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APPLICATION NUMBER:

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MEDICAL REVIEW(S)

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From: Joseph E. Gootenberg, MD, DTBOP/ODE VI

Subject: Clinical Review of BLA STN 125031
Amgen, Inc.
Pegfilgrastim (NEULASTA™)

Through: Patricia Keegan, Director, DTBOP

To: BLA STN 125031 File

This document is the Medical Officer Clinical Review for BLA STN 125031

Sponsor: Amgen, Inc.

Product: Pegfilgrastim (NEULASTA™)

Proposed Indication:

"NEULASTA™ is indicated to decrease the incidence of infection, as manifested by febrile neutropenia in patients with non-myeloid malignancies receiving myelosuppressive anti-cancer drugs associated with a clinically significant incidence of febrile neutropenia."

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Executive Summary:

The licensure of Pegfilgrastim (tradename, NEULASTA™) as a 6 mg fixed dose administered subcutaneously once per cycle of myeolosuppressive chemotherapy is recommended for the following indication: *"NEULASTA™ is indicated to decrease the incidence of infection, as manifested by febrile neutropenia in patients with non-myeloid malignancies receiving myelo-suppressive anti-cancer drugs associated with a clinically significant incidence of febrile neutropenia."*

Efficacy

In two large multicenter trials, the duration of severe neutropenia (DSN) following a single dose of either 100 µg/kg or fixed dose 6 mg of subcutaneous Pegfilgrastim administered during the first cycle of chemotherapy met prespecified criteria for non-inferiority to daily subcutaneous Filgrastim (NEUPOGEN®). In both trials, the results of secondary endpoints of DSN in cycles 2 through 4, depth of absolute neutrophil count (ANC) nadir in cycles 1 through 4, rates of febrile neutropenia (FN) and time to ANC recovery supported the findings regarding the primary endpoint. Four supportive trials that included a range of both solid and hematologic malignancies yielded similar results.

In a series of subgroup analyses, there was no evidence of an interaction between treatment and subject age, gender, race, or body weight. Clinical efficacy was similar irrespective of subjects' weight indicating that a Pegfilgrastim fixed dose of 6 mg is a reasonable alternative to eight-adjusted dosing in adults.

Safety

Pegfilgrastim was tested at doses of 30 to 300 µg/kg and at a fixed dose of 6 mg. At doses of 100 µg/kg and 6 mg, the safety profile of Pegfilgrastim was comparable to that of Filgrastim. In the fixed-dose study, the 6 mg fixed dose of Pegfilgrastim was well tolerated by subjects with a wide range of body weights.

The most common dose-related adverse event was bone pain in 26% of subjects. Elevated alkaline phosphatase and LDH occurred in fewer than 20% of subjects receiving Pegfilgrastim and were not associated with clinical symptoms

No subject who received Pegfilgrastim became seropositive for antibodies to the drug, nor was there evidence of neutralizing antibodies.

Pegfilgrastim was well tolerated in every subject population and subgroup tested. There are no contraindications to the use of Pegfilgrastim.

Introduction

General

Pegfilgrastim is a chemically modified human granulocyte colony-stimulating factor (G-CSF). Pegfilgrastim is the USAN-designated name for recombinant methionyl human granulocyte colony-stimulating factor (r-metHuG-CSF) (Filgrastim) covalently bound to a 20-kd polyethylene glycol (PEG) molecule. NEULASTA™ is the Amgen Inc. trademark for Pegfilgrastim.

Natural colony-stimulating factors (CSFs) are glycoproteins that act on hematopoietic cells by binding to specific cell surface receptors and stimulating proliferation, differentiation, commitment and end-cell functional activation. Endogenous G-CSF (Granulocyte-CSF) is a lineage specific CSF that is produced by monocytes, fibroblasts, and endothelial cells. G-CSF regulates the production of neutrophils within the bone marrow, and affects neutrophil progenitor proliferation and differentiation as well as selected end-cell functional activation. G-CSF has been shown to have minimal direct effects in vivo or in vitro on the production of hematopoietic cell types other than the neutrophil lineage. Recombinant methionyl human G-CSF (r-metHuG-CSF) has been shown to enhance neutrophil production and to mobilize hematopoietic stem cells from the bone marrow to the blood in human subjects.

Filgrastim is the USAN-designated name for Amgen. Inc.'s recombinant methionyl human G-CSF (r-metHuG-CSF) which is marketed in the United States under the trade name NEUPOGEN®. Filgrastim was originally approved by the US FDA in 1991 to decrease the incidence of infection, as manifested by febrile neutropenia (FN), in patients with non-myeloid malignancies receiving myelosuppressive anticancer drugs associated with a significant incidence of severe neutropenia with fever. NEUPOGEN® is marketed in 93 countries for this indication. In addition, Filgrastim has been approved in many countries, including the United States, for reduction of the time to neutrophil recovery following autologous or allogeneic bone marrow transplantation (BMT), treatment of severe chronic neutropenia (SCN), mobilization of peripheral blood progenitor cells (PBPC), reducing the time to neutrophil recovery following induction or consolidation chemotherapy for acute myeloid leukemia (AML), and for treatment of neutropenia in patients with HIV infection and/or acquired immunodeficiency syndrome. From December 1986 to March 2001, approximately 26,000 subjects have been enrolled in Amgen-sponsored clinical trials of Filgrastim. Over [redacted] patients have received Filgrastim during the more than 10 years that it has been marketed.

Filgrastim is predominately eliminated from the body by two routes: the major excretion mechanism via renal filtration, and secondarily via neutrophil-mediated clearance. Because of its short (approximately 3 hour) half-life in the circulation, Filgrastim requires multiple daily injections to exert an extended pharmacological effect. Pegfilgrastim was developed by Amgen, Inc. in an attempt to produce a chemically modified Filgrastim with a longer circulating half-life which, therefore, would require fewer injections to exert an extended pharmacological effect. To produce Pegfilgrastim, Filgrastim is chemically modified by covalently binding a 20-kd polyethylene glycol (PEG) molecule to the amino terminal (N-terminal) residue. This results in a molecule with a hydrodynamic size greater than the threshold for glomerular filtration, and virtually eliminates renal filtration as a route of excretion. As a result, Pegfilgrastim remains in the circulation for a prolonged duration, and fewer injections are required for pharmacological effect. The major route of elimination for Pegfilgrastim is via specific binding to myeloid cell surface G-CSF receptors, and subsequent clearance.

The biologically active moiety of Pegfilgrastim is identical to Filgrastim, and it has the same mechanism of action, i.e., binding to the G-CSF receptor on myeloid cells. Non-clinical studies have demonstrated that Pegfilgrastim appears to have a similar, or only slightly lower, receptor binding affinity than that of Filgrastim and the same pharmacological properties.

Proposed Indication

Amgen Proposed Indication

[redacted]

CBER Proposed Indication

"NEULASTA™ is indicated to decrease the incidence of infection, as manifested by febrile neutropenia, in patients with non-myeloid malignancies receiving myelosuppressive anti-cancer drugs associated with a clinically significant incidence of febrile neutropenia."

Regulatory History of BLA 125031

Chronology of BLA 125031

Table 1: Chronology of BLA 125031 Milestones

Date	Milestone
April 1997	IND — original submission
September 1997	Clinical Trials Initiated
May 1999	End of Phase 2 Meeting
October 2000 November 2000 December 2000	Pre-BLA Discussions
March 2001	BLA 125031/0 Submitted
January 31, 2002	NEULASTA™ Approval

Major Clinical Regulatory Agreements During Development of Pegfilgrastim

The following major clinical regulatory agreements were reached during the development of Pegfilgrastim:

During Phase 2

- Reduction in duration of severe (grade 4; ANC less than 0.5 x 10^9/L) neutropenia (DSN) is an acceptable endpoint in the licensure study for Pegfilgrastim provided that in the chemotherapy regimen being studied, the incidence of FN was approximately 40% in subjects not receiving cytokine support. This is consistent with ASCO practice guidelines for appropriate use of hematopoietic growth factors.

