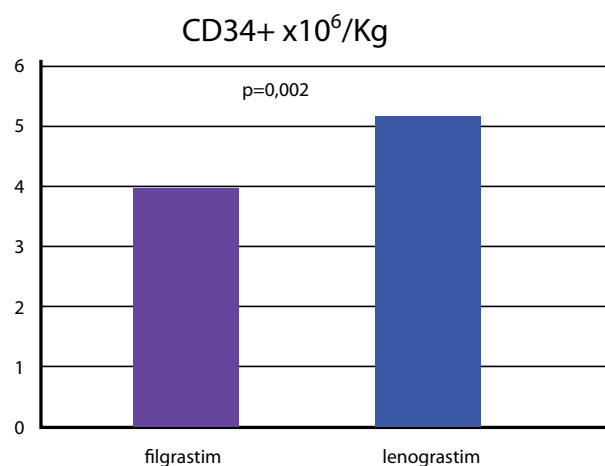
The wide use of G-CSFs in ordinary clinical practice and prospective trials, both on cancer patients and healthy donors results in a growing concern on the safety profile of available molecules. The exact characterization of the long-term safety profile of these G-CSFs is perhaps the single greatest aspect one focuses attention on when selecting a drug as part of a tailored treatment regimen. This topic assumes greater importance if G-CSFs are administered to “tumor free” subjects, such as a healthy donor, or patients undergoing adjuvant care for breast cancer, on in the case of patients with longer life expectancies (e.g. lymphomas). Theoretically, the use of a

G-CSF whose stimulation resembles a natural physiological mechanism as much as possible is one way of protecting patients and donors, which is guaranteeing them a more safety therapy. In this theoretical context, lenograstim may be advantaged with respect to filgrastim and pegfilgrastim since, as above discussed, it interacts with G-CSF receptor in such a way that it is undistinguishable from the natural endogenous ligand [2, 12, 13].

Development of acute myeloid leukemia and/or myelodysplastic syndrome is the main long-term side effect of G-CSF, which requires an accurate monitoring assessment in prospective trials for a long-lasting follow-up [37-42]. In this context, trials investigating this side effect in healthy donors are more suitable than studies focused on cancer patients; essentially, this type of trials exclude myelodysplastic and leukemogenic effects of antineoplastic drugs, radiotherapy and other medications, which are not currently used in healthy donors [43]. Currently, the only such wide scale studies have been carried out on lenograstim use. A prospective trial aimed to evaluate the long-term effects of lenograstim in 184 CD34+ cells donors has confirmed safety profile of this G-CSF [44]. In fact, after a median follow-up of 62 months, no subject has developed hematological neoplasia. In a cumulative study on 3 928 healthy donors stimulated with lenograstim ( $7.5 \mu\text{g}/\text{kg}$  per day) and observed for 12 years, this G-CSF has showed a safe and effective profile, with a hematopoietic

FIGURA 8

MOBILIZATION OF CD34+ CELLS IN HEALTHY DONORS TREATED WITH FILGRASTIM (n=116) OR LENOGRASTIM (n=147) AT A SUB-DERMAL DOSE OF  $10 \mu\text{G}/\text{KG}/\text{DAY}$



stem cells mobilizing rate of 97%, no cases of mobilization interruption, and only with transitory and mild short-term adverse events [45]. Bone pain and headache were the most common side effects of lenograstim. Central venous access was required for donations in 0.6% of cases. Throughout the follow-up, the ANC's were slightly lower the initial baseline values but remained within the normal range. The majority of the donors reported good or very good health. Only four donors have developed hematological tumors (acute myeloid leukemia, chronic lymphatic leukemia, two cases of Hodgkin lymphoma); only the incidence of Hodgkin lymphoma differed significantly from those reported in an age-adjusted population [45]. On the other hand, reliable data on long-term effects of filgrastim are not available. This is more relevant for its long-acting analog, pegfilgrastim, which remains in the bloodstream for at least about 16 days after a single administration [46]. The overexposure to G-CSF may heighten the associated risks [45]. Thus, administration of G-CSF should be as short as possible to ensure chemotherapy dose intensity, with a mean duration of treatment of  $\approx 5$  days, suspending G-CSF administration when ANC is  $\geq 1000$  cells/ $\mu\text{l}$  after the nadir [47-49]. Similarly, G-CSF administration should be as short as possible to collect adequate amounts of hematopoietic stem cells in healthy donors, with the aim to maintain safety profile.

## COST-EFFECTIVE DIFFERENCES

Available rHuG-CSFs and pegylated equivalent are important cost-effective resources in cancer treatment. Overall, prophylaxis with G-CSF shortens the duration of chemotherapy-induced neutropenia, hospitalization for infection and intravenous antimicrobial therapy in cancer patients treated both with conventional-dose chemotherapy and/or hematopoietic stem-cells transplantation, with a consequent positive effect on health costs. Sensitivity analyses have demonstrated that the total cost of treatment rises as the probability of febrile neutropenia increases, but the costs increase more rapidly in patients who do not receive G-CSF [50-55]. At the threshold risk of febrile neutropenia, the added cost of rHuG-CSF is equaled by a reduction in costs associated with hospitalization, which

constitutes the major expenses associated with febrile neutropenia. Above the risk threshold, the cost is less when rHuG-CSF is used. The above-discussed goals of G-CSF use, that is fast neutrophil recovery, neutropenia prophylaxis and stem cells mobilization, facilitate the administration of dose-intense or dose-dense chemotherapy regimens, with improved clinical outcomes, which is an important parameter in cost-effectiveness estimations. In addition, rHuG-CSFs are generally well tolerated in most treatment settings, rarely requiring the use of drugs to treat related side effects, with an insignificant impact on cost-effectiveness balance.

