Original article

Lipegfilgrastim: pharmacodynamics and pharmacokinetics for body-weight-adjusted and 6 mg fixed doses in two randomized studies in healthy volunteers

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Abstract

Objective:

Two phase I, single-blind (subject blinded to treatment), randomized studies were conducted to assess the pharmacodynamics, pharmacokinetics, safety, and tolerability of lipegfilgrastim compared with pegfilgrastim in healthy adult volunteers.

Methods:

Study 1 consisted of a pilot safety phase ($N = 8$) during which subjects received a single body-weight-adjusted subcutaneous dose of lipegfilgrastim 25 μg/kg and a dose escalation phase ($N = 45$) wherein subjects received lipegfilgrastim 50 or 100 μg/kg or pegfilgrastim 100 μg/kg. Study 2 was a single-blind, fixed-dose study ($N = 36$) comparing subcutaneous lipegfilgrastim 6 mg and pegfilgrastim 6 mg.

Results:

Cumulative exposure ($AUC_{0-tlast}$ and $AUC_{0-\infty}$) and peak exposure (C_{max}) were higher for lipegfilgrastim than pegfilgrastim after both weight-adjusted and fixed dosing. In both studies, the terminal elimination half-life of lipegfilgrastim was 5–10 hours longer than the terminal elimination half-life for pegfilgrastim at the maximum dose, and the time to maximum serum concentration (t_{max}) was observed later for lipegfilgrastim than for pegfilgrastim. The area over the baseline effect curve ($AOBEC$) for absolute neutrophil count (ANC) was approximately 30% greater after lipegfilgrastim dosing compared with the same dose of pegfilgrastim at the maximum dose. Both drugs were well tolerated, with a similar occurrence of adverse events between treatment groups. Key limitations of these studies include the small numbers of subjects and differences in dosage regimens between the two studies.

Conclusions:

In these studies, lipegfilgrastim provided a longer-lasting increase in ANC compared with pegfilgrastim at an equivalent dose, without increasing the peak ANC values. This may reflect the higher cumulative exposure and slower clearance (therefore longer body residence) of lipegfilgrastim. These data support the use of single-dose lipegfilgrastim 6 mg in subsequent phase III trials as prophylactic treatment for patients receiving myelosuppressive chemotherapy.

Introduction

Neutropenia is a commonly reported dose-limiting toxicity associated with cyto-toxic cancer chemotherapy regimens[1]. Defined as a decrease in white blood cell (WBC) counts of the neutrophil granulocytic lineage, it is associated with an increased risk for opportunistic infection and fever, in part because of the

reduced ability of the immune system to respond to disturbances of the gut mucosa and the gut microflora and related structures[2]. The development of fever in neutropenic patients, i.e., febrile neutropenia (FN), is indicative of potentially fatal opportunistic infections, and often requires hospitalization and the use of intravenous antibiotics[3–5]. The risk of initial infection and subsequent complications is inversely proportional to a patient's absolute neutrophil count (ANC), and when the ANC is $<1.5 \times 10^9$/L, the risk begins to increase[3]. Therefore, preventing FN is a crucial consideration when treating cancer patients with chemotherapy.

Recombinant granulocyte colony-stimulating factor (G-CSF) and granulocyte–macrophage colony-stimulating factor products have emerged as effective therapies for reducing the duration and incidence of chemotherapy-induced neutropenia and FN by stimulating neutrophil proliferation and differentiation[6–9]. These growth factors are now well established as prophylactic therapies for the prevention of FN and are recommended in treatment guidelines for patients receiving chemotherapy whose risk for developing FN is $\geq 20\%$[10,11]. Filgrastim, a recombinant methionyl human G-CSF (r-metHuG-CSF) produced in *Escherichia coli* (*E coli*), was introduced to clinical practice in 1991 under the brand name Neupogen*. It was followed by a long-acting formulation, pegfilgrastim (Neulasta†), in 2002.

Unlike standard r-metHuG-CSFs, such as filgrastim, which require daily subcutaneous (SC) injections, the attachment of a polyethylene glycol (PEG) molecule to filgrastim (e.g., pegfilgrastim, a covalent conjugate of r-metHuG-CSF and PEG)[12], decreases plasma clearance and extends the drug's terminal elimination half-life ($t_{1/2}$) in the body, allowing for less frequent dosing[13].

Lipegfilgrastim (previously known as XM22) was approved by the European Medicines Agency in July 2013 and launched in Germany in November 2013 and in the United Kingdom in February 2014 for the reduction of the duration of severe neutropenia (DSN) and the incidence of FN in patients receiving chemotherapy[14,15]. Approval was based on findings from three double-blind, randomized studies of lipegfilgrastim administered once per cycle to patients receiving four cycles of chemotherapy (study duration 12 weeks): a phase II dose-finding study in patients with breast cancer receiving lipegfilgrastim doses 3, 4.5, or 6 mg ($n = 154$) or pegfilgrastim 6 mg ($n = 54$)[16], a phase III study in patients with breast cancer receiving lipegfilgrastim 6 mg ($n = 101$) or pegfilgrastim 6 mg ($n = 101$)[17], and a phase III study in patients with non-small-cell lung cancer receiving lipegfilgrastim 6 mg ($n = 250$) or placebo ($n = 125$)[18]. In the breast cancer studies, DSN in cycle 1 (the primary end point) was similar in

the lipegfilgrastim and pegfilgrastim groups. In the lung cancer study, febrile neutropenia in cycle 1 (the primary end point) was not significantly different between patients receiving lipegfilgrastim and placebo, but was numerically lower in patients receiving lipegfilgrastim (2.4% vs 5.6%, respectively).

Lipegfilgrastim differs from pegfilgrastim in its modification, as it is a covalent conjugate of filgrastim with a single methoxyPEG molecule at the natural glycosylation site of the protein attached via a carbohydrate linker consisting of glycine, N-acetylneuraminic acid, and N-acetylgalactosamine. Specifically, the methoxyPEG moiety of lipegfilgrastim is linked to the threonine 134, whereas the PEG moiety of pegfilgrastim is chemically linked to the N-terminus of filgrastim[19,20].