- Provided that comparable reductions in DSN and incidence of febrile neutropenia were observed, fewer injections represent an obvious improvement in Quality of Life and would not require specific evaluation.

Amgen's Proposal for Registration Trials

Amgen's clinical development of Pegfilgrastim focused on demonstrating comparable, or non-inferior, safety and efficacy of a single injection of Pegfilgrastim per cycle of chemotherapy compared to daily injections of Filgrastim. The pivotal trial program proposed at the conclusion of Phase 2 consisted of 2 randomized, double-blind, placebo-controlled non-inferiority studies in advanced and metastatic breast cancer, one treating on a per weight-dose basis and the other on a fixed-dose basis. These trials were to be conducted in the US, Canada, Europe, and Australia.

The primary endpoint in the 2 pivotal trials was proposed to be duration of severe neutropenia (defined as grade 4 neutropenia) based on the following rationale:

- The duration of neutropenia is strongly predictive of the incidence of febrile neutropenia in chemotherapy-induced neutropenia settings.
- The clinical efficacy of Filgrastim, based on reduction in the duration of neutropenia (which in turn results in reduced incidence of febrile neutropenia, infectious episodes, IV antibiotic use, and hospitalization) has been thoroughly demonstrated.
- The mechanism of action of Pegfilgrastim is identical to that of Filgrastim, namely, preferential stimulation of the growth, differentiation, and function of neutrophils.
- ANC is a quantitative, standardized, reproducible, and clinically meaningful measure.

End-of-Phase 2 Meeting

- The definition of time to ANC recovery in the two pivotal studies should be the first of 2 consecutive days with an ANC of greater than or equal to 2 x 10^9/L.
- Differences in the incidence of FN, severity and duration of thrombocytopenia, and differences in mortality and survival would be important in the review and approval of Pegfilgrastim.

- Patient follow-up would be extended to 3 months after last injection of study drug and an assessment of hematologic toxicity and incidence of immunogenicity of Pegfilgrastim would be performed.

- The phase 3 statistical analysis plan (SAP) was acceptable including the proposal for the handling of missing data.

- Nonparametric analysis of confidence intervals would be performed to evaluate non-inferiority of the primary endpoint.

- A separate study would be conducted to investigate the safety, pharmacodynamic effect, and immunogenicity of delayed re-exposure to Pegfilgrastim.

- In accordance with ICH guidelines, a safety database of 300 to 600 subjects would be acceptable for submission of the BLA.

Follow-up to End-of-Phase 2 Meeting

- Time to disease progression, overall survival up to 2 years, and long term safety data would only be required from 1 of the 2 pivotal studies.

- The BLA may be submitted with 6 months of follow-up data (time to disease progression and overall survival). Thereafter, regular safety updates will be provided to the BLA file for a length of time to be determined by the adverse event profile relative to Filgrastim.

Phase 3 Analysis, Pivotal Fixed-dose Study, and Pediatric Study

- Amgen would amend the statistical analysis plan to include and define outliers and specify the stratification variables for comparing survival and time-to-progression of disease.

- The proposed analysis method of resampling for duration of neutropenia is acceptable.

- Amgen would explore more traditional parameters of pharmacokinetics, including clearance in the 990749 fixed-dose protocol.

- Amgen would include in the analysis the weight ranges and trends with regards to whether a higher or lower dose is needed.

- Amgen would revise the pediatric study protocol to

- The BLA filing would be accepted with deferral of the pediatric data, if the pediatric trial were initiated and good faith efforts toward collection of data were underway.

Pre-BLA Discussions

- Data from the pediatric and retreatment studies could be provided as text within the ISS only.

- Data from the investigator IND study

could be presented within the BLA in the format in which it was received from the investigator

Preclinical Development Program

General

The biologically active moiety of Pegfilgrastim is identical to Filgrastim, and it has the same mechanism of action, i.e., binding to the G-CSF receptor on myeloid cells. Preclinical studies established that Pegfilgrastim appears to have a similar, or slightly lower, receptor binding affinity than that of Filgrastim and has similar pharmacological properties. In animal studies, Pegfilgrastim demonstrated a longer circulating half-life due to the covalent binding of the PEG molecule to Filgrastim, which virtually eliminates renal clearance (the primary factor for the short half-life of Filgrastim of approximately 3 hours), leaving neutrophil-mediated clearance as the primary mechanism for elimination. This resulted in a prolonged duration of pharmacologic activity as compared to Filgrastim. Comparative studies in animal models have shown that a single dose of Pegfilgrastim produces prolonged neutrophilia (elevation in absolute neutrophil count [ANC]) compared to a single dose of Filgrastim.

The major findings of the toxicology studies of Pegfilgrastim were qualitatively and quantitatively similar to those observed previously and concurrently with Filgrastim and included the following:

- Pegfilgrastim did not cause mortality or evidence of toxicity in rats at single doses as high as 10,000 µg/kg, 100-fold the clinical dose of Pegfilgrastim. Extramedullary hematopoiesis was evident in the enlarged spleens of rats at 10,000 µg/kg.
- In sub-chronic/chronic repeat-dose studies, Pegfilgrastim was well-tolerated at weekly doses of 1000 µg/kg and 750 µg/kg in rats and monkeys, respectively, with no treatment-related clinical signs or mortality. Laboratory and pathologic findings included dose-related increases in numbers and functional abilities of neutrophils, morphological and maturational changes in neutrophils, decreased marrow M/E ratio, decreased red cell mass, increased Alkaline Phosphatase levels, splenomegaly, and extramedullary hematopoiesis in the liver and spleen. The extramedullary hematopoiesis in the spleen and liver were postulated to reflect a secondary pharmacological effect of mobilization of hematopoietic stem cells from the bone marrow.
- In reproductive toxicology studies, adverse effects were principally observed in embryo-fetal development studies in rabbits, in which decreased fetal body weights, increased post-implantation loss due to early resorptions and decreased numbers of live fetuses were noted.

Table 2: 125031 *in vivo* Preclinical Trials

Study Designation/ Source	Study Title	GLP Status
Study Ang00-149 / Amgen	Subcutaneous injection of Pegfilgrastim or Filgrastim in BDF1 mice	n
Study Ang00-152 / Amgen	Daily fluctuations in neutrophils following daily Filgrastim or a single injection of Pegfilgrastim in BDF1 mice	n
Study Ang00-150 / Amgen	Continuous infusion of Pegfilgrastim in BDF1 splenectomized mice	n
Study Ang00-146 / Amgen	Timing of Pegfilgrastim 1 relative to chemotherapy in BDF1 mice	n
Study Ang00-151 / Amgen	IV versus SC administration of Pegfilgrastim in BDF1 mice	n
Study Ang00-153 / Amgen	Mobilization and Transplant of PBPC by Pegfilgrastim or Filgrastim in BDF1 mice	n
Study Ang00-154 / Amgen	Immunological response to rhuG-CSF or Pegfilgrastim in dogs	n
Study 00-8748 /	To examine the efficacy and immunogenicity of Pegfilgrastim relative to Filgrastim in chimpanzees	n
Study 100965 /	Single dose intravenous 2 week toxicity study of Pegfilgrastim in rats	y
Study 100204 / Amgen	A 9-day screening toxicology study of subcutaneous Pegfilgrastim and Filgrastim-SD/02-157 in Sprague-Dawley rats	n