Despite the unquestionable cost-effectiveness value of rHuG-CSFs, differences in economic parameters among available molecules have not been demonstrated by formal randomized trials. However, some indirect evidence supports a cost advantage in favor of lenograstim. In fact, several randomized trials have showed no significant differences between lenograstim and filgrastim in terms of mobilization efficacy [27, 30, 31, 56, 57], but it is important to underline that these results were achieved by using lenograstim dosages that were  $\approx 25\text{--}30\%$  lower than the filgrastim dosages [27, 31, 56]. These dosages have been considered equipotent given that lenograstim demonstrated greater *in vitro* potency than filgrastim. Moreover, the median duration of G-CSFs administration to achieve the prefixed CD34+ cell endpoint was significantly shorter for lenograstim 5  $\mu\text{g}/\text{kg}/\text{day}$  than for filgrastim [57], with was significantly shorter median time to an ANC  $> 0.5 \times 10^9/\text{L}$  in subjects managed with lenograstim (3 vs. 4 days;  $p=0.0005$ ). Importantly, lenograstim 10  $\mu\text{g}/\text{kg}/\text{day}$  has been more effective than filgrastim 10  $\mu\text{g}/\text{kg}/\text{day}$  in terms of the proportion of patients with successful mobilization and the CD34+ cell harvest in a nonrandomized trial on patients with lymphoma or myeloma [29]. In a second nonrandomized trial [58], the median number of CD34+ cells harvested did not significantly differ between lymphoma patients who received lenograstim 263  $\mu\text{g}/\text{day}$  and those who received filgrastim 10  $\mu\text{g}/\text{kg}/\text{day}$ , despite the lower lenograstim dosage. All this evidence analyzed together seems to suggest that lenograstim and filgrastim are associated with similar quantitative results in terms of stem cells mobilization and neutrophil recovery, but that lenograstim produces these results with a lower daily dose and shorter administration duration, which is necessarily associated with

a better cost-effectiveness effect. Accordingly, we have to underscore that recommended dose of lenograstim in hemopoietic stem cell transplantation is  $150 \mu\text{g}/\text{m}^2$ , which is similar to the dose suggested to prevent iatrogenic neutropenia, whereas recommended dose of filgrastim is  $10 \mu\text{g}/\text{kg}$ , which is twice the dose required to reduce iatrogenic neutropenia [59]. In other words, in most cases, approximately one vial of lenograstim is necessary, while two vials of filgrastim are approximately requested for identical efficacy. In these conditions, the overall cost of patient care is increased proportionally. Nevertheless, the best economic criteria when choosing a G-CSF remain to be defined. Actually both molecules are not identical, and do not have the same molecular weight, and, accordingly, it is not logical to compare them by their price per microgram. Thus, economic criteria to select G-CSF largely depend on the habits of people using these drugs in the hospital [13]. The choice of G-CSF must be made according to the daily cost of treatment [ $255 \mu\text{g}$  for lenograstim and  $325 \mu\text{g}$  for filgrastim for an average patient) in hospitals where the exact dosages are used ( $150 \mu\text{g}/\text{m}^2$  for lenograstim or  $5 \mu\text{g}/\text{kg}$  for filgrastim). In hospitals where one vial per patient per day is used, whatever be their weight of body surface area, both the prices per million units and per vial should be considered together, putting into perspective the potential therapeutic benefit for patients, one vial of lenograstim containing more million units than one vial of filgrastim [13].

## CONCLUSIONS

The wide use of G-CSFs in oncology and hematology resulted in treatment delivery with maintained dose intensity, significant reduction of febrile neutropenia and related septic complications and hematopoietic stem cells collection both in cancer patients

eligible for autologous transplantation and healthy donors. These applications resulted in remarkably improved outcome in several subgroups of patients with hematological malignancies and solid tumors. All G-CSFs are efficacious in producing an increase in the circulating neutrophils count; however, these molecules exhibit relevant pharmacokinetic, safety, stability, and pharmacodynamic differences, with lenograstim having a mechanism identical to those of native human G-CSF. Conversely, filgrastim, and its long-acting analog pegfilgrastim, do not have a counterpart existing in nature. Consequently to its physiologic mechanism, lenograstim delivery is followed by the expansion of mature neutrophils that function normally, whilst filgrastim and pegfilgrastim, perhaps due to anomalous receptor interactions, produces immature neutrophils which tend to be underactive. This feature reflects in a better protection from febrile episodes of lenograstim-induced neutrophils. Lenograstim has also shown to be more active than filgrastim in the mobilization of stem cells both in cancer patients and healthy donors, with suitable long-term safety data. Currently, one should rely on the minimal G-CSF dose necessary to induce adequate amounts of mature neutrophils, with minimal risk of late hematological complications, which are of paramount importance in healthy donors and cancer patients with long-life expectancy. Finally, several issues regarding G-CSF administration modalities and indications deserve to be addressed in future studies, and further development of these molecules will result in new indications and improved outcome in the best candidates.

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