

# Pharmacokinetic/Pharmacodynamic Modeling of Pegfilgrastim in Healthy Subjects

Lorin K. Roskos, PhD, Peggy Lum, BS, Pamela Lockbaum, MBA,  
Gisela Schwab, MD, and Bing-Bing Yang, PhD

*This analysis was conducted to characterize the pharmacokinetics and pharmacodynamics of pegfilgrastim and to develop a pharmacokinetic-pharmacodynamic model to describe the granulopoietic effects of pegfilgrastim and the homeostatic regulation of pegfilgrastim clearance in healthy subjects. Pegfilgrastim serum concentration data and differential white cell counts were obtained from an open-label, single-dose, dose escalation study. Healthy subjects (8 subjects/dose group) received a single subcutaneous dose of 30, 60, 100, or 300 µg/kg pegfilgrastim. Pegfilgrastim exhibited nonlinear pharmacokinetics; clearance decreased with increasing dose. A dose-dependent increase in absolute*

*neutrophil count with an increase in the percentage of band cells was observed. A pharmacokinetic-pharmacodynamic model was developed that adequately described the nonlinear pharmacokinetics of pegfilgrastim, feedback regulation of pegfilgrastim clearance by neutrophils, and the differential effects of pegfilgrastim on neutrophil populations in blood.*

**Keywords:** PK/PD modeling; G-CSF; nonlinear kinetics; pegfilgrastim; pharmacodynamics

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Colony-stimulating factors are proteins that act on hematopoietic cells by binding to specific receptors on the surface of precursor cells, resulting in increased proliferation, differentiation commitment, and some end-cell function activation.<sup>1</sup> Filgrastim is a recombinant methionyl form of human granulocyte colony-stimulating factor (G-CSF) derived from *Escherichia coli*.<sup>2</sup> The biological activity of filgrastim is identical to that of endogenous G-CSF, which stimulates the activation, proliferation, and differentiation of neutrophil progenitor cells and enhances the functions of mature neutrophils.<sup>3</sup> Clinically, filgrastim is used to enhance the recovery of neutrophils after chemotherapy and to mobilize transplantable hematopoietic progenitor populations to the blood.<sup>4</sup> Because of the short half-life (2-4 hours) of filgrastim in humans,<sup>5</sup> daily injections of filgrastim are required to achieve therapeutic effects.

From Amgen, Inc, Thousand Oaks, California. Dr Roskos, Ms Lum, Ms Lockbaum, Dr Schwab, and Dr Yang are currently or were previously employed at Amgen, Inc and are shareholders of Amgen stock. Submitted for publication December 9, 2005; revised version accepted March 16, 2006. Address for reprints: Bing-Bing Yang, PhD, Amgen, Inc, One Amgen Center Drive, Thousand Oaks, CA 91320; e-mail: byang@amgen.com. DOI: 10.1177/0091270006288731

Pegfilgrastim is a sustained-duration form of filgrastim, produced by covalently binding a 20-kd polyethylene glycol molecule to the N-terminal methionine residue of filgrastim.<sup>6</sup> Results from *in vitro* proliferation, receptor binding, and neutrophil function studies demonstrate that filgrastim and pegfilgrastim have the same mechanism of action.<sup>7</sup> However, pegfilgrastim *in vivo* has sustained effects on granulopoiesis compared with filgrastim.<sup>8</sup> Filgrastim is eliminated predominantly by neutrophil G-CSF receptor-mediated clearance<sup>9</sup> and by renal clearance.<sup>10</sup> During chemotherapy-induced neutropenia, non-receptor-mediated clearance is the primary route for filgrastim and pegfilgrastim elimination. The sustained effects of pegfilgrastim have been attributed to decreased renal clearance of the pegylated molecule with a higher hydrodynamic radius.<sup>10</sup>

A phase I study was conducted to evaluate the pharmacokinetics, pharmacodynamics, and safety of pegfilgrastim in healthy subjects. To assess the relationship between serum concentrations of pegfilgrastim and granulopoietic effects of the drug, as well as to define the relationship between neutrophil counts and pegfilgrastim clearance, a semimechanistic pharmacokinetic-pharmacodynamic model was established. Pharmacokinetic data and pharmacokinetic-pharmacodynamic modeling are presented herein,

expanding on descriptive pharmacodynamics and safety results previously reported.<sup>11</sup>

**METHODS**

**Study Management**

This study was conducted at PPD Pharmaco (706-A Ben White Boulevard West, Austin, Tex), in accordance with the principles stated in the Declaration of Helsinki, the US Food and Drug Administration regulations, and the International Committee for Harmonisation Good Clinical Practice guidelines. All subjects provided written informed consent before entering the study. Subject confidentiality was maintained throughout the study.

**Study Drug**

Pegfilgrastim (Neulasta®) was manufactured by Amgen, Inc (Thousand Oaks, Calif) and was supplied in single-use, preservative-free vials as a sterile, clear 1.2-mL solution at 10 mg/mL. To equalize the volume of study drug administered to each subject, pegfilgrastim was diluted with diluent to a 3-mL solution before injection to the subjects.

**Study Design**

This was an open-label, single-dose, dose escalation study. Thirty-two healthy subjects received a single dose of 30, 60, 100, or 300 µg protein/kg pegfilgrastim (8 subjects/dose group) on day 1. Pegfilgrastim was administered as two 1.5-mL subcutaneous injections on day 1 at 2 different injection sites (the deltoid area of both arms) within 1 minute of each other. Blood samples for pegfilgrastim concentration measurement were collected before pegfilgrastim administration and at 0.25, 0.5, 0.75, 1, 2, 3, 4, 6, 8, 12, 16, 24, 36, and 48 hours after the second injection of pegfilgrastim and then daily until day 15. Serum samples were stored at -70°C until assayed for pegfilgrastim. Blood samples for differential white cell counts were collected concurrently with all pharmacokinetic samples.