Study Designation/ Source	Study Title	GLP Status
Study 970002 / [redacted]	2-week subcutaneous toxicity study of G-20K in the rat	y
Study 100062 / [redacted]	A 3/6-month subcutaneous/intravenous toxicity study of Pegfilgrastim in rats with recovery	y
Study 100298 / [redacted]	A 1-month repeat-dose subcutaneous toxicity study of Pegfilgrastim in cynomologus monkeys with a 1-month recovery	y
Study 100782 / [redacted]	The effect of Pegfilgrastim administered subcutaneously on fertility and early embryonic development to implantation in rats	y
Study 100199 / [redacted]	A range finding developmental toxicity study in rabbits with Pegfilgrastim via subcutaneous administration	y
Study 100297 / [redacted]	The effects on embryo-fetal development of subcutaneous administration of Pegfilgrastim to rabbits	y
Study 100168 / [redacted]	A study of the effects of Pegfilgrastim administered subcutaneously on embryo-fetal development in rats.	y
Study 100439 / [redacted]	A study of the effects of Pegfilgrastim administered subcutaneously on pre- and postnatal development, including maternal function in the rat	y
Study PK97010 / Amgen	Pharmacokinetic and pharmacodynamic dose-ranging study of single intravenous doses of 20 KD pegylated, granulocyte colony stimulating factor (PEG-GCSF) in male CD-1 mice	n
Study PK97005 / Amgen	Pharmacokinetic and pharmacodynamic dose-ranging study of 20 KD mono, pegylated-granulocyte colony stimulating factor (PEG-GCSF) in male Sprague-Dawley rats	n

Study Designation/ Source	Study Title	GLP Status
Study 100338 / [redacted]	Pharmacokinetics, pharmacodynamics, and placental transfer of r-metHuG-CSF-SD/01 in pregnant and non-pregnant rats via subcutaneous administration	y
Study 100626 / Amgen	A pharmacokinetic study of Filgrastim and Pegfilgrastim following intravenous administration in bilateral nephrectomized male Sprague-Dawley rats	n
Study 101170 / Amgen	Pharmacokinetics of Pegfilgrastim after a subcutaneous injection of 2 different concentrations of dose solution to male Sprague-Dawley rats	n
Study PK 94006 / [redacted]	Pharmacokinetics and pharmacodynamics of PEG (20 K) G-CSF given at 2 dose levels intravenously and subcutaneously to rhesus monkeys	n
Study PK97018 / Amgen	A 10-Day pharmacodynamic study of G-20K mono PEG G-CSF administered by subcutaneous injection to cynomolgus monkeys	n
Study 100841 / Amgen	A pharmacokinetic and pharmacodynamic study in myeloablated rhesus monkeys followed by bone marrow transplant	n

Clinical Development Program

Pegfilgrastim was developed as a longer-acting chemically-modified Filgrastim that would exert effective pharmacologic action utilizing a single injection of drug per cycle of chemotherapy, as opposed to daily injections continuing until ANC recovery.

The Pegfilgrastim oncology clinical development program included 10 Amgen-sponsored studies conducted in the US, Europe, Australia, and Canada in accordance with Good Clinical Practice (GCP). The phase 1 program consisted of 2 dose-escalation and pharmacokinetic studies in healthy volunteers (studies 970230 and 980230). The completed phase 2 program consisted of 2 randomized, dose-ranging, multicycle chemotherapy trials in adult solid tumors (thoracic [study 970144] and breast [study 980147]), and 2 open-label, randomized trials in hematologic malignancies: a non-Hodgkins lymphoma (NHL)/Hodgkins lymphoma study (study 990177) and a dose-ranging study in elderly subjects with NHL(study 990118). The pivotal trial program comprised 2 randomized, double-blind, placebo-controlled studies in advanced and metastatic breast cancer, one administrating Pegfilgrastim as a weight-adjusted dose (study 980226) and the other as a fixed-dose (study 990749),carried out in the US, Canada, Europe, and Australia. These completed trials are described in table 3 below. In addition, a pediatric sarcoma study (study 990130) and a study retreating subjects with Pegfilgrastim after previous exposure (study 990736) were ongoing at the time of submission of this BLA and are described in table 4 below.

Of the 882 subjects enrolled in the entire Pegfilgrastim oncology clinical development program, 540 received Pegfilgrastim and 342 received Filgrastim as the comparator drug. Of the 540 subjects who received Pegfilgrastim, 65 subjects were healthy volunteers; the remaining subjects were patients with cancer. A diagram of the Pegfilgrastim clinical development program is provided in Figure 1 below, and a chronological summary of the completed Pegfilgrastim clinical program is provided in table 3 which follows.

In addition to the trials included in the Pegfilgrastim clinical development plan outlined above, 1 Amgen-sponsored study and 2 physician-sponsored studies were ongoing during the period of this review. These included an Amgen-sponsored trial investigating pharmacokinetics and pharmacodynamics of administration on a fixed-dose basis (study 2000131), and physician-sponsored studies of [redacted]. These trials are described in table 4 below.

Figure 1: Pegfilgrastim Clinical Development Program

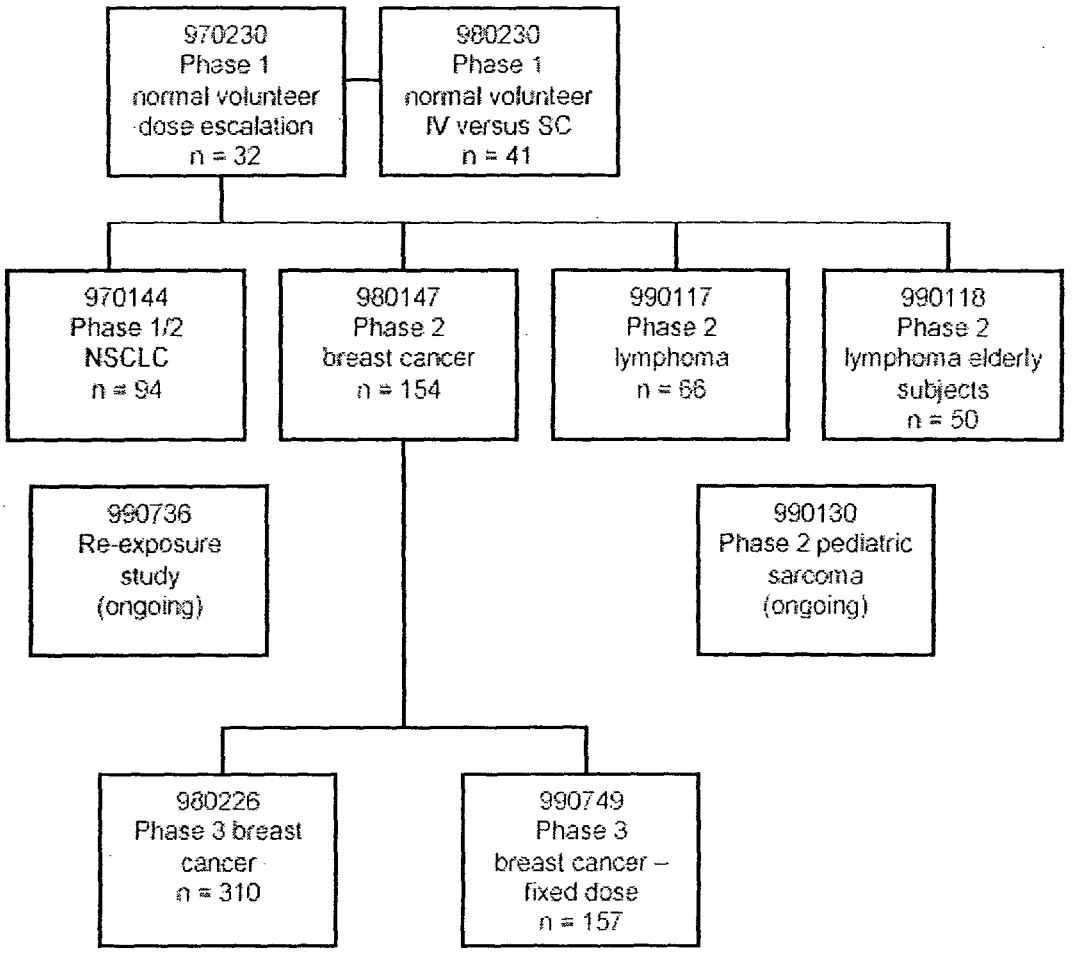

Table 3: Chronology of Completed Clinical Trials Submitted to BLA 125031

Study Number	Clinical Trial Title	Study Characteristics			Date Initiated	Date Completed
		N	Randomized	Double-Blinded		
970144	*A Study of Pegfilgrastim in Patients with Non-small Cell Lung Cancer or Other Thoracic Tumors Treated with Carboplatin and Paclitaxel.*	94	Yes	No	September 1997	October 1999
970230	*A Phase 1 Study of Pegfilgrastim in Normal Volunteers.*	32	No	No	November 1997	February 1998
980147	*A Randomized Study of Single Administration Pegfilgrastim or Daily Filgrastim as an Adjunct to Chemotherapy in Patients with High-risk Stage II or Stage III/IV Breast Cancer*	154	Yes	Yes/No	July 1998	October 1999
980230	*A Phase I Study Comparing Subcutaneous Injection and Intravenous Infusion of Pegfilgrastim or Subcutaneous Daily Filgrastim in Healthy Volunteers*	41	Yes	No	September 1998	January 1999