Two phase I studies were conducted to determine the pharmacokinetic (PK) and pharmacodynamic (PD) properties of lipegfilgrastim in healthy subjects and to compare the properties with those of pegfilgrastim. In study 1, two body-weight-adjusted ascending doses of lipegfilgrastim (50 µg/kg and 100 µg/kg) were administered SC to healthy volunteers to assess the PD effects, PK parameters, safety, and tolerability of lipegfilgrastim. A body-weight-adjusted dose of pegfilgrastim (100 µg/kg) was used as a positive control. Study 2 compared a fixed 6 mg SC dose of lipegfilgrastim with a 6 mg SC dose of pegfilgrastim to determine further PK and PD data for lipegfilgrastim. These doses were based on the pegfilgrastim dose recommended for use in clinical practice. While assessment in healthy volunteers versus cancer patients may differ in terms of benefit to risk ratio owing to the effects of the patients' underlying disease, cytotoxic chemotherapy, or neutropenia-associated complications, this dose level is well suited to assess PK/PD relationships, without these confounding factors.

Methods

Subjects

Healthy female and male subjects were recruited at a single study center in Switzerland. Subjects were deemed healthy on the basis of a medical history, physical examination, clinical laboratory tests, serology, urinalysis, urine pregnancy test (β-HCG test) in females, and an electrocardiogram (ECG). All subjects were aged 18 to 45 years, had a body weight of 50 to 95 kg, and had a body mass index of 18.5 to 29.9 kg/m².

Exclusion criteria included: evidence of infection with hepatitis B or C virus or human immunodeficiency virus; impaired hepatic function (alanine aminotransferase or aspartate aminotransferase $>2 \times$ upper limit of normal [ULN] and gamma-glutamyl transpeptidase $>3 \times$ ULN); impaired renal function (serum creatinine >210 µmol/L

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*Neupogen is a registered trade name of Amgen Inc., Thousand Oaks, CA, USA
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[males], $>190\,\mu$mol/L [females], or $>1.5 \times$ ULN); drugs or alcohol abuse; a donation of >500 mL of blood during the previous 3 months; treatment with any prescription medication within 14 days of administration of the study drug; use of over-the-counter medication in the previous 7 days; or a history of severe allergic disease. All subjects had no prior exposure to filgrastim, pegfilgrastim, or lenograstim and had no history of hypersensitivity to pegfilgrastim, filgrastim, or *E coli* derived proteins. Subjects were hospitalized for 12 hours before dosing (day 0) and remained in the hospital until day 7. Screening and study days 10, 14, 17, and 21 (follow-up) were ambulatory visits. Subjects were allowed to withdraw from the study at any time. Table S1 (available online) summarizes the subjects and the treatment schedules.

Study design

Study 1 was a single-blind (i.e., the subject was blinded to treatment), dose escalation study in which subjects received a single dose of lipegfilgrastim $50\,\mu$g/kg or $100\,\mu$g/kg or pegfilgrastim $100\,\mu$g/kg, with 21 days of follow-up. A dose of lipegfilgrastim $200\,\mu$g/kg was planned if needed or in case of acceptable tolerability of the $100\,\mu$g/kg dose. Study 2 was a single-blind (i.e., the subject was blinded to treatment), fixed-dose study in which subjects received a 6 mg SC dose of either lipegfilgrastim or pegfilgrastim, with a follow-up period of 21 days. In both studies, each eligible subject was assigned a randomization number in the sequence of study entry. In study 1, subjects were stratified by body weight and sex to one of four strata and were assigned to treatment using random permutations; in study 2, subjects were stratified by body weight and sex to one of six strata, and half of the subjects within each stratum were administered each study treatment using random permutations.

Female and male subjects were included in each study to evaluate PD and PK properties in both sexes. Weight stratification aimed to ensure the representation of subjects with low and high body weight. The weight-dependent dose of pegfilgrastim $100\,\mu$g/kg was determined in previous phase II and III studies in breast cancer patients undergoing chemotherapy[1,5]. The pegfilgrastim fixed dose of 6 mg was determined in a separate phase III trial in breast cancer patients undergoing chemotherapy, stratified by body weight[21]. Subjects in study 2 also were stratified according to sex and weight to assess the effects of these parameters on the pharmacokinetics of fixed-dose 6 mg SC lipegfilgrastim (Table S2, available online).

Several measures were taken to ensure that no potential adverse events (AEs) for humans, which may have been underestimated or missed because of unavoidable limitations of preclinical safety testing, posed unacceptable risks to the study subjects. Before the dose escalation in study 1, a pilot cohort of eight subjects received a $25\,\mu$g/kg dose of lipegfilgrastim in a staggered fashion to ensure that the number of subjects exposed to unavoidable risks was kept to a minimum. Staggering involved the following: one subject was dosed; after 1 week of observation, two more subjects were dosed, and after 1 week of observation of the second and third subjects, the remaining five subjects were dosed. To further ensure the safety of each subject, the $100\,\mu$g/kg dose in study 1 and the 6 mg fixed dose in study 2 were tested only after the safety parameters of the $50\,\mu$g/kg dose were fully evaluated in treated subjects.

Both studies were conducted in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice Guidelines and were approved by the Independent Ethics Committee of Basel, Switzerland. All subjects provided written informed consent before enrollment.

Pharmacodynamic assessments

In both studies, blood samples for PD evaluation were taken immediately after PK sampling at overlapping time points. Blood samples for the determination of ANC-derived parameters were collected immediately before dosing (<2 hours) and at 2, 4, 8, 12, 24, 48, 72, 96, 120, 144, and 168 hours after dosing, as well as in the morning of days 10, 14, 17, and 21. ANC was determined by the Advia 120 differential automated hematology analyzer at Bioscientia Institut für Medizinische Diagnostic GmbH (Ingelheim, Germany).