**Sample Analysis**

*Pegfilgrastim serum concentration measurement.* Pegfilgrastim concentrations in serum were analyzed by an enzyme-linked immunosorbent assay (ELISA, Quantikine™ Human G-CSF Immunoassay Kit, R&D

Systems, Inc, Minneapolis, Minn). Microtiter immunoassay plates were coated with murine monoclonal anti-G-CSF antibody. Standard and quality control samples containing known amounts of pegfilgrastim along with samples containing unknown amounts of pegfilgrastim were added to the wells. After a 2-hour incubation at room temperature, the plates were washed with a buffer to remove unbound protein. Then, a goat polyclonal antibody against G-CSF conjugated with horseradish peroxidase was added to the wells for a 2-hour incubation at room temperature. After another washing step, a substrate solution (tetramethylbenzidine peroxidase and peroxidase solution) for color development was added. The reaction was stopped with 2 N sulfuric acid, and the intensity of the color was measured by a spectrophotometric plate reader at a wavelength of 450 nm and at a reference wavelength of 650 nm. A log-log fit was used to estimate pegfilgrastim concentration in human serum. The lower limit of quantitation of the assay was 0.031 ng/mL serum. The precision ranged from 1% to 14% within an assay and from 5% to 9% between assays. The accuracy ranged from 95% to 119% within an assay and from 93% to 107% between assays.

*Hematology evaluation.* Whole-blood samples were analyzed immediately after collection at the testing site (PPD Pharmaco, Austin, Tex). The absolute neutrophil count (ANC) was calculated as the total white blood cell count times the percentage of total neutrophils.

**Data Analyses**

*Noncompartmental pharmacokinetic analysis.* Pharmacokinetic parameters were calculated for each subject using noncompartmental analysis of serum pegfilgrastim concentration-time data (WinNonlin Professional, Pharsight Corp, Mountain View, Calif). The maximum concentration ( $C_{max}$ ) and the time it occurred ( $t_{max}$ ) after dosing were recorded as observed. The area under the serum concentration-time curve,  $AUC_{(0-last)}$ , was estimated using the linear trapezoidal method from time 0 to last, the time of the last quantifiable concentration ( $C_{last}$ ). The first-order terminal rate constant ( $k_{el}$ ) was estimated using linear regression of the terminal log-linear decay phase. The terminal half-life ( $t_{1/2}$ ) was calculated as  $\ln(2)$  divided by  $k_{el}$ . The area under the curve calculated from time 0 to infinity,  $AUC_{(0-\infty)}$ , was estimated as the sum of corresponding  $AUC_{(0-last)}$  and  $C_{last}/k_{el}$  values. Apparent clearance (CL/F) was estimated as dose divided by  $AUC_{(0-\infty)}$ .

*Pharmacokinetic/pharmacodynamic modeling and simulation.* Mean pegfilgrastim serum concentration data and peripheral blood band cell and segmented neutrophil counts from all dose groups were modeled simultaneously. Pegfilgrastim serum concentration data were modeled using a 1-compartment model with a first-order, delayed absorption process; the elimination of pegfilgrastim was described by parallel G-CSF receptor-mediated clearance ( $CL_N/F$ ) and linear clearance ( $CL_{lin}/F$ ) (Figure 1). The G-CSF receptor-mediated clearance was described as follows:

$$CL_N/F = \frac{(k_{cat}/F)(B_p + S_p)}{K_m + C}. \quad (1)$$

$B_p$  is the peripheral blood band cell count,  $S_p$  is the peripheral blood-segmented neutrophil count (the sum of  $B_p$  and  $S_p$  is the ANC), and  $C$  is the serum pegfilgrastim concentration at any given time,  $t$ . The G-CSF receptor-mediated clearance is a Michaelis-Menten process; the product of  $k_{cat}/F$  and  $(B_p + S_p)$  is the maximum velocity ( $V_{max}$ ) of drug elimination through this pathway, and  $K_m$  is the Michaelis constant. The term,  $F$ , represents the absolute bioavailability of the subcutaneous dose and indicates that the estimates of the  $CL_N/F$  and  $k_{cat}/F$  parameters will be inflated by an absolute bioavailability less than 100%. Because absolute bioavailability of protein therapeutics may be affected by dose and dosing volume, relative bioavailability parameters,  $F_{60}$ ,  $F_{100}$ , and  $F_{300}$ , were applied to the 60-, 100-, and 300- $\mu\text{g}/\text{kg}$  doses to adjust for potential bioavailability differences relative to the 30- $\mu\text{g}/\text{kg}$  dose. Mean absorption time (MAT) was estimated by the sum of the absorption lag time ( $t_{lag}$ ) and the reciprocal of the first-order absorption rate constant ( $k_a$ ).

A maturation-structured cytokinetic model was developed to describe the granulopoietic effects of pegfilgrastim and the feedback regulation of pegfilgrastim clearance (Figure 2). The model construct was based on the biology of normal human granulopoiesis.<sup>12</sup> The starting point of the pharmacodynamic model was the production of metamyelocytes from the last maturational step of mitotic precursors. The most distal effect of the drug, expansion of the mitotic promyelocyte and myelocyte pools, was empirically described by a Hill equation. Serum concentrations of pegfilgrastim were assumed to stimulate mitosis and mobilization of band cells and segmented neutrophils in bone marrow and increase adhesion of peripheral blood band cells ( $B_p$ ) and segmented neutrophils ( $S_p$ ) to blood vessels

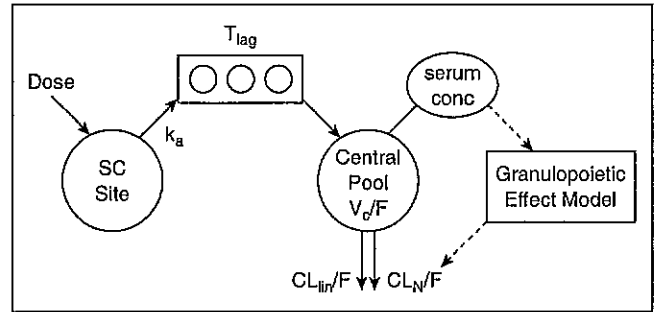


Figure 1. Compartmental model describing the pharmacokinetics of pegfilgrastim. The subcutaneous dose undergoes first-order, delayed absorption. The solid box containing catenary-linked compartments represents a transit time ( $t_{lag}$ ) associated with delayed absorption. Absorbed pegfilgrastim distributes into a central compartment with an apparent volume of  $V_c/F$ . Serum pegfilgrastim concentration modulates neutrophil counts (granulopoietic effect model), which in turn regulate drug clearance. Drug clearance is by 2 parallel processes: a Michaelis-Menten, G-CSF receptor-mediated process,  $CL_N/F$ , and a linear mechanism,  $CL_{lin}/F$ , that is independent of neutrophil counts. G-CSF, granulocyte colony-stimulating factor.