Study Number	Clinical Trial Title	Study Characteristics			Date Initiated	Date Completed
		N	Randomized	Double-Blinded		
990118	*An Open-label, Randomized, Dose-ranging Study of a Single Administration Pegfilgrastim versus Daily Filgrastim as an Adjunct to Chemotherapy in Elderly Subjects with Non-Hodgkin's Lymphoma*	50	Yes	No	June 1999	November 2000
980226	*A Blinded, Randomized, Multicenter Study to Evaluate Single Administration Pegfilgrastim per Cycle Versus Daily Filgrastim as an Adjunct to Chemotherapy in Subjects With High-risk Stage II or Stage III/IV Breast Cancer.*	310	Yes	Yes	August 1999	January 2001
990117	*An Open-label, Randomized, Dose-ranging Study of a Single Administration Pegfilgrastim versus Daily Filgrastim as an Adjunct to Chemotherapy in Subjects with Non-Hodgkin's Lymphoma*	66	Yes	No	September 1999	November 2000
990749	*A Blinded, Randomized, Multicenter Study to Evaluate Fixed Dose Single Administration Pegfilgrastim per Cycle Versus Daily Filgrastim as an Adjunct to Chemotherapy in Subjects With High-risk Stage II or Stage III/IV Breast Cancer*	157	Yes	Yes	November 1999	November 2000

Phase 1 and Phase 2 Trials

Phase 1 Trials

Study 970230

Title: A Phase 1 Study of Pegfilgrastim in Normal Volunteers.

Study Period: November 1997 to February 1998

Center:

This phase 1, open-label, dose-escalation, single-center study was designed to evaluate the safety, pharmacodynamics (PD), and pharmacokinetics (PK) of Pegfilgrastim administered as a single subcutaneous (SC) dose in healthy subjects.

Objectives:
The primary objectives of this study were to evaluate the safety, PD, and PK of Pegfilgrastim administered as a single subcutaneous (SC) dose in healthy subjects

Design:
This was a phase 1, open-label, dose-escalation, single-center study designed to evaluate the safety, PD, and PK of a single dose of Pegfilgrastim in healthy subjects. The doses of Pegfilgrastim selected for this study were based on comparative efficacy studies in animal models. The study was initially designed to investigate Pegfilgrastim as a single dose at 30, 100, and 300 µg/kg in 8 subjects per cohort; however, the protocol was amended to include an additional intermediate dose of 60 µg/kg based on an acceptable safety and efficacy profile at similar doses in a concurrent study in subjects with non-small cell lung cancer (Amgen Study 970144).

Subjects were sequentially enrolled in 1 of 4 Pegfilgrastim dose cohorts and received a single dose of Pegfilgrastim at 30, 60, 100, or 300 µg/kg. Adverse events, concomitant medications, and changes in vital signs and laboratory values were monitored through day 15. Blood samples were collected concurrently for PK and PD analyses through day 15. An additional blood sample for PK, PD, and immunogenicity analyses was collected in a subset of subjects at later time points.

Results:
Thirty-two healthy subjects were enrolled in this study. Pegfilgrastim was well tolerated at single doses ranging from 30 to 300 µg/kg. Neutralizing antibodies were not detected at baseline, during the 15 day observation period, nor in the 15 subjects who returned for late evaluation between days 77 and 86. A single dose of Pegfilgrastim demonstrated a sustained and dose-dependent increase in peak absolute neutrophil counts (ANC) and circulating CD34+ cells. Mean maximum ANC was reached from 1.2 to 4.0 days, and increased with increasing dose of Pegfilgrastim. Values ranged from 29.9 to 55.2 x 10^9/L for Pegfilgrastim doses ranging from 30 µg/kg to 300 µg/kg. Circulating CD34+ cells reached peak levels at day 4 with means of approximately 40 and 120 x 10^3 CD34+ cells/ml at the 100 µg/kg and 300 µg/kg dose levels, respectively. The pharmacokinetic profile of Pegfilgrastim was nonlinear, consistent with G-CSF receptor-mediated clearance at high serum concentrations.

Study 980230

Title: A Phase I Study Comparing Subcutaneous Injection and Intravenous Infusion of Pegfilgrastim or Subcutaneous Daily Filgrastim in Healthy Volunteers.

Study Period: September 1998 to January 1999

Center:

This phase-1, single-center, open-label PK/PD study in healthy volunteers compared a single administration of either SC or intravenous (IV) Pegfilgrastim (30 or 60 µg/kg) to daily SC Filgrastim (NEUPOGEN®) 5 µg/kg/day for 10 days.

Objectives:
The primary objectives of this study were to compare the safety, PK, PD, and effect on neutrophil function of Pegfilgrastim (30 or 60 µg/kg) administered as a single SC or IV dose to daily SC Filgrastim 5 µg/kg/day for 10 days in healthy subjects

Design:
This was a phase-1, single-center, 5 arm, randomized open-label PK/PD study in healthy volunteers designed to compare a single administration of either SC or IV Pegfilgrastim (30 or 60 µg/kg) to daily SC Filgrastim (NEUPOGEN®); 5 µg/kg/day for 10 days. Subjects were randomized in a 1:1:1:1:1 ratio to the 5 treatment groups. Subjects in groups 1 through 4 received a single dose of Pegfilgrastim on day 1 delivered per the following assignment: group 1, 30 µg/kg SC; group 2, 30 µg/kg IV; group 3, 60 µg/kg SC ;and group 4, 60 µg/kg IV. Subjects in group 5 received daily SC Filgrastim (5 µg/kg/day) on days 1 through 10 or until absolute neutrophil count (ANC) $\geq 75 \times 10^9$/L, whichever came first. Daily blood sampling was conducted days 1 to 14 for PK, PD, blood chemistry, and antibody measurements. Follow-up visits were scheduled within 30 days from the last day of study drug.

Results:
Forty-one healthy subjects were enrolled in this study; 40 subjects (8 subjects per treatment group × 5 treatment groups) completed the study. One Pegfilgrastim subject was withdrawn from the treatment phase because of an adverse event of moderate severity considered not related to the study drug by the investigator; this subject completed all study assessments and was considered evaluable for safety.

Pegfilgrastim, administered either IV or SC at either 30 or 60 µg/kg, was well tolerated in the 33 healthy subjects studied, with an adverse event profile similar to that observed in the 8 subjects receiving daily SC Filgrastim. A single dose of Pegfilgrastim produced a sustained elevation of ANC comparable to multiple daily doses of Filgrastim. Although IV administration yielded greater systemic exposure of Pegfilgrastim than SC administration, the magnitude of the peak ANC and the duration of ANC elevation were greater after SC administration than after IV administration. A single dose of Pegfilgrastim produced changes in neutrophil function similar to those observed with daily SC Filgrastim.