The primary end point of both studies was the change in ANC with lipegfilgrastim compared with pegfilgrastim, summarized by its area over the baseline effect curve (*AOBEC*). The use of ANC as the main PD outcome was chosen based on the mechanism of action of the drug, whereby the aim is to prevent or reduce neutropenia. The use of *AOBEC*, unlike the maximum increase in ANC, allowed the integration of the magnitude and duration of the drug effect in a single parameter and was less affected by uncertainties related to the timing of sampling and unavoidable imprecision. *AOBEC* was calculated as follows: the individual baseline value obtained before dosing was subtracted from ANC values obtained after dosing. Using these values, the area under the curve (*AUC*) versus time expressed in hours was calculated using the linear trapezoidal rule for all time points or the last value before the subject was below his/her baseline ANC. Negative values were set either to zero before the last value over the individual baseline or to missing after this value. Secondary PD end points included ANC_{max} (maximum observed ANC value); $ANCt_{max}$ (time point

at which ANC_{max} was found); and ANC time to return to baseline.

Blood samples for the determination of CD34+ cell counts (i.e., stem cells and progenitor cells carrying the antigen CD34 on their surface) were collected immediately before dosing (<2 hours) and at 24, 48, 72, 96, 120, 144, and 168 hours after dosing, as well as in the morning of days 10, 14, 17, and 21. Flow cytometry for CD34+ cell counting was performed on a FacsCalibur instrument (Becton, Dickinson & Co., Franklin Lakes, NJ, USA) located at the Bioscientia Institut für Medizinische Diagnostic GmbH. Summary statistics of CD34+ cell count derived PD parameters included $CD34+_{max}$ (maximum measured value of CD34+ cell count after dosing), $CD34+$ $AOBEC$, and CD34+ peak serum concentration (t_{max}; time point after dosing at which $CD34+_{max}$ is achieved). The $AOBEC$ for blood CD34+ cell count was calculated in a fashion similar to the ANC $AOBEC$. The protocol of the International Society of Hematotherapy and Graft Engineering (ISHAGE)[22], which standardizes the measurement of CD34+ cells across flow cytometers, antibodies, and sites, was not employed in the current study protocols because all CD34+ measurements were conducted using the same methodology, in the same laboratory, and using the same equipment. Blood samples were also taken at screening and at follow-up in both studies to assess the formation of antibodies against G-CSF.

Pharmacokinetic assessments

In both studies, venous blood samples were collected according to a predetermined schedule: immediately before study drug administration (<0.5 hours) and at 1, 2, 3, 4, 8, 12, 18, 24, 30, 36, 48, 72, 96, 120, 144, and 168 hours after dosing, as well as in the morning of days 10, 14, 17, and 21. The PK assessments of the active treatments and the calculation of PK characteristics were based on G-CSF serum concentration measurements over time. Analysis of G-CSF concentrations in blood serum was performed using a validated Good Laboratory Practice method based on an enzyme-linked immunosorbent assay (ELISA; Quantikine, R&D Systems, Minneapolis, MN, USA). The following noncompartmental PK parameters were assessed: area under the serum concentration versus time curve from time 0 to the last data point ($AUC_{0-tlast}$), AUC versus time curve extrapolated to infinity ($AUC_{0-\infty}$), clearance (CL), maximum observed serum concentration (C_{max}), time to reach C_{max} (t_{max}), terminal half-life ($t_{1/2}$), the rate constant associated with the terminal phase (λ_z), mean residence time (MRT; the average time a molecule spends in the body), and relative bioavailability. No baseline corrections were applied for the calculation of the PK parameters.

Safety assessments

All clinical AEs and laboratory abnormalities were evaluated by the investigators for a potential relationship to lipegfilgrastim or pegfilgrastim. Blood pressure, heart rate, respiratory rate, and body temperature were monitored before dosing, 12 hours after dosing, and in the morning of days 1 through 7 and days 10, 14, 17, and 21. An ECG was recorded before dosing and on days 1, 2, 3, 4, 7, and 21. Laboratory parameters were assessed before dosing (day 0) and on follow-up days 3, 7, and 21. A sonographic examination of the spleen, with special attention to its size, was performed within 96 hours before and 24, 48, and 96 hours after dosing, before discharge from the study center (1 week after dosing), at follow-up examination, and if a subject complained of pain in the left upper quadrant of the abdomen or in the left shoulder.

Statistical analysis

For both studies, a formal sample size calculation was not feasible. The statistical analysis of the PD and PK end points was based on the per-protocol population, which included all subjects who received study drug and completed sampling for ANC determination, as stated in the protocol, without major deviations. Statistical evaluations were based on the use of shortest confidence intervals (CIs; at the 90% level for PK parameters and at the 95% level for PD parameters) for the geometric means ratio of each different parameter after the 50 µg/kg, 100 µg/kg, and 6 mg lipegfilgrastim doses divided by the corresponding value after the reference 100 µg/kg or 6 mg dose of pegfilgrastim. The CI limits were compared with the conventional bioequivalence limits (80%–125%) or to expanded limits (70%–143%) for the CD34+ cell counts because of its high variability. A difference was considered statistically significant if the CIs rejected a value of 1 for the ratio of geometric means or a value of 0.00 for the difference of the medians. A factorial analysis of variance (ANOVA) model using type I (unadjusted) and type III (adjusted for other effects) tests was used to evaluate the impact of lipegfilgrastim treatment on PD and PK ($AUC_{0-\infty}$, $AUC_{0-tlast}$, and C_{max}) parameters compared with pegfilgrastim, treatment, sex, and body weight as fixed effects in study 2.

Results

Study population

Study 1 was conducted from October 2006 to June 2007, and study 2 was conducted from March 2007 to June 2007. In total, 89 healthy volunteers were enrolled: 8 subjects in the pilot phase, 45 subjects in the body-weight-adjusted

single-dose study (study 1 in Table 1), and 36 subjects in the fixed single-dose study (study 2 in Table 1). All 89 subjects received one dose of lipegfilgrastim or pegfilgrastim, and all completed the study. Most subjects were white males (Table S3; available online).

Pharmacodynamic analysis

In both studies, the ANC AOBEC was approximately 30% greater after administration of lipegfilgrastim compared with the same dose of pegfilgrastim. Baseline-corrected ANC values for studies 1 and 2 are plotted in Figure 1. In study 1, the geometric mean ANC AOBEC was approximately 32% higher (point estimate for the ratio: 1.3246) in subjects who received lipegfilgrastim 100 µg/kg compared with the same dose of pegfilgrastim. The difference was significant, as demonstrated by the shortest 95% CI (1.1545–1.5198), rejecting an equal or lower value for the 100 µg/kg dose of lipegfilgrastim. Similarly, in study 2, the geometric mean ANC AOBEC was approximately 30% higher (point estimate for the ratio 1.2970) following treatment with lipegfilgrastim 6 mg compared with pegfilgrastim. This difference was significant, as demonstrated by the shortest 95% CI (1.1376–1.4786), rejecting an equal or lower value for lipegfilgrastim. The ANC secondary PD parameters, including maximum measured ANC value after dosing (ANC_{max}), time point at which ANC_{max} was found ($ANC\ t_{max}$), and time to return to ANC baseline, were also assessed (Table 1). The geometric mean ANC_{max} following treatment with lipegfilgrastim was at least 6% higher in both study 1 (point estimate for the ratio 1.0605) and study 2 (point estimate for the ratio 1.0788) compared with that of pegfilgrastim. In both studies, the shortest 95% CIs (0.9306–1.2084 for study 1 and 0.9586–1.2141 for study 2) were within the conventional equivalence limits. While the median $ANCt_{max}$ was similar for lipegfilgrastim 100 µg/kg and pegfilgrastim in study 1, $ANCt_{max}$ was observed later following treatment with lipegfilgrastim compared with pegfilgrastim in study 2 (point estimate for the median difference: 23.98 hours) and the 95% CI rejected a lower but not equal $ANCt_{max}$ value.

Dose escalation in study 1 was not continued beyond the 100 µg/kg dose because two subjects experienced ANC_{max} levels >70 neutrophils/nL, which is considered to be the limit for excessive hyperleukocytosis, which should be avoided in healthy subjects[23]. In addition, one subject who received pegfilgrastim in study 2 also experienced ANC_{max} levels higher than 70 neutrophils/nL; levels did not exceed 58.90 neutrophils/nL in the lipegfilgrastim group in any subject in study 2.

In study 2, simultaneous evaluation of the influence of sex and body weight on the ANC AOBEC and ANC secondary parameters demonstrated no significant effect of

Table 1. Summary statistics of absolute neutrophil count pharmacodynamic parameters from studies 1 and 2.

Parameter	Study 1				Study 2	
	Lipegfilgrastim 25 µg/kg (n = 8)	Lipegfilgrastim 50 µg/kg (n = 15)	Lipegfilgrastim 100 µg/kg (n = 15)	Pegfilgrastim 100 µg/kg (n = 15)	Lipegfilgrastim 6 mg (n = 18)	Pegfilgrastim 6 mg (n = 18)
ANC AOBEC, h·neut/n)* (90% CI)	3086.99 N/A	4853.03 (4247.34–5545.10)	6818.55 (5893.22–7889.17)	5147.45 (4743.89–5585.33)	5913.89 (5503.00–6355.46)	4559.84 (3958.73–5252.23)
ANC_{max}, neut/n)* (90% CI)	25.00 N/A	33.58 (30.61–36.84)	42.93 (37.38–49.30)	40.48 (37.44–43.76)	40.07 (36.33–44.20)	37.15 (33.47–41.22)
$ANCt_{max}$ (h)†	24.00	48.00	72.00	72.00	72.00	60.01
ANC time to return to baseline (h)†	504.00	504.00	504.00	504.00	504.00	484.17

ANC, absolute neutrophil count; AOBEC, area over the baseline effect curve; ANC_{max} maximum measured ANC value after dosing; $ANCt_{max}$ time point at which ANC_{max} was found; N/A, not available; neut, neutrophils.
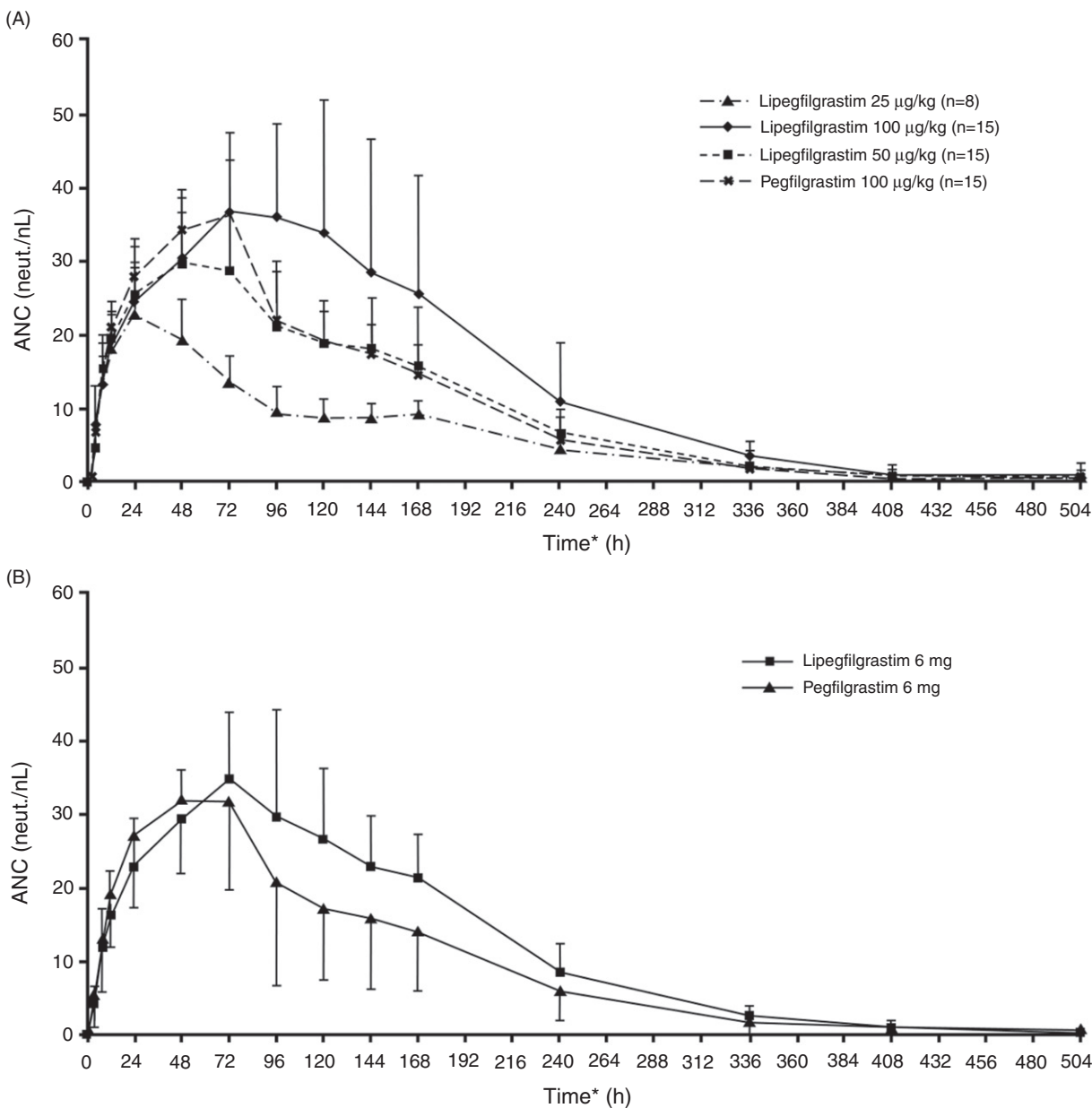*Geometric mean.
†Median value.

Figure 1. Baseline-corrected absolute neutrophil count (*ANC*). (A) Dose-escalated lipegfilgrastim and pegfilgrastim 100 µg/kg, from study 1. (B) Lipegfilgrastim and pegfilgrastim 6 mg, from study 2. Baseline corrected values were calculated as follows: (*ANC* [at specific time point]) − (*ANC* [at predose]). Baseline corrected values <0 were set to 0. *Scheduled time.

these factors (Tables S4 and S5, available online). Treatment was the only factor found to have a significant ($P \leq 0.0241$) effect on ANC AOBEC. The mean ANC AOBEC was 11% higher (point estimate for the ratio: 1.1060) for male subjects who received lipegfilgrastim compared with those who received pegfilgrastim, although this difference was not significant (shortest 95% CI: 0.9422–1.2983). The mean ANC AOBEC was 56% higher (point estimate for the ratio: 1.5826) for female subjects who received lipegfilgrastim compared with those who received pegfilgrastim reaching statistical significance according to the shortest 95% CI (1.2863–

1.9472), which rejected an equal value (Table S4, available online). For the body weight stratum less than 60 kg, the ANC AOBEC was 19% higher (point estimate for the ratio 1.1928) after dosing with lipegfilgrastim than after dosing with pegfilgrastim (no significant difference, shortest 95% CI: 1.0285–1.3834). For the weight stratum between 60 and 80 kg, the 27% difference (point estimate for the ratio: 1.2686) in the ANC AOBEC was not significantly different (95% CI: 0.9212–1.7469), and for the body weight stratum of 80 kg or higher, the ANC AOBEC was significantly higher (49%; point estimate for the ratio: 1.4887) for lipegfilgrastim compared
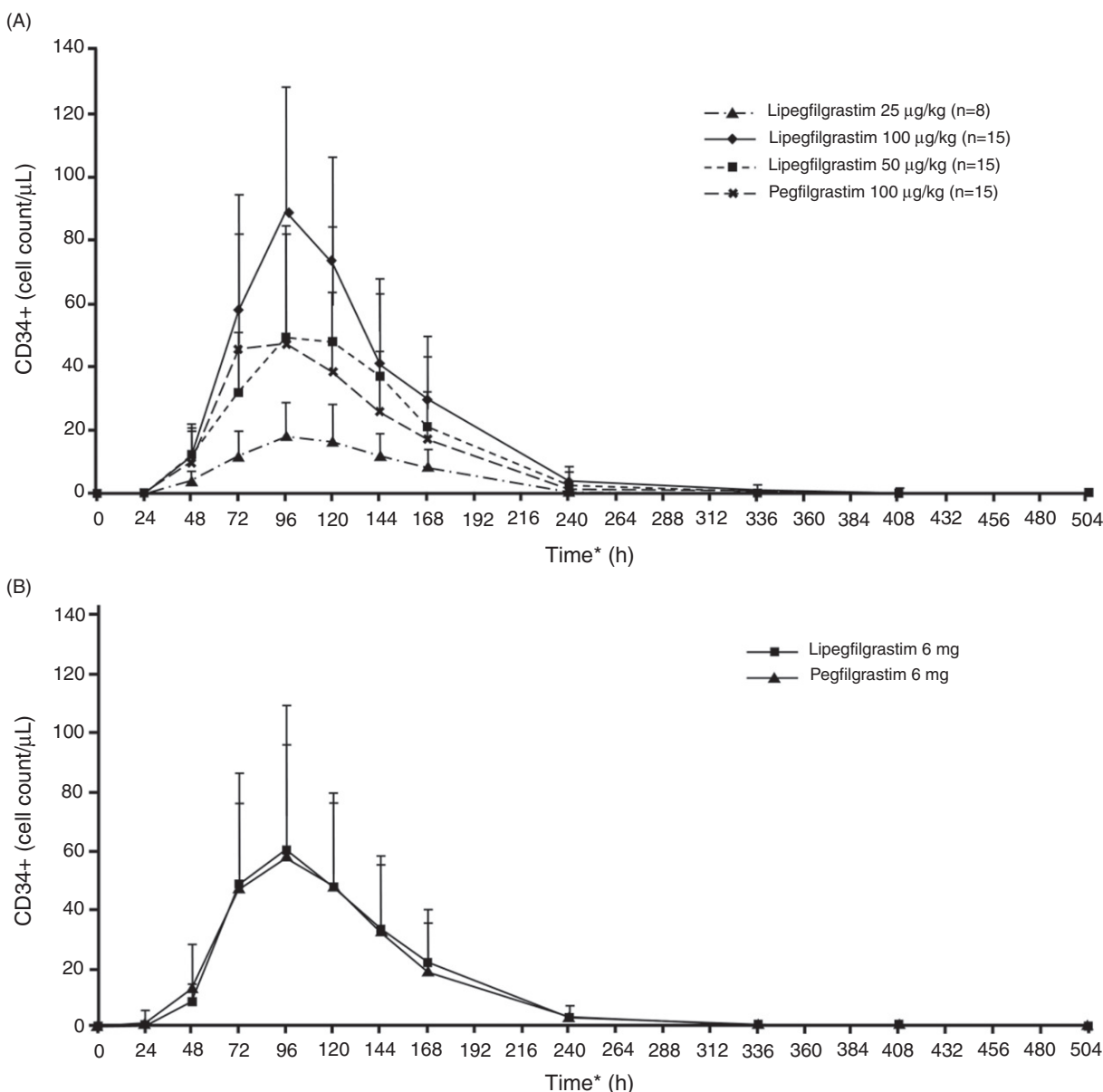
Figure 2. Mean baseline-corrected values of CD34+ cell count over time. (A) Study 1. (B) Study 2. Baseline corrected values were calculated as follows: (CD34+ cell count [at specific time point]) − (CD34+ cell count [at predose]). Baseline corrected values <0 were set to 0. *Scheduled time.

with pegfilgrastim (95% CI: 1.2786–1.7333) (Table S5, available online).

To compare the PD response of lipegfilgrastim with pegfilgrastim further, CD34+ cell counts were measured by flow cytometry. Baseline-corrected CD34+ cell counts for studies 1 and 2 are shown in Figure 2. In study 1, higher CD34+ cell counts were found following treatment with lipegfilgrastim versus the same dose of pegfilgrastim. When lipegfilgrastim and pegfilgrastim were administered at equal doses of 100 µg/kg, both CD34+ AOBEC and CD34+ C_{max} were 83% (point estimate for the ratio 1.8283) and 98% (point estimate for the ratio 1.9843) higher, respectively, for

lipegfilgrastim (significant difference based on the shortest 95% CI: 1.3651–2.4486 and 1.4947–2.6341, respectively). In contrast, CD34+ t_{max} values were similar between the lipegfilgrastim and pegfilgrastim groups (Table 2; Figure 2A). In study 2, the CD34+ AOBEC and CD34+ C_{max} were 9% (point estimate for the ratio: 1.0889) and 16% (point estimate for the ratio: 1.1642) higher, respectively, in subjects who received lipegfilgrastim versus pegfilgrastim, with wide 95% CI limits that did not reject an equal, higher, or lower value. CD34+ t_{max} was observed at approximately the same time in subjects who received lipegfilgrastim or pegfilgrastim (Table 2; Figure 2B).

Pharmacokinetic analysis

For both studies, the mean lipegfilgrastim and pegfilgrastim serum concentrations were assessed over time by treatment group (Figures S1 and S2, available online). In study 1, the PK parameters of AUC, C_{max}, and t_{max} increased with increasing doses of lipegfilgrastim (Table S6, available online). Evaluation of the dose linearity of lipegfilgrastim demonstrated that $AUC_{0-\infty}$, $AUC_{0-tlast}$, and C_{max} following SC administration in the 25 µg/kg to 100 µg/kg range increased in more than a linear proportion with the dose. The ANOVA-based analysis suggested that this is mainly because of the difference from the 50 µg/kg to the 100 µg/kg dose.

None of the lipegfilgrastim doses were pharmacokinetically equivalent to the reference dose of pegfilgrastim. In study 1, the $AUC_{0-tlast}$, $AUC_{0-\infty}$, and C_{max} geometric mean values for lipegfilgrastim 100 µg/kg were 56%, 57%, and 22% higher (point estimate for the ratio 1.5709, 1.5596, and 1.2188), respectively, versus pegfilgrastim, demonstrating a higher total cumulative exposure and peak exposure. The shortest 90% CI for AUC rejected an equal value, demonstrating a significant difference from pegfilgrastim, whereas the shortest 90% CI for C_{max} did not reject an equal value and therefore did not indicate a significant difference. In study 1, t_{max} in subjects who received lipegfilgrastim increased with increasing lipegfilgrastim doses. In subjects receiving pegfilgrastim 100 µg/kg, t_{max} was approximately midway between the value for subjects who received 50 µg/kg and 100 µg/kg of lipegfilgrastim. The $t_{1/2}$ and MRT geometric mean values were approximately 7 to 10 hours longer for lipegfilgrastim 100 µg/kg compared with pegfilgrastim 100 µg/kg.

Similar results were observed when comparing fixed doses of lipegfilgrastim 6 mg and pegfilgrastim 6 mg in study 2, wherein the peak exposure (C_{max}) was 36% higher and the cumulative exposure ($AUC_{0-tlast}$ and $AUC_{0-\infty}$) was 63% to 64% higher in subjects treated with lipegfilgrastim compared with subjects treated with pegfilgrastim (Table S6, available online). In addition, t_{max} was observed later (30 hours) and the $t_{1/2}$ was longer (32.41 hours) for subjects treated with lipegfilgrastim compared with those treated with pegfilgrastim (21 hours and 27.21 hours, respectively).

Subjects in study 2 also were stratified according to sex and body weight to assess the effects of these parameters on the PK of fixed-dose lipegfilgrastim 6 mg SC (Tables S7 and S8, available online). In subjects who received lipegfilgrastim compared with those who received pegfilgrastim, the effect of body weight was significant ($P \leq 0.001$) for $AUC_{0-\infty}$, $AUC_{0-tlast}$, and C_{max} in both the adjusted and unadjusted analyses. The overall effect of increasing body weight was a decrease in $AUC_{0-\infty}$, $AUC_{0-tlast}$, and C_{max} for both lipegfilgrastim and pegfilgrastim (Table S8). The effect of sex on $AUC_{0-\infty}$, $AUC_{0-tlast}$ and C_{max} was not

Table 2. Summary statistics of CD34+ pharmacodynamic parameters from studies 1 and 2.

Parameter	Study 1					Study 2	
	Lipegfilgrastim 25 µg/kg (n = 8)	Lipegfilgrastim 50 µg/kg (n = 15)	Lipegfilgrastim 100 µg/kg (n = 15)	Pegfilgrastim 100 µg/kg (n = 15)		Lipegfilgrastim 6 mg (n = 18)	Pegfilgrastim 6 mg (n = 18)
CD34+ cell count $AOBEC$ (h·cell count/µL)* (90% CI)	1290.49 N/A	4089.93 (2822.74–5925.98)	7340.16 (5785.75–9312.17)	4014.85 (3084.04–5226.58)		5115.99 (4060.70–6445.53)	4698.53 (3615.86–6105.36)
$CD34+_{max}$ (cell count/µL)* (90% CI)	17.38 N/A	45.55 (32.31–64.21)	86.87 (70.93–106.39)	43.78 (33.14–57.84)		58.86 (47.79–72.49)	50.56 (38.14–67.02)
CD34+ t_{max} (h)†	96.00	120.00	96.00	96.00		96.00	96.00

$AOBEC$, area over the baseline effect curve; $CD34+_{max}$, maximum measured value after dosing of CD34+ cell count; CD34+ t_{max}, time point after dosing at which $CD34+_{max}$ is found; CI, confidence interval; N/A, not available.
*Geometric mean.
†Median value.

significant in the adjusted analysis, and was significant ($P = 0.0267$) only for C_{max} in the unadjusted analysis, most likely a random effect.

Safety

In both studies, the overall safety of the study drugs was good, and no serious AEs were reported. The occurrence of AEs was comparable between the different treatment groups, and tolerability was appropriate and consistent with historical data for pegfilgrastim in healthy subjects[24,25]. No clinically significant alterations were observed in ECGs, blood pressure, pulse rate, respiratory rate, or sonographic examination of the spleen. No subject tested positive for antibodies against lipegfilgrastim, pegfilgrastim, or unpegylated G-CSF at the screening and follow-up visits in either study. Local tolerability was good, with no ecchymosis or induration reported by any subject. Erythema was reported only in study 2 by one subject per treatment group and registered as AEs. No clinically significant alterations in laboratory parameters occurred in either study.

In study 1, 35 lipegfilgrastim-treated subjects (92.1%) ($n = 7$, 25 µg/kg; $n = 14$, 50 µg/kg; $n = 14$, 100 µg/kg) experienced 94 AEs (44 mild, 49 moderate, and 1 severe); a total of 15 pegfilgrastim-treated subjects (93.3%) experienced 37 AEs (6 mild, 29 moderate, and 2 severe). With the exception of two AEs (sore throat and gastroenteritis), all of the reported AEs in study 1 were suspected to be related to study drug. In study 2, 15 lipegfilgrastim-treated subjects (88.3%) experienced 38 treatment-emergent AEs (6 mild, 31 moderate, and 1 severe); 17 pegfilgrastim-treated subjects (94.4%) experienced 36 treatment-emergent AEs (8 mild, 26 moderate, and 2 severe). All treatment-emergent AEs in study 2 were considered to be suspected adverse drug reactions. In study 1, across all treatment groups, the most frequently reported AEs were arthralgia ($n = 43$, 82.7%), headache ($n = 28$, 52.8%), rhinitis ($n = 5$, 9.4%), and back pain ($n = 4$, 7.5%). In study 2, the most frequently reported AEs were bone pain (27 of 36 subjects, 75.0%) and headache (52.8%), followed by abdominal pain, dizziness, constipation, and musculoskeletal chest pain (5.6%). The incidence of these AEs was similar across treatment groups within each study (Table S9).

Discussion

The present studies evaluated PD, PK, and safety parameters in healthy subjects who received lipegfilgrastim, using the only commercially available product from the same therapeutic class (pegfilgrastim) as a positive control, to objectively assess drug effects and select an appropriate dose for phase II evaluation.

No significant effects of sex or body weight on ANC AOBEC were observed. Importantly, the ANC AOBEC in study 2 did not significantly differ between the body weight strata after lipegfilgrastim fixed-dose treatment. Additionally, there was no effect of treatment, sex, or body weight on any secondary PD parameter to further support a fixed-dose strategy for lipegfilgrastim. The ANOVA testing of the PK parameters showed significant effects of treatment and body weight but not of sex for both $AUC_{0-\infty}$ and $AUC_{0-tlast}$. The overall effect of body weight was a decrease in $AUC_{0-\infty}$, $AUC_{0-tlast}$, and C_{max} that coincided with increasing body weight for subjects who received lipegfilgrastim and pegfilgrastim. The lipegfilgrastim:pegfilgrastim ratios tended to decrease with increasing body weight. However, the small number of subjects in each weight stratum and treatment group made these estimations imprecise, as reflected by the wide and overlapping shortest 90% CIs. The effect of sex was either not significant (cumulative exposure) or depended on adjustment by body weight and was likely due to random fluctuations.

In both studies, the primary end point of ANC AOBEC was significantly higher ($\approx 30\%$) with lipegfilgrastim than with an equal dose of pegfilgrastim, demonstrating that the efficacy of lipegfilgrastim for the target effect of increased neutrophil counts in healthy subjects was higher than that of pegfilgrastim. The ANC AOBEC obtained for the 100 µg/kg dose of pegfilgrastim was higher than previously reported[25]. Although the reasons for this discrepancy are not clear (and may be related to differences in analytical methods, sampling schedules, or calculation details), the relatively high ANC response to pegfilgrastim confirms that the higher response to lipegfilgrastim is not due to an unusually low response to pegfilgrastim. In subjects treated with lipegfilgrastim, ANC_{max} was similar ($\approx 6\%$–8% higher) to that of subjects who received pegfilgrastim. The discrepancy between the higher ANC AOBEC and the similar ANC_{max} for the same dose of pegfilgrastim is likely due to a longer duration of action for lipegfilgrastim. Indeed, ANC t_{max} tends to be observed approximately 24 hours later with lipegfilgrastim compared with the same dose of pegfilgrastim. In both studies, the $t_{1/2}$ of lipegfilgrastim was significantly longer than the $t_{1/2}$ of pegfilgrastim, reflecting a longer body residence for lipegfilgrastim, which likely explains the approximately 60% higher cumulative exposure of lipegfilgrastim (100 µg/kg and 6 mg fixed dose) versus pegfilgrastim. Although the MRT values for subjects treated with lipegfilgrastim were longer in study 1 compared with subjects treated with pegfilgrastim, MRT values were marginally shorter for subjects who received lipegfilgrastim in study 2 and were likely influenced by a limited number of pegfilgrastim-treated subjects with very high MRT values. In both studies, t_{max} was observed later with lipegfilgrastim compared with

pegfilgrastim, suggesting a slower clearance process. The differences in clearance times for subjects who received lipegfilgrastim versus those who received pegfilgrastim via neutrophil-mediated clearance mechanisms (e.g., receptor binding and internalization and/or the release of neutrophil elastase into the blood) may account for the higher cumulative exposure for lipegfilgrastim (56%–64% higher) compared with pegfilgrastim.

The longer body residence also led to a longer lipegfilgrastim duration of action, accounting for the significantly higher ANC $AOBEC$ for the same doses of lipegfilgrastim and pegfilgrastim. Furthermore, ANC_{max} values were similar, demonstrating that higher ANC $AOBEC$ after lipegfilgrastim was due to a more sustained ANC increase rather than to a higher peak value. The more sustained ANC increase with lipegfilgrastim may be related to various factors. The significantly longer time to t_{max} for lipegfilgrastim might be related to a slower clearance process versus pegfilgrastim or greater resistance to elastase degradation, which is an established clearance pathway for G-CSF[26]. In support of the latter speculated mechanism, in vitro data suggest that lipegfilgrastim is indeed more resistant to elastase degradation compared with pegfilgrastim[27].

In a recently published, multinational, multicenter, randomized, double-blind, active-control phase III study by Bondarenko and colleagues, the clinical efficacy of a single 6 mg fixed dose of lipegfilgrastim was demonstrated in patients with breast cancer receiving doxorubicin/docetaxel chemotherapy[17]. Patients treated with lipegfilgrastim ($n = 101$) had a similar or lower incidence and DSN as did patients treated with pegfilgrastim ($n = 101$), the same active comparator used in the present study. In a PK subanalysis, the geometric means of $AUC_{0–last}$ and $AUC_{0–\infty}$ were higher for lipegfilgrastim compared with pegfilgrastim in chemotherapy cycles 1 and 4. These results are consistent with the present study in which cumulative ($AUC_{0–tlast}$ and $AUC_{0–\infty}$) and peak (C_{max}) exposures were higher for lipegfilgrastim compared with pegfilgrastim.

In a multinational, multicenter, randomized, double-blind, placebo-controlled phase III study in 375 patients with non-small-cell lung cancer, a post hoc analysis demonstrated the clinical efficacy of a single 6 mg fixed dose of lipegfilgrastim in patients receiving cisplatin/etoposide. Patients in the lipegfilgrastim group had a reduced incidence of FN compared with that of patients in the placebo group, which reached statistical significance in elderly patients (>65 years)[28].

Some safety concerns have been raised about the use of growth factors in healthy volunteers. There have been case reports of healthy individuals developing hematologic malignancies several years after treatment with G-CSF or pegylated recombinant human megakaryocyte growth and development factor (PEG-rHuMGDF)[29] or spontaneous splenic rupture[30]. There was no evidence of clinically significant splenic or hematologic abnormalities in the present studies, but these hematologic AEs were long-term safety concerns that would not be apparent in the current studies. Long-term safety studies would be needed to evaluate whether there were similar safety concerns with lipegfilgrastim.

Although the clinical significance of differences in PD and PK parameters with lipegfilgrastim and pegfilgrastim has not been determined, the similar incidence of AEs observed in both treatment groups suggests that these differences do not affect safety. Overall, the safety profile for both drugs was similar and no new safety concerns were observed for this class of therapy in these studies of healthy volunteers. The incidence of bone-pain related AEs was similar between the lipegfilgrastim and pegfilgrastim groups within each study.

Conclusions

In the present studies conducted in healthy volunteers, lipegfilgrastim resulted in a longer-lasting increase in ANC compared with pegfilgrastim, without a significant increase in ANC peak values. Subjects treated with lipegfilgrastim had a higher cumulative exposure as a result of a longer body residence, as evidenced by a longer $t_{1/2}$ and t_{max}, potentially the reason for this prolonged duration of action. These data provided support for the evaluation of lipegfilgrastim 6 mg in phase III trials as prophylactic treatment for patients receiving myelosuppressive chemotherapy.

Transparency

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