(a process known as margination), causing a change in the volume of distribution of the circulating neutrophils.

Change in metamyelocyte mass after stimulation of mitotic precursors was described by 3 transit compartments represented by the following differential equations:

$$\frac{dM_1}{dt} = S_0 + \frac{E_{mit}C}{EC_{50} + C} - \frac{3}{\tau_{meta} \left(1 - \frac{f_{mmt}C}{EC_{50} + C}\right)} M_1 \quad (2)$$

$$\frac{dM_2}{dt} = \frac{3}{\tau_{meta} \left(1 - \frac{f_{mmt}C}{EC_{50} + C}\right)} M_1 - \frac{3}{\tau_{meta} \left(1 - \frac{f_{mmt}C}{EC_{50} + C}\right)} M_2 \quad (3)$$

$$\frac{dM_3}{dt} = \frac{3}{\tau_{meta} \left(1 - \frac{f_{mmt}C}{EC_{50} + C}\right)} M_2 - \frac{3}{\tau_{meta} \left(1 - \frac{f_{mmt}C}{EC_{50} + C}\right)} M_3 \quad (4)$$

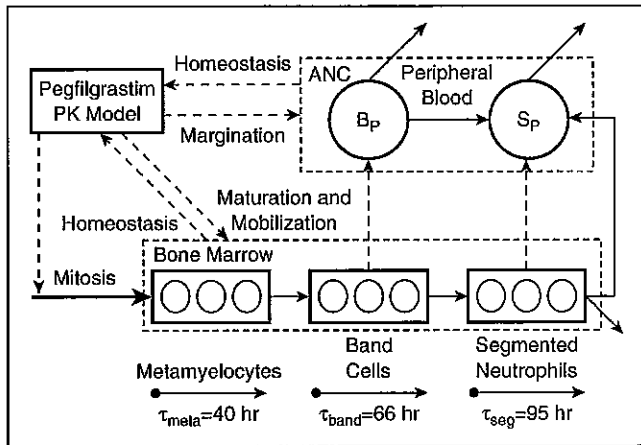


Figure 2. Pharmacodynamic model describing the granulopoietic effects of pegfilgrastim. Serum concentrations of pegfilgrastim stimulate mitosis and mobilization of band cells and segmented neutrophils in bone marrow, decrease maturation times for postmitotic cells in marrow, and affect margination of the peripheral blood band cell ( $B_p$ ) and segmented neutrophil ( $S_p$ ) populations, the sum of which is the total absolute neutrophil count (ANC). Changes in neutrophil counts in peripheral blood provide feedback regulation of pegfilgrastim clearance.

where  $M_i$  is the mass in the  $i$ th metamyelocyte compartment,  $S_0$  is the baseline metamyelocyte influx rate,  $E_{mit}$  is the maximum rate of metamyelocyte influx that can be elicited by pegfilgrastim,  $\tau_{meta}$  is the baseline mean maturation time of the metamyelocytes, and the constant, "3," represents the number of transit compartments; the number of transit compartments modulates the variance of the maturation time. The parameter  $f_{mmt}$  is the maximum fraction by which the baseline mean maturation time can be decreased by pegfilgrastim.  $EC_{50}$  is the serum concentration of pegfilgrastim that exerts half-maximum granulopoietic effects and was assumed to be the same for effects on mitosis, maturation, mobilization, and margination. The  $EC_{90}$  for mitosis, maturation, and mobilization was calculated by multiplying the  $EC_{50}$  by 9.

Change in the band cell mass in bone marrow was described by the following differential equations:

$$\frac{dB_1}{dt} = \frac{3}{\tau_{meta} \left(1 - \frac{f_{mmt}C}{EC_{50} + C}\right)} M_3 - \left( \frac{3}{\tau_{band} \left(1 - \frac{f_{mmt}C}{EC_{50} + C}\right)} + \frac{E_{band}C}{EC_{50} + C} \right) B_1 \quad (5)$$

$$\frac{dB_2}{dt} = \frac{3}{\tau_{band} \left(1 - \frac{f_{mmt}C}{EC_{50} + C}\right)} B_1 - \left( \frac{3}{\tau_{band} \left(1 - \frac{f_{mmt}C}{EC_{50} + C}\right)} + \frac{E_{band}C}{EC_{50} + C} \right) B_2 \quad (6)$$

$$\frac{dB_3}{dt} = \frac{3}{\tau_{band} \left(1 - \frac{f_{mmt}C}{EC_{50} + C}\right)} B_2 - \left( \frac{3}{\tau_{band} \left(1 - \frac{f_{mmt}C}{EC_{50} + C}\right)} + \frac{E_{band}C}{EC_{50} + C} \right) B_3 \quad (7)$$

where  $B_i$  is the mass in the  $i$ th band cell compartment,  $E_{band}$  is the maximum rate of band cell flux from the bone marrow pool to the circulating neutrophil pool that can be elicited by pegfilgrastim, and  $\tau_{band}$  is the mean maturation time of bone marrow band cells, which was fixed at 66 hours based on published values.<sup>13-15</sup>

Change in the segmented neutrophil mass in bone marrow was described by the following differential equations:

$$\frac{dS_1}{dt} = \frac{3}{\tau_{band} \left(1 - \frac{f_{mmt}C}{EC_{50} + C}\right)} B_3 - \left( \frac{3}{\tau_{seg} \left(1 - \frac{f_{mmt}C}{EC_{50} + C}\right)} + \frac{E_{seg}C}{EC_{50} + C} \right) S_1 \quad (8)$$

$$\frac{dS_2}{dt} = \frac{3}{\tau_{seg} \left(1 - \frac{f_{mmt}C}{EC_{50} + C}\right)} S_1 - \left( \frac{3}{\tau_{seg} \left(1 - \frac{f_{mmt}C}{EC_{50} + C}\right)} + \frac{E_{seg}C}{EC_{50} + C} \right) S_2 \quad (9)$$

$$\frac{dS_3}{dt} = \frac{3}{\tau_{seg} \left(1 - \frac{f_{mmt}C}{EC_{50} + C}\right)} S_2 - \left( \frac{3}{\tau_{seg} \left(1 - \frac{f_{mmt}C}{EC_{50} + C}\right)} + \frac{E_{seg}C}{EC_{50} + C} + k_{ig} \right) S_3 \quad (10)$$

where  $S_i$  is the mass in the  $i$ th bone marrow segmented neutrophil compartment,  $E_{\text{seg}}$  is the maximum rate of segmented neutrophil flux from the bone marrow pool to the circulating neutrophil pool that can be elicited by pegfilgrastim,  $\tau_{\text{seg}}$  is the mean maturation time of segmented neutrophils in bone marrow, and  $k_{\text{ig}}$  is a sink that describes ineffective granulopoiesis, or loss of cells in bone marrow before mobilization to circulation.<sup>16</sup> The maturation times of bone marrow-segmented neutrophils was fixed at 95 hours based on published values.<sup>13-15</sup>

The change in band cell and segmented neutrophil counts in peripheral blood was described as

$$\frac{dB_p}{dt} = \sum_{i=1}^3 \left[ \left( \frac{E_{\text{band}} C}{EC_{50} + C} \right) B_i \right] - k_{\lambda} B_p - k_{\text{bpmat}} B_p \quad (11)$$

$$\frac{dS_p}{dt} = \sum_{i=1}^3 \left[ \left( \frac{E_{\text{seg}} C}{EC_{50} + C} \right) S_i \right] + k_{\text{bpmat}} B_p - k_{\lambda} S_p \quad (12)$$

where  $B_p$  and  $S_p$  are neutrophil counts unadjusted for changes in neutrophil margination,  $k_{\text{bpmat}}$  is the rate constant for maturation of peripheral blood band cells to segmented neutrophils, and  $k_{\lambda}$  is the rate constant for neutrophil elimination from peripheral blood, which was assumed to be the same for band cells and segmented neutrophils. Margination was modeled as a rapid expansion of the relative, blood neutrophil dilution volume,  $V_N$ , where

$$V_N = \left( 1 + \frac{E_{\text{marg}} C^{\gamma_N}}{EC_{50}^{\gamma_N} + C^{\gamma_N}} \right) \quad (13)$$

$E_{\text{marg}}$  is the increase in baseline neutrophil dilution volume that can be induced by pegfilgrastim, and  $\gamma_N$  is the Hill coefficient. The half-life of the circulating segmented neutrophil,  $t_{1/2, \text{seg}}$ , was calculated as  $\ln(2)$  divided by  $k_{\lambda}$ .

### Model Fitting and Parameter Estimation

Modeling was conducted using SAAM II (SAAM Institute, Seattle, Wash). Numerical integration was conducted in SAAM II using the Rosenbrock method with an adjustable step size.<sup>17</sup> Goodness of fit was evaluated by asymptotic standard deviations for estimated parameters and by weighted residual plots. The relative goodness of fit for different structural models was evaluated using the objective function value or the Akaike information criterion.<sup>18</sup>

## RESULTS

### Subjects

Thirty-two subjects were enrolled in and completed the study. The demographic data were similar across all dose groups (Table I).

### Pharmacokinetics of Pegfilgrastim

Mean concentration-time profiles of pegfilgrastim after subcutaneous administration are presented in Figure 3. The mean  $C_{\text{max}}$  value increased more than dose proportionally; a 10-fold increase in the dose resulted in an approximately 25-fold increase in mean ( $\pm$ SD)  $C_{\text{max}}$  from  $43.6 \pm 20.0$  ng/mL to  $1070 \pm 360$  ng/mL (Table II). The median  $t_{\text{max}}$  occurred later as the dose increased from 8 hours at 30  $\mu\text{g}/\text{kg}$  to 24 hours at 300  $\mu\text{g}/\text{kg}$ . The mean serum clearance of pegfilgrastim decreased from  $38.6 \pm 15.4$  mL/h/kg to  $5.19 \pm 2.31$  mL/h/kg, as the dose increased from 30 to 300  $\mu\text{g}/\text{kg}$ . At the terminal phase, the serum concentrations of pegfilgrastim for all groups declined in parallel with a mean terminal  $t_{1/2}$  ranging from  $46.3 \pm 10.3$  to  $62.1 \pm 5.8$  hours.

### Pharmacodynamics of Pegfilgrastim

Mean ANC-time profiles after subcutaneous administration are presented in Figure 3. During the first hour of pegfilgrastim administration, a transient decrease in ANC was observed. This decrease was followed by a rapid and significant increase in ANC. The ANC profiles demonstrated a clear dose response both in the magnitude and in the duration of elevation. The ANC parameters were presented previously.<sup>11</sup> In brief, the median maximum ANC value increased with increasing dose and was reached in 1.5 days at 30  $\mu\text{g}/\text{kg}$  and in approximately 2 days at 60  $\mu\text{g}/\text{kg}$  and 100  $\mu\text{g}/\text{kg}$ ; ANC continued to increase at the 300- $\mu\text{g}/\text{kg}$  group until reaching a maximum at 4 days postdose. The median area over the baseline effect curve increased with increasing dose from  $101 \times 10^9$  cells-day/L at 30  $\mu\text{g}/\text{kg}$  to  $223 \times 10^9$  cells-day/L at 300  $\mu\text{g}/\text{kg}$ .

### Pharmacokinetic/Pharmacodynamic Modeling

Mean pegfilgrastim serum concentration data, band cell counts, and segmented neutrophil counts from all dose groups were modeled simultaneously. The

**Table I** Demographic Data

Parameter	30 µg/kg (n = 8)	60 µg/kg (n = 8)	100 µg/kg (n = 8)	300 µg/kg (n = 8)
Age, years	27 (7)	26 (7)	28 (7)	25 (4)
Height, cm	170 (10)	171 (8)	171 (10)	173 (5)
Weight, kg	62.6 (3.4)	63.4 (10.4)	64.5 (11.0)	67.5 (6.1)
Sex (male/female)	3/5	3/5	3/5	3/5

Data are presented as mean (SD).

**Table II** Pharmacokinetic Parameter Values in Healthy Subjects After Single Subcutaneous Administration of 30, 60, 100, and 300 µg/kg Pegfilgrastim

Parameter	30 µg/kg (n = 8)	60 µg/kg (n = 8)	100 µg/kg (n = 8)	300 µg/kg (n = 8)
$C_{max}$ , ng/mL	43.6 (20.0)	104 (63)	305 (93)	1070 (360)
$t_{max}$ , h	8 (8-16)	12 (6-24)	16 (16-24)	24 (24-48)
$t_{1/2}$ , h	50.9 (10.7)	62.1 (5.8)	50.1 (12.0)	46.3 (10.3)
AUC <sub>(0-∞)</sub> , ng·h/mL	887 (336)	3160 (2090)	13 100 (5800)	66 500 (24 400)
CL/F, mL/h/kg	38.6 (15.4)	24.9 (12.1)	8.88 (3.59)	5.19 (2.31)

Data are presented as mean (SD), except for  $t_{max}$ , which is presented as median (range).

fit of the model to the pegfilgrastim serum concentration and ANC profiles is illustrated in Figure 4. The model fit to the differential neutrophil counts is shown in Figure 5. The model accurately described the dose-dependent pharmacokinetic and pharmacodynamic behavior of pegfilgrastim.

Pharmacokinetic parameter estimates are listed in Table III. Pegfilgrastim exhibited first-order, delayed absorption with a mean absorption time of 1.84 days. The bioavailability of pegfilgrastim relative to the 30-µg/kg dose after subcutaneous administration was approximately 94%, 101%, and 80% for the 60-, 100-, and 300-µg/kg doses, respectively. Drug was absorbed into a central compartment with an apparent volume of distribution of 72.4 mL/kg. The Michaelis constant ( $K_m$ ) estimate for G-CSF receptor-mediated clearance was 5.93 ng/mL. The apparent clearance of pegfilgrastim by the linear pathway was 28.2 mL/d/kg.

Pharmacodynamic parameter estimates are listed in Table IV. The pharmacodynamic model included effects of pegfilgrastim on margination, mitosis, and mobilization. Margination was described as a pegfilgrastim-dependent expansion of the dilution volume of peripheral blood neutrophils. Based on the model estimate of  $E_{marg}$ , pegfilgrastim could elicit a 4-fold increase in the apparent neutrophil dilution volume. Pegfilgrastim was assumed to increase the production rate of metamyelocytes in marrow through effects on mitotic precursor cells. A comparison of the

baseline apparent production rate ( $S_0$ ) to the maximum production rate elicited by pegfilgrastim ( $E_{mit}$ ) suggests that pegfilgrastim could amplify myelocyte mass by a maximum factor of 4. Accelerated emergence of neutrophils into peripheral blood was modeled as the pegfilgrastim-dependent release of marrow band cells and segmented neutrophils into blood; the model predicted the increased proportion of band cells in circulation. The model estimate for the maximum rate of segmented neutrophil flux into blood ( $E_{seg}$ ) was nearly 3 times as fast as the maximum flux rate for band cells ( $E_{band}$ ), which contributed to maintenance of a higher proportion of segmented neutrophils in blood at all dose levels. The model fit was not improved by assuming different  $EC_{50}$  values for margination, mitosis, mobilization, and maturation; therefore, the same  $EC_{50}$  was applied to all processes. The  $EC_{50}$  estimate for pegfilgrastim-mediated effects was 9.86 ng/mL.

Elimination of neutrophils from peripheral blood was modeled using various assumptions, including different elimination rate constants for band cells and segmented neutrophils and the presence or absence of band cell maturation in peripheral blood. The best model fit was obtained when assuming an identical, first-order elimination rate constant for both neutrophil populations and assuming peripheral maturation of band cells into segmented neutrophils. The estimated half-life for mature, segmented neutrophils in peripheral blood was approximately 9 hours.

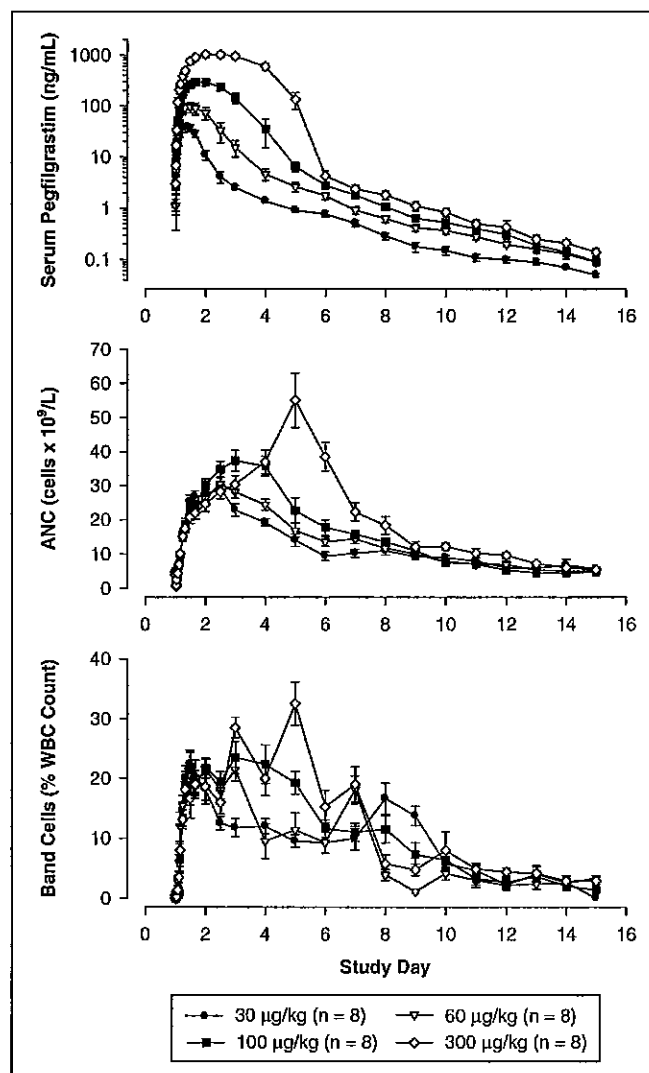


Figure 3. Pegfilgrastim concentration-time, ANC-time, percent band cell-time profiles in healthy subjects after a single subcutaneous administration of 30, 60, 100, and 300  $\mu\text{g}/\text{kg}$  pegfilgrastim ( $n = 8/\text{dose group}$ ). Data are presented as mean  $\pm$  SEM. ANC, absolute neutrophil count.

## DISCUSSION

Pegfilgrastim is a sustained-duration form of filgrastim and is indicated to decrease the incidence of infection, as manifested by febrile neutropenia, in patients with nonmyeloid malignancies receiving myelosuppressive chemotherapies. The pharmacokinetic properties enable the administration of 1 subcutaneous injection of pegfilgrastim per cycle of chemotherapy, as opposed to multiple daily injections of filgrastim, to prevent

chemotherapy-induced febrile neutropenia in patients with nonmyeloid malignancies.<sup>19,20</sup> The pharmacokinetics and pharmacodynamics of pegfilgrastim have been evaluated in healthy men and women to further characterize clearance mechanisms and concentration-response relationships for granulopoietic effects.

In healthy subjects, the pharmacokinetics of pegfilgrastim was nonlinear in a dose range of 30 to 300  $\mu\text{g}/\text{kg}$ ; the clearance of pegfilgrastim decreased with increasing dose, which is attributed to the neutrophil G-CSF receptor-mediated pathway. The terminal half-life was independent of dose (Table II), suggesting that pegfilgrastim serum concentrations at the terminal phase fell below levels saturating G-CSF receptors. The nonlinear pharmacokinetic profiles across doses could not be modeled with a constant  $V_{\text{max}}$  for the Michaelis-Menten clearance process. Pharmacokinetic modeling required that the  $V_{\text{max}}$  change in proportion to peripheral neutrophil counts, consistent with a G-CSF receptor-mediated clearance mechanism. The low rate of pegfilgrastim clearance by a neutrophil-independent, linear pathway may explain the prolonged exposure to drug and sustained effects during chemotherapy-induced neutropenia.<sup>8</sup> During neutropenia, the linear clearance pathway is expected to be the predominant elimination pathway for pegfilgrastim because of the decreased numbers of neutrophils and neutrophil precursors in blood and marrow. In contrast, filgrastim has a more rapid linear clearance rate, attributed to renal clearance of the smaller molecule,<sup>10</sup> which necessitates daily administration of the drug to maintain therapeutic concentrations in serum.

After administration of pegfilgrastim, a transient decrease in ANC followed by a rapid, dose-dependent increase in ANC was observed. The transient decrease in ANC, which occurred within the first hour after dosing, has also been observed following administration of filgrastim.<sup>21</sup> The initial transient decrease in ANC may be the result of neutrophil margination to endothelial cells, followed by neutrophil demargination and, finally, by an increase in neutrophil proliferation and accelerated release of mature neutrophils from bone marrow.<sup>22,23</sup> A dose-dependent increase in the percentage of band cells in peripheral blood was observed. The appearance of the younger, band cell population in peripheral blood—also observed after dosing with filgrastim<sup>5</sup>—suggests that pegfilgrastim stimulates early release of neutrophils from marrow.

A maturation-structured model of granulopoiesis was established to describe the relationship between pegfilgrastim serum concentrations and neutrophil counts in peripheral blood (Figures 1 and 2). Modeling

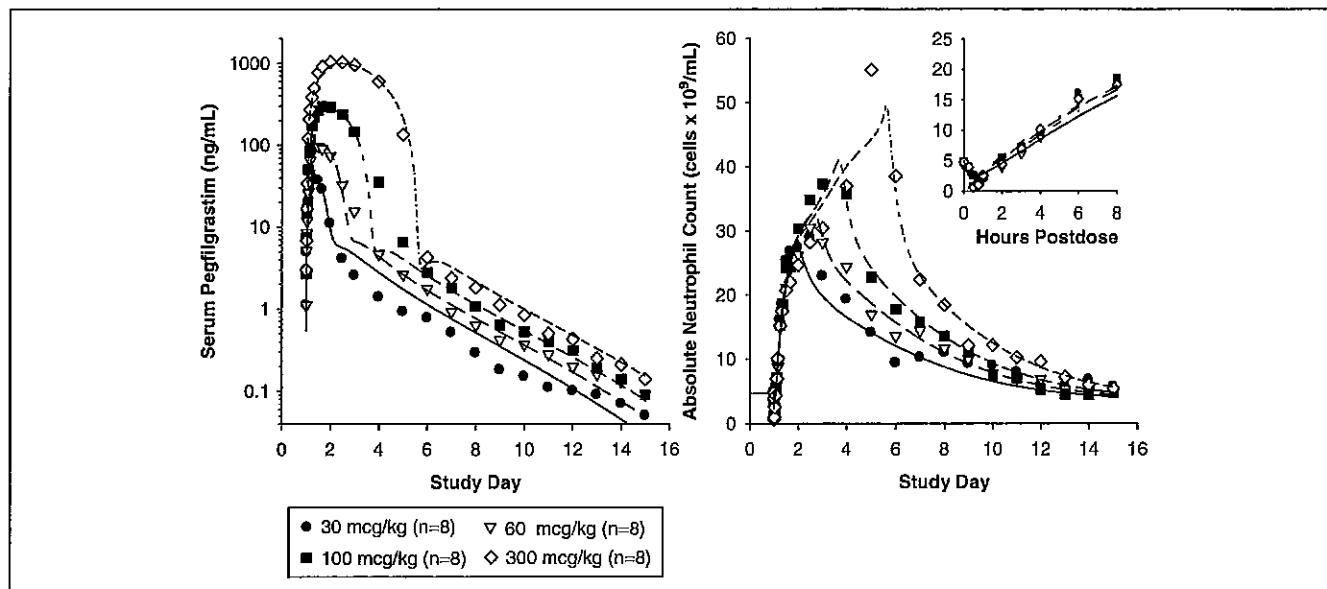


Figure 4. Modeled (lines) and observed (symbols) mean serum pegfilgrastim concentrations and absolute neutrophil counts (ANCs) after subcutaneous administration of 30, 60, 100, and 300  $\mu\text{g}/\text{kg}$  pegfilgrastim ( $n = 8/\text{dose group}$ ).

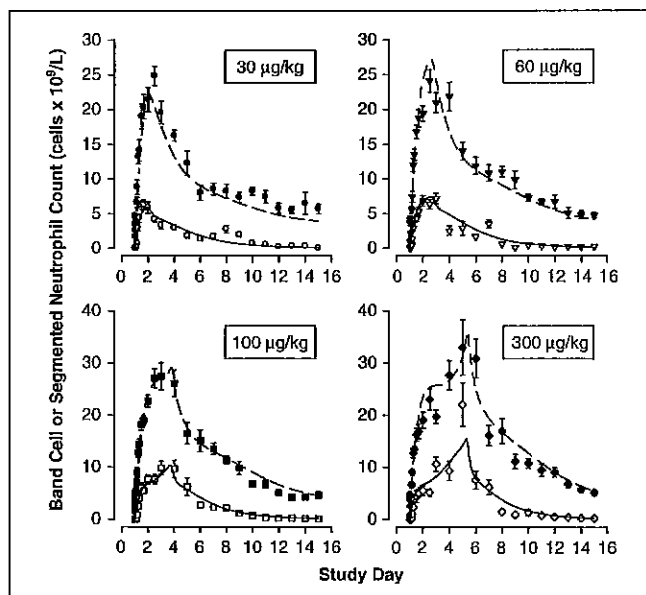


Figure 5. Modeled (lines) and observed mean band cell (open symbols) and segmented neutrophil (closed symbols) counts in peripheral blood after subcutaneous administration of 30, 60, 100, and 300  $\mu\text{g}/\text{kg}$  pegfilgrastim ( $n = 8/\text{dose group}$ ). Observed data are presented as mean  $\pm$  SEM.

predicted that pegfilgrastim could elicit a maximum 4-fold increase in the rate of metamyelocyte production. Consistent with the modeling results, neutrophil

kinetic studies of filgrastim in healthy subjects have shown that filgrastim exerts mitotic effects primarily on promyelocytes and myelocytes, with a 2- to 3-fold amplification of mitosis and a minimal delay before influx of metamyelocytes.<sup>24,25</sup>

Differential effects of pegfilgrastim on blood neutrophil populations were modeled as a pegfilgrastim-dependent flux of younger neutrophils, including band cells and segmented neutrophils through early release from marrow and a decrease in maturation time. Band cells are juvenile neutrophils and differ from mature, segmented neutrophils primarily by the shape of the nucleus. Modeling the differential neutrophil counts provides insight into mechanisms by which pegfilgrastim mobilizes neutrophils from marrow and stimulates neutrophil recovery following chemotherapy. Daily filgrastim doses of 30 and 300  $\mu\text{g}$  in healthy subjects have been demonstrated to shorten the neutrophil marrow transit time to  $4.3 \pm 0.2$  days and  $2.9 \pm 0.1$  days, respectively, compared with a  $6.3 \pm 0.3$ -day transit time in subjects in the control group, as determined by a flash labeling study with  $^3\text{H-TdR}$ .<sup>25</sup> As suggested by the model, this decreased transit time may be due, in part, to the early release of band cells and segmented neutrophils from bone marrow. The early release of neutrophils and accelerated precursor maturation may be important contributors to accelerated ANC recovery after myelosuppressive chemotherapy.

**Table III** Pharmacokinetic Model Parameter Estimates in Healthy Subjects After Single Subcutaneous Administration of 30, 60, 100, and 300 µg/kg Pegfilgrastim

Parameter	Estimate	CV (%)	95% Confidence Interval	
			Lower	Upper
$V_c/F$ , mL/kg	72.4	3.87	66.9	77.9
$k_{cat}/F$ , ng/d/kg/( $10^9$ cells/L)	276	16.3	187	364
$K_m$ , ng/mL	5.93	13.8	4.32	7.55
$CL_{lin}/F$ , mL/d/kg	28.2	8.04	23.7	32.6
$k_a$ , $d^{-1}$	0.547	2.88	0.516	0.578
$t_{lag}$ , d	0.00928	9.84	0.00748	0.0111
$F_{60}$ , %	0.943	2.70	0.893	0.994
$F_{100}$ , %	1.01	2.76	0.954	1.06
$F_{300}$ , %	0.798	3.20	0.748	0.848
MAT, d	1.84	2.86	1.73	1.94

$V_c/F$ , apparent volume of distribution;  $k_{cat}/F$ , apparent catabolic rate constant;  $K_m$ , Michaelis constant;  $CL_{lin}$ , linear clearance rate;  $k_a$ , absorption rate constant;  $t_{lag}$ , time delay for absorption;  $F_{60}$ , bioavailability of the 60-µg/kg dose relative to 30 µg/kg;  $F_{100}$ , bioavailability of the 100-µg/kg dose relative to 30 µg/kg;  $F_{300}$ , bioavailability of the 300-µg/kg dose relative to 30 µg/kg; MAT, mean absorption time.

**Table IV** Pharmacodynamic Model Parameter Estimates in Healthy Subjects After Single Subcutaneous Administration of 30, 60, 100, and 300 µg/kg Pegfilgrastim

Parameter	Estimate	CV (%)	95% Confidence Interval	
			Lower	Upper
$EC_{50}$ , ng/mL	9.86	7.17	8.47	11.3
$S_0$ , $10^9$ cells/L/d	86.4	24.9	44.1	129
$E_{mit}$ , $10^9$ cells/L/d	273	19.4	169	378
$E_{band}$ , $10^9$ cells/L/d	0.274	24.2	0.144	0.405
$E_{seg}$ , $10^9$ cells/L/d	0.769	21.9	0.439	1.10
$f_{mtt}$	0.434	18.0	0.281	0.588
$\tau_{meta}$ , d	0.947	28.2	0.421	1.47
$k_{ig}$ , $d^{-1}$	6.23	21.3	3.62	8.85
$E_{marg}$	3.08	25.0	1.56	4.59
$\gamma_N$	0.276	15.8	0.190	0.362
$k_{bpmat}$ , $d^{-1}$	1.03	28.5	0.453	1.61
$k_{\lambda}$ , $d^{-1}$	1.89	5.19	1.70	2.09
$t_{1/2,seg}$ , h	8.79	5.19	7.89	9.68

$EC_{50}$ , serum concentration of pegfilgrastim eliciting a half-maximal effect;  $S_0$ , baseline production rate of neutrophils;  $E_{mit}$ , maximum pegfilgrastim-stimulated production rate of neutrophils;  $E_{band}$ , maximum pegfilgrastim-stimulated influx rate of band cells into blood;  $E_{seg}$ , maximum pegfilgrastim-stimulated influx rate of segmented neutrophils into blood;  $f_{mtt}$ , maximum fraction by which mean maturation time can be decreased by pegfilgrastim;  $\tau_{meta}$ , baseline mean maturation time of metamyelocytes;  $k_{ig}$ , rate constant for precursor cell loss in marrow by ineffective granulopoiesis;  $E_{marg}$ , maximum effect on neutrophil margination;  $\gamma_N$ , Hill coefficient for neutrophil margination;  $k_{bpmat}$ , rate constant for maturation of band cells in blood to segmented neutrophils;  $k_{\lambda}$ , rate constant for random loss of band cells and segmented neutrophils from blood;  $t_{1/2,seg}$ , half-life of segmented neutrophils in blood.

Pegfilgrastim promoted rapid margination of peripheral blood neutrophils (Figure 4); this effect was modeled as an expansion of neutrophil dilution volume, consistent with a study of filgrastim in rats.<sup>26</sup> In rats receiving a single dose of filgrastim before an intravenous (IV) tracer injection of labeled neutrophils, the initial concentration of the labeled

neutrophils was reduced to about one third of that in control animals, consistent with a rapid effect on the initial dilution volume of the blood neutrophil pool. The margination of neutrophils after administration of filgrastim to healthy subjects has also been modeled using the assumption of an expanded neutrophil dilution volume.<sup>27</sup> Following administration of

the 300- $\mu\text{g}/\text{kg}$  dose, a sharp cusp in the neutrophil counts was observed on day 5. Based on modeling, this sharp peak appears to be due to demargination of neutrophils as serum pegfilgrastim concentrations decline rapidly and the effects on neutrophil dilution volume are reversed. The cusp was not predicted by the model if the margination effects were not included. Elimination of neutrophils from blood was modeled as a first-order loss, as numerous kinetic studies of labeled autologous neutrophils have demonstrated that neutrophils are eliminated randomly from circulation and not by senescence; the model estimate of segmented neutrophil half-life was consistent with these published results.<sup>13,25</sup>

The modeling-derived  $\text{EC}_{50}$  (9.86 ng/mL) and  $\text{EC}_{90}$  (88.7 ng/mL) for the mitotic, mobilization, and maturational effects of pegfilgrastim in healthy subjects agree with exposure-response data collected in patients receiving chemotherapy. In patients with non-small-cell lung cancer who received carboplatin and paclitaxel, patients receiving 30  $\mu\text{g}/\text{kg}$  pegfilgrastim experienced a lower ANC nadir and a slower ANC recovery compared to patients receiving 100- and 300- $\mu\text{g}/\text{kg}$  doses.<sup>8</sup> The 100- and 300- $\mu\text{g}/\text{kg}$  doses produced similar ANC profiles, suggesting that those doses produced serum levels on the plateau of the concentration-response curve. Consistent with this observation, the serum levels during the period of neutropenia in patients receiving 30  $\mu\text{g}/\text{kg}$  were less than 10 ng/mL (below the  $\text{EC}_{50}$ ) and were approximately 100 ng/mL (above the  $\text{EC}_{90}$ ) in patients receiving a 100- $\mu\text{g}/\text{kg}$  dose. In patients with breast cancer who received doxorubicin and docetaxel, a 60- $\mu\text{g}/\text{kg}$  dose of pegfilgrastim resulted in a slower ANC recovery and a longer duration of grade 4 neutropenia compared to patients receiving a 100- $\mu\text{g}/\text{kg}$  dose.<sup>19</sup> Serum levels in the breast cancer patients receiving the 60- $\mu\text{g}/\text{kg}$  dose were approximately 10 to 20 ng/mL (just above the  $\text{EC}_{50}$ ) during neutropenia and were above the  $\text{EC}_{90}$  in patients receiving 100  $\mu\text{g}/\text{kg}$  pegfilgrastim. The pharmacokinetic-pharmacodynamic relationship in healthy subjects appears to predict the optimum serum concentrations of pegfilgrastim in cancer patients receiving different chemotherapy regimens.

In summary, pegfilgrastim exhibited nonlinear pharmacokinetics in healthy subjects and produced dose-dependent increases in band cell and segmented neutrophil counts in peripheral blood, consistent with the biology of G-CSF-mediated granulopoiesis. A mechanistic pharmacokinetic-pharmacodynamic model incorporating feedback regulation of pegfilgrastim clearance by neutrophils accurately described the dose-dependent pharmacokinetics and

pharmacodynamics of the drug in healthy volunteers. The pharmacokinetic-pharmacodynamic model may have future applicability in the modeling and simulation of pegfilgrastim therapy in various settings of chemotherapy-induced neutropenia.

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