Two screening assays, a radio-immune assay (RIA) and an enzyme immunoassay (EIA) were used to detect antibodies binding to Pegfilgrastim. Although nonspecific seroreactivity was detected in 16 of 33 Pegfilgrastim subjects (48%) and 2 of 8 Filgrastim subjects (25%) in at least 1 of the 2 screening immunoassays, no neutralizing antibodies were detected at baseline, during the 14 day observation period, or at the 30 day follow-up.

On the basis of these findings, subcutaneous administration of Pegfilgrastim was chosen as the route of administration to be taken forward in clinical studies.

Completed Phase 2 trials

Study 970144

Title: A Study of Pegfilgrastim in Patients with Non-small Cell Lung Cancer or Other Thoracic Tumors Treated with Carboplatin and Paclitaxel."

Study Period: September 1997 to October 1999

Centers: Parts A and B, Single center

Parts C and D, Multi-center in the U.S

This phase 1/2 study represented the first clinical investigation of Pegfilgrastim. Parts A and B (phase 1) were designed to determine preliminary safety, PD, and PK properties of Pegfilgrastim over a range of doses in subjects with non-small cell lung cancer (NSCLC). A single SC injection of Pegfilgrastim was compared to multiple daily SC injections of Filgrastim in a dose escalation design during both pre- and post chemotherapy periods.

Parts C and D represent the randomized, open-label, dose ranging phase 2 portion of the study. The duration of severe neutropenia was assessed in subjects undergoing multiple-cycle chemotherapy with carboplatin and paclitaxel for the treatment of nonsmall-cell lung cancer or other thoracic tumors. The activity of a single SC injection of Pegfilgrastim was compared to multiple daily SC injections of Filgrastim.

Objectives:
The primary objectives of Parts A and B (phase 1) were to compare safety, PD, and PK properties of a single SC injection of Pegfilgrastim, over a range of doses, to multiple daily SC injections of Filgrastim in subjects with non-small cell lung cancer (NSCLC). Part A was conducted prior to the administration of chemotherapy, and Part B was conducted following a single cycle of myelosuppressive chemotherapy.

The primary objectives of Parts C and D were to compare the safety, and PD properties (DSN) of a single SC injection of Pegfilgrastim over a range of doses

to those of multiple daily SC injections of Filgrastim in subjects with non-small cell lung cancer (NSCLC) undergoing multiple cycles of myelosuppressive chemotherapy.

Design:
This was a phase 1/2, multi-center, 4 phase, randomized open-label PK/PD study in subjects with nonsmall-cell lung cancer or other thoracic tumors designed to compare a single administration of various doses of SC Pegfilgrastim to daily SC Filgrastim (NEUPOGEN®); 5 µg/kg/day. Part A and B were two components of a dose escalation trial in which subjects were randomized 1:1:1:1 to single SC doses of Pegfilgrastim at 30, 100, or 300 µg/kg versus daily doses of Filgrastim. Part A was a 14-day prechemotherapy phase (cycle 0), and part B was a 21-day first cycle postchemotherapy phase for eligible subjects who had completed part A. Changes in ANC, levels of hematopoietic progenitor cells in the peripheral blood and Pegfilgrastim PK were determined in the prechemotherapy phase. After cycle 1 of chemotherapy, changes in ANC, levels of hematopoietic progenitor cells in the peripheral blood, Pegfilgrastim PK, duration of severe neutropenia and incidence of febrile neutropenia were assessed

The study was amended in February and October 1998 to include parts C and D, which extended dosing in subsequent subjects to multiple (4) cycles of chemotherapy in a dose ranging design. In part C, subjects were randomized 1:1:1 to receive Pegfilgrastim 60 or 100 µg/kg, or Filgrastim 5 µg/kg/day (n = 20 per group). In part D, subjects were randomized 3:1 to receive Pegfilgrastim 30 µg/kg (n = 15) or Filgrastim 5 µg/kg/day (n = 5). All subjects underwent multiple-cycle chemotherapy with carboplatin and paclitaxel. Duration of severe neutropenia, incidence of febrile neutropenia and time to ANC recovery were studied following a maximum of 4 cycles per subject.

Results:
A total of 94 subjects were enrolled in this study; 29 subjects were randomized to receive Filgrastim, and 65 subjects were randomized to receive Pegfilgrastim.

Pharmacodynamic response to a single injection of Pegfilgrastim in the pre-chemotherapy phase (part A, cycle 0) was demonstrated by a rapid and dose-dependent increase in ANC. In part B, after a single cycle of chemotherapy, mean duration of severe neutropenia was comparable between Pegfilgrastim subjects and Filgrastim subjects. Administration of Pegfilgrastim resulted in CD34+ cell mobilization to the peripheral blood in both the pre- and postchemotherapy cycles.

In parts C and D, single injections of Pegfilgrastim at a dose of 60 µg/kg or 100 µg/kg yielded mean durations of severe neutropenia and mean times to neutrophil recovery similar to those obtained with daily Filgrastim. However, there was a trend toward increased PD effect with higher doses.

The incidence and severity of adverse events were similar between Pegfilgrastim-treated and Filgrastim-treated subjects. Most adverse events appeared to be due to concomitant chemotherapy or underlying disease. The incidence of immune responses to Filgrastim and Pegfilgrastim, as detected in RIA and EIA screening assays, was similar between treatment groups, and no neutralizing antibodies were detected at baseline or during the treatment period.

The pharmacokinetics of Pegfilgrastim in subjects with NSCLC or thoracic tumors was dose- and ANC-dependent, consistent with a self-regulating, receptor-mediated clearance mechanism.

On the basis of these findings, the Pegfilgrastim dose of 100 µg/kg was chosen as the dose to be taken forward in clinical studies.

Study 980147

Title: A Randomized Study of Single Administration Pegfilgrastim or Daily Filgrastim as an Adjunct to Chemotherapy in Patients with High-risk Stage II or Stage III/IV Breast Cancer

Study Period: July 1998 to October 1999

Centers: Multi-center,
8 centers (including 30 subcenters) in the U.S.

This randomized, double-blind and open label dose ranging trial was the phase 2 pilot for the two phase 3 pivotal trials and was conducted to identify the Pegfilgrastim dosing regimens to be taken into pivotal trials. It utilized the same patient population and chemotherapy as the pivotal trials and compared the DSN, ANC profile, and PK in subjects receiving a single SC dose of Pegfilgrastim at 30, 60, or 100 µg/kg (followed by daily placebo injections) per cycle of chemotherapy with those of subjects receiving daily SC doses of Filgrastim 5 µg/kg /day.

Objectives:
The primary objective was to assess the DSN in cycle 1 of chemotherapy after treatment with a single injection of Pegfilgrastim or multiple daily injections of Filgrastim. The secondary objectives included assessing the DSN in cycles 2 through 4, the ANC profile in cycles 1 through 4, the time to ANC recovery in cycles 1 through 4, PK in cycle 1, the safety profile in cycles 1 through 4, and rates of febrile neutropenia (FN).

Design:
This was a phase 2, multi-center, randomized, two phase (the first double-blind and the second open-label) dose ranging clinical activity, safety and PK study in subjects with high-risk stage II or stage III/IV breast cancer designed to compare a single administration of various doses of SC Pegfilgrastim to daily SC Filgrastim (NEUPOGEN®) 5 µg/kg/day. Both the originally planned double-blind cohorts (3 treatment groups) and the two subsequently added (Amendment, December 1998) open-label cohorts (4 treatment groups) were evaluated in this study.

Subjects in the originally planned double-blind cohorts were randomized 1:1:1 to receive either Filgrastim 5 µg/kg/day or Pegfilgrastim 60 or 100 µg/kg. Based on the results of this phase, subjects in the first open-label cohort were randomized 1:1 to receive either Pegfilgrastim 30 µg/kg or Pegfilgrastim 60 µg/kg. Subjects in the second open-label cohort were randomized 1:1 to receive either Pegfilgrastim 60 µg/kg or Pegfilgrastim 100 µg/kg. After March 1999, the study was amended to allow subjects randomized to the 30 µg/kg Pegfilgrastim dose to receive 60 µg/kg in all subsequent cycles based on interim data from this study that suggested that subjects receiving 30 µg/kg had longer DSN than subjects receiving higher doses.

All subjects were administered chemotherapy (intravenous bolus infusion of doxorubicin 60 mg/m^2 followed 1 hour later by a 1-hour intravenous infusion of docetaxel 75 mg/m^2) on day 1 of each cycle for a maximum of 4 cycles.

In the double-blind phase, subjects were randomized to receive either

- Pegfilgrastim (60 or 100 µg/kg) administered as a single SC injection on day 2 of each chemotherapy cycle followed by daily SC injections of placebo beginning on day 3 of each cycle or
- Filgrastim 5 µg/kg/day administered as multiple daily SC injections beginning on day 2.

Dosing was continued until either the ANC reached 10 x 10^9/L or for up to 14 days after the expected nadir, whichever came first.

In the open-label phase, Pegfilgrastim (30, 60, or 100 µg/kg) was administered as a single SC injection on day 2 of each chemotherapy cycle.

Complete blood counts (CBCs) with 5-part differential were performed daily until ANC reached 10 x 10^9/L after the expected nadir, then 3 times weekly thereafter. Pharmacokinetic samples were collected concurrently with CBC samples. A blood chemistry panel was performed weekly in cycle 1 and before each chemotherapy dose in cycles 2 through 4. Samples were obtained on day 2 of cycle 1 before study drug administration and on day 1 of cycles 2, 3, and 4 for determination of antibodies directed against Filgrastim.

Results:
A total of 154 subjects were enrolled: 127 subjects received Pegfilgrastim, 25 received Filgrastim, and 2 never received study drug. Ninety-one percent (91%) of all subjects who enrolled completed the study; including 90% of all Pegfilgrastim subjects and 96% of Filgrastim subjects. Intolerable adverse events, none of which were related to study drug, were the primary reason for subject withdrawal.

A single dose of either Pegfilgrastim 60 or 100 µg/kg administered once per cycle was well tolerated and provided a reduction in DSN similar to that provided by daily Filgrastim. The mean DSN in cycle 1 for the Pegfilgrastim 30-, 60-, and 100 µg/kg dose groups was 3.2, 2.2, and 1.5 days, respectively, compared with 2.2 days in the Filgrastim group. The one-sided upper 90% confidence limits for the difference in the mean DSN between the Pegfilgrastim dose groups and the Filgrastim group indicated that the mean DSN was unlikely to exceed that of Filgrastim by > 1.56 days with Pegfilgrastim 30 µg/kg, by > 0.43 days with Pegfilgrastim 60 µg/kg, or by any margin with a single injection of Pegfilgrastim 100 µg/kg. Pegfilgrastim 100 µg/kg was selected as the optimal dose for either weight-adjusted dosing or as the basis for determining a fixed dose for phase 3 study development.

The incidence and severity of adverse events were similar between Pegfilgrastim-treated and Filgrastim-treated subjects. Most adverse events appeared to be due to concomitant chemotherapy or underlying disease. Anti-Filgrastim antibodies were detected by RIA in 3 subjects (2 Pegfilgrastim and 1 Filgrastim), and no neutralizing antibodies were detected at baseline or during the treatment period.

The PK of Pegfilgrastim in subjects with high-risk stage II or stage III/IV breast cancer was dose and ANC dependent, consistent with a self-regulating, receptor-mediated clearance mechanism.

Study 990117

Title: An Open-label, Randomized, Dose-ranging Study of a Single Administration Pegfilgrastim versus Daily Filgrastim as an Adjunct to Chemotherapy in Subjects with Non-Hodgkin's Lymphoma

Study Period: September 1999 to November 2000

Centers: Multi-center,
17 centers in the U.S. and Canada

This study was a phase 2, open-label, randomized trial designed to compare the effects of a single SC injection of Pegfilgrastim 100 µg/kg per chemotherapy cycle to daily SC injections of Filgrastim 5 µg/kg /day in subjects with Hodgkin's or non-Hodgkin's lymphoma (NHL)receiving ESHAP chemotherapy.

Objectives:
The primary objective was to assess the duration of severe neutropenia (DSN) in cycle 1 of ESHAP chemotherapy after treatment with a single injection of Pegfilgrastim or multiple daily injections of Filgrastim. The secondary objectives included assessing the DSN in cycles 2 through 4, the ANC profile in cycles 1 through 4, the time to ANC recovery in cycles 1 through 4, PK in cycle 1, and the safety profile in cycles 1 through 4, and rates of febrile neutropenia (FN).

Design:
This was a phase 2, multi-center, randomized, open-label activity and PK study in subjects with Hodgkin's or Non-Hodgkin's Lymphoma designed to compare a single administration of SC Pegfilgrastim to daily SC Filgrastim (NEUPOGEN®). Originally designed to enroll only patients with Non-Hodgkin's Lymphoma, the trial was amended to include Hodgkin's Lymphoma patients in March 2000.

Subjects were randomized 1:1 to receive either Pegfilgrastim 100 µg/kg or Filgrastim 5 µg/kg/day. ESHAP chemotherapy was administered during days 1 through 5, and study drug (Pegfilgrastim or Filgrastim) was initiated on day 6, 1 day after completion of chemotherapy. Pegfilgrastim subjects received a single SC injection per chemotherapy cycle; Filgrastim subjects received daily SC injections for 12 days or until ANC $\geq 10 \times 10^9$/L after chemotherapy-induced ANC nadir, whichever occurred first. Chemotherapy cycles were repeated every 3 weeks. Subjects were expected to complete a minimum of 2 and a maximum of 4 chemotherapy cycles and were to return for follow-up 1 month and 3 months after the end of treatment.

Blood samples for CBC were collected on day 1 before chemotherapy administration, daily during the treatment period beginning on day 6, and at follow-up visits. Serum samples were collected for PK analyses daily beginning on day 6 during cycle 1 only. Blood samples were collected for serum chemistry and antibody analyses at the beginning of each chemotherapy cycle; blood samples for antibody analysis also were collected during the 3-month follow-up visit. End of study was defined as the last follow-up visit at 3 months.

Results:

A total of 66 subjects were enrolled: 29 subjects received Pegfilgrastim, 31 received Filgrastim, and 6 never received study drug. Since, as intended, most subjects continued on to other therapies or to BMT or PBPC transplant after completing cycle 2, the number of subjects remaining in cycles 3 and 4 was too small to draw any clinically meaningful conclusions.

A single dose of Pegfilgrastim 100 µg/kg administered once per cycle was well tolerated and provided a reduction in DSN similar to that provided by daily Filgrastim. The mean DSN in cycle 1 was 2.8 days for the Pegfilgrastim subjects compared with 2.4 days in the Filgrastim group. The observed difference in the means was 0.43 days and the 90% confidence interval for the difference between means was (-0.72, 1.58). The incidence of severe neutropenia (SN) in cycle 1 in the Pegfilgrastim and Filgrastim groups was 69% and 68%, respectively. Results of the secondary endpoint analyses (DSN in cycles 2 through 4, incidence of FN, ANC profile, and mean time to ANC recovery in cycles 1 through 4) were similar between treatment groups in cycles 1 and 2. The cumulative incidence of FN in cycles 1 and 2 was 21% and 19% for Pegfilgrastim and Filgrastim subjects, respectively. The mean time to ANC recovery to 2 $x10^9$/L in cycle 1 was 15.5 days and 14.4 days for Pegfilgrastim and Filgrastim subjects, respectively. The geometric mean ANC nadir in cycle 1 was 0.161 $x10^9$/L and 0.208 $x10^9$/L for Pegfilgrastim and Filgrastim subjects, respectively.

The incidence and severity of adverse events were similar between Pegfilgrastim subjects and Filgrastim subjects. Most adverse events appeared to be due to concomitant chemotherapy or underlying disease. Antibodies to Pegfilgrastim and Filgrastim were detected by the [redacted] screening assay at baseline in 7 subjects (4 Pegfilgrastim and 3 Filgrastim) and in 1 Filgrastim subject on day 1 of cycle 2. No neutralizing antibodies were detected in any subject at baseline, during the treatment period or at follow-up.

The PK of Pegfilgrastim in subjects with Hodgkin's or non-Hodgkin's Lymphoma was dose and ANC dependent, consistent with a self-regulating, receptor-mediated clearance mechanism.

Study 990118

Title: An Open-label, Randomized, Dose-ranging Study of a Single Administration Pegfilgrastim versus Daily Filgrastim as an Adjunct to Chemotherapy in Elderly Subjects with Non-Hodgkin's Lymphoma

Study Period: June 1999 to November 2000

Centers: Multi-center,
13 centers in Europe and Australia

This study was a phase 2, open-label, randomized dose ranging trial designed to compare the clinical activity and safety of a single SC injection of Pegfilgrastim 60 or 100 µg/kg per chemotherapy cycle to daily SC injections of Filgrastim 5 µg/kg /day in elderly subjects (> 60 years old) with non-Hodgkin's lymphoma (NHL) receiving multiple cycles of CHOP chemotherapy. One group received no cytokine support in cycle 1 followed, in cycles 2 through 6, by Filgrastim 5 µg/kg/day.

Objectives:
The primary objective was to assess the DSN in cycle 1 of CHOP chemotherapy after treatment with a single injection of Pegfilgrastim or multiple daily injections of Filgrastim. The secondary objectives included assessing the DSN in cycles 3 and 6, the incidence and duration of neutropenia (ANC $<1.0 \times 10^9$/L) in cycles 1, 3 and 6, PK in cycle 1, safety profiles in cycles 1 through 6, times to ANC recovery, and rates of febrile neutropenia (FN). One treatment group, who received no-cytokine support in cycle 1 but who received Filgrastim 5 µg/kg /day in the remaining cycles, was evaluated to provide information regarding DSN and ANC profile of the chemotherapy regimen in the absence of cytokine support.

Design:
This was a phase 2, multi-center, randomized, open-label dose ranging activity and PK study in elderly subjects (> 60 years old) with Non-Hodgkin's Lymphoma designed to compare a single administration of SC Pegfilgrastim to daily SC Filgrastim (NEUPOGEN®).

Subjects were randomized 1:1:1:1 to receive either Pegfilgrastim 60 µg/kg, Pegfilgrastim 100 µg/kg , Filgrastim 5 µg/kg/day, or no cytokine support in cycle 1 followed by Filgrastim 5 µg/kg/day in cycles 2 through 6. CHOP chemotherapy was administered during day 1, and study drug (Pegfilgrastim or Filgrastim) was administered on day 2, 1 day after completion of chemotherapy. Pegfilgrastim

subjects received a single SC injection per chemotherapy cycle; Filgrastim subjects received daily SC injections for 14 days or until ANC ≥ 10×10^9/L after chemotherapy-induced ANC nadir, whichever occurred first. Chemotherapy cycles were repeated every 3 weeks. Subjects were expected to complete 6 chemotherapy cycles and were to return for follow-up 1 month and 3 months after the end of treatment.

In cycle 1, blood samples were collected for a CBC with full differential on cycle day 1, 3, and daily from days 7 to 14 or until ANC ≥ 10×10^9/L after the expected nadir, whichever came first. In cycles 2 through 6, blood samples were collected for CBC with full differential on cycle day 1, 3, and then 3-times-a-week in cycles 3 and 6, and twice-a-week in cycles 2, 4, and 5 until the end of the cycle. Serum samples for PK analyses were collected concurrently with the CBCs during cycle 1 only. Blood samples were collected for serum chemistry and antibody analyses at the beginning of each chemotherapy cycle; blood samples for CBC and antibody analysis also were collected at the 1 month and 3 month follow-up visits. End of study was defined as the last follow-up visit at 3 months.

Results:
A total of 50 subjects aged 60 to 82 years were enrolled: 13 subjects received Pegfilgrastim 60 µg/kg, 14 received Pegfilgrastim 100 µg/kg, 14 received Filgrastim, and 9 had a "no cytokine" cycle followed by Filgrastim.

Single doses of Pegfilgrastim of 60 or 100 µg/kg administered once per cycle were well tolerated and the higher dose provided a reduction in DSN similar to that provided by daily Filgrastim. The mean DSN in cycle 1 was 1.5 days for the Pegfilgrastim 100 µg/kg subjects, 2.2 days for the Pegfilgrastim 60 µg/kg subjects, and 0.8 days for the Filgrastim subjects compared with 5.0 days in the no cytokine group. The incidence of FN across all-cycles was low, occurring in 5 subjects (1 Filgrastim, 4 Pegfilgrastim 60 µg/kg) during the study. For other endpoints, treatment group differences varied between cycles and no overall trends were observed.

The incidence and severity of adverse events in this elderly subject population were similar between Pegfilgrastim subjects and Filgrastim subjects. Most adverse events appeared to be due to concomitant chemotherapy or underlying disease. Antibodies to Pegfilgrastim and Filgrastim were detected by the [redacted] screening assay in 1 Filgrastim subject on day 1 of cycle 1. No neutralizing antibodies were detected at baseline, during the treatment period or at follow-up.

The PK of Pegfilgrastim in elderly subjects with non-Hodgkin's Lymphoma was dose and ANC dependent, consistent with a self-regulating, receptor-mediated clearance mechanism.

Phase 3 (Pivotal) Trials

Introduction and Summary

Pegfilgrastim was evaluated in two randomized, double-blind, active control phase 3 studies employing doxorubicin 60 mg/m^2 and docetaxel 75 mg/m^2 administered every 21 days for up to 4 cycles for the treatment of metastatic breast cancer:

- Study 980226
  "A Blinded, Randomized, Multicenter Study to Evaluate Single Administration Pegfilgrastim per Cycle Versus Daily Filgrastim as an Adjunct to Chemotherapy in Subjects With High-risk Stage II or Stage III/IV Breast Cancer."
  (conducted in the United States and Canada)

  and

- Study 990749
  "A Blinded, Randomized, Multicenter Study to Evaluate Fixed Dose Single Administration Pegfilgrastim per Cycle Versus Daily Filgrastim as an Adjunct to Chemotherapy in Subjects With High-risk Stage II or Stage III/IV Breast Cancer."
  (conducted in Europe, Australia, and the United States)

The design of the two studies was essentially identical except that Study 980226 investigated the utility of a 100 µg/kg weight-adjusted dose of Pegfilgrastim and Study 990147 employed a 6 mg fixed dose. In the absence of growth factor support, chemotherapy regimens similar to the doxorubicin 60 mg/m^2 and docetaxel 75 mg/m^2 utilized in both studies have been reported to result in a 100% incidence of severe neutropenia with a mean duration of 5-7 days, and a 30-40% incidence of febrile neutropenia. Studies with Filgrastim, the parent compound of Pegfilgrastim, have demonstrated the correlation between the duration of severe neutropenia and the incidence of febrile neutropenia. Based on these findings, a decrease in duration of severe neutropenia was considered a valid surrogate for the clinical benefit of reduction in the incidence of febrile neutropenia and was chosen as the primary endpoint in both studies. Both studies were designed as non-inferiority trials, and the efficacy of Pegfilgrastim was to be demonstrated by establishing comparability to Filgrastim-treated subjects in the mean days of severe neutropenia.

In Study 980226, 310 subjects were randomized to receive a single SC injection of Pegfilgrastim at 100 µg/kg on day 2 or Filgrastim at 5 µg/kg /day beginning on day 2 of each cycle of chemotherapy. In Study 990749, 157 subjects were

randomized to receive a single subcutaneous (SC) dose of 6 mg of Pegfilgrastim on day 2 of each chemotherapy cycle or Filgrastim at 5 µg/kg /day SC beginning on day 2 of each cycle.

Both studies met their primary goals of demonstrating that the mean days of severe neutropenia of Pegfilgrastim-treated patients could not have exceeded that of Filgrastim-treated patients by more than one day in cycle 1 of chemotherapy based on a 95% Confidence Interval (CI) (see Tables 8 and 13). The rates of febrile neutropenia in the two studies were comparable for Pegfilgrastim and Filgrastim (in the range of 10 to 20%). No differences were observed in other secondary endpoints, such as the days of severe neutropenia in cycles 2-4, the depth of ANC nadir in cycles 1-4, or the time to ANC recovery after nadir; however, the studies were not adequately powered to exclude that such differences might exist.

Pivotal Trial Design

Since the design of the two phase 3 trials was essentially identical except for the doses of Pegfilgrastim utilized, the common elements of their design will be discussed together. Any significant trial design differences will be pointed out. The conduct and results of each trial will then be discussed separately.

Overview of the 2 Pivotal Trials

These phase 3, multicenter, randomized, double-blind, active-control studies were designed to determine whether a single subcutaneous (SC) injection of Pegfilgrastim per chemotherapy cycle is as safe and effective as daily SC injections of Filgrastim 5 µg/kg/day in providing neutrophil support of chemotherapy-induced neutropenia in subjects with high-risk stage II or stage III/IV breast cancer treated with up to 4 cycles of myelosuppressive doxorubicin/docetaxel chemotherapy. As previously noted, the design of the two trials was essentially identical except that Study 980226 investigated the utility of a 100 µg/kg weight-adjusted dose of Pegfilgrastim and Study 990147 employed a 6 mg fixed dose

Design of the 2 Pivotal Trials

Rationale for Non-inferiority Study Design

An active-control, non-inferiority study design was chosen for both pivotal trials because Filgrastim (NEUPOGEN®) is licensed as an effective therapy for prophylactic treatment of severe, prolonged neutropenia in patients undergoing myelosuppressive chemotherapy. A double-blind study design was chosen to

minimize investigator and subject bias. A chemotherapy regimen was selected that would produce a significant incidence of febrile neutropenia (approximately 40% as outlined in the ASCO guidelines for use of Hematopoietic Growth Factors). The non-inferiority margin, the amount of the therapeutic effect of Filgrastim which could be lost yet still result in a determination of non-inferiority, was set as one day, which was felt to be clinically insignificant. A more detailed discussion of the basis for the non-inferiority design, including the predicted rate of Febrile Neutropenia, predicted DSN, and justification of the one day non-inferiority margin, is included in this review under "Special Topics: Non-Inferiority Design".

Rationale for Selection of Growth Factor Doses in the Studies

Amgen Study 980147, the phase 2 pilot for these studies, examined the same patient population receiving the same chemotherapy regimen: subjects with high-risk stage II or stage III/IV breast cancer who were slated to receive a chemotherapeutic regimen consisting of doxorubicin and docetaxel for a maximum of 4 cycles. In that study, Pegfilgrastim 100 µg/kg produced a mean and median cycle 1 DSN of 1.5 days and 1.5 days, respectively, similar to those observed with daily Filgrastim (2.2 days and 2.0 days, respectively). Comparable results were observed in Amgen Study 970144 in subjects with non-small cell-lung cancer. In Amgen Study 980147, a single dose of either 60 or 100 µg/kg of Pegfilgrastim administered once per chemotherapy cycle was safe and well tolerated, and provided a duration of severe neutropenia similar to that provided by daily Filgrastim. However, analysis of the primary efficacy endpoint (cycle 1 DSN) indicated that the 100 µg/kg dose of Pegfilgrastim provided the greatest assurance of comparability to Filgrastim. Therefore, a by-weight dose of Pegfilgrastim of 100 µg/kg administered once per chemotherapy cycle was selected as the dose for investigation in Study 980226.

For Study 990749, conducted in the same tumor setting and with the same chemotherapy regimen as Study 980147, a fixed dose of Pegfilgrastim of 6 mg administered once per chemotherapy cycle was studied. This fixed dose level was calculated based on the average dose received in the 60- and 100-µg/kg cohorts in Amgen Study 980147, which were approximately 4 mg and 8 mg, respectively. The profile of severe neutropenia, including mean duration, was compared in subjects who received approximately 4, 6, and 8 mg of Pegfilgrastim (regardless of the per-weight dose group to which they were actually assigned). Subjects who received approximately 4 mg (i.e., 3 to 4.99 mg) had a longer DSN compared with subjects who received approximately 6 mg (i.e., 5 to 6.99 mg) or Filgrastim, with a mean DSN of 2.3 days, 1.7 days, and 2.2 days respectively. Therefore, it was felt that a 4 mg dose would not have provided a high level of assurance of non-inferiority to Filgrastim. There was little added benefit at 8 mg (i.e., 7 to 8.99 mg) with a mean DSN of 1.6 days, and the profile of DSN in subjects who received total doses of Pegfilgrastim between 5 and 7 mg was most comparable in profile to the 100-µg/kg cohort. Thus, a fixed Pegfilgrastim dose

of 6 mg was chosen. With a 6-mg fixed dose, subjects between 60 kg and 100 kg (most patients with cancer) would receive an equivalent per body weight dose of between 100 µg/kg and 60 µg/kg respectively.

Both studies compared Pegfilgrastim to multiple daily SC injections of Filgrastim 5 µg/kg/day

Rationale for Selection of Chemotherapy regimen
The dose-intensive combination chemotherapy regimen of docetaxel 75 mg/m^2 and doxorubicin 60 mg/m^2 every 21 days was chosen for both studies because this regimen has yielded a high disease-response rate in patients with metastatic breast cancer, and because it is associated with significant myelosuppression in the absence of myeloid growth factor support. In a number of studies utilizing the same chemotherapeutic agents at somewhat lower doses, a median DSN of 5 to 7 days and a febrile neutropenia (FN) incidence rate of 33% to 80% have been observed in the absence of myeloid growth factor support. (Dieras et al, 1998; Dieras, 1997; Kennedy et al, 1997). (For more detailed discussion, see "Basis for Non-Inferiority Trial Design" under Special Topics)

Primary objective and secondary objectives
The primary objective of both studies was to assess the efficacy of Pegfilgrastim compared to Filgrastim with respect to the mean duration of severe neutropenia (DSN; the number of days of observed or imputed ANC less than 0.5 x 10^9/L) in cycle 1 of myelosuppressive chemotherapy.

The secondary objectives were to further assess the relative efficacy of Pegfilgrastim with respect to the following: the mean DSN in each of cycles 2 through 4, the depth of ANC nadir in each of cycles 1 through 4, and the safety profile in each of cycles 1 through 4. Rates of FN and the time to ANC recovery to 2 x 10^9/L after the expected nadir in each cycle also were to be analyzed. In Study 980226, the additional secondary objective of the ANC-time profile by cycle was to be assessed.

Primary Criteria for Eligibility
In both studies
Eligible subjects were to be

- males or females
- greater than or equal to 18 years old
- diagnosed with high-risk stage II or stage III/IV breast cancer
- either chemotherapy naive or could have received adjuvant therapy and/or completed no more than 1 regimen of chemotherapy for metastatic disease.

Subjects were to have

- an Eastern Cooperative Oncology Group performance status less than or equal to 2
- an ANC greater than or equal to 1.5 x 109/L
- platelets greater than or equal to 100 x 109/L
- adequate renal function (creatinine less than 1.5 x upper limit of normal)

Females of childbearing potential were to use adequate birth control to prevent pregnancy.

Subjects were to give written informed consent before any study specific procedure.

Any previous chemotherapy exposure was to have been completed less than 4 weeks before randomization into the study.

Subjects were to be excluded if they had

- abnormal liver function
- clinically significant cardiac disease
- prior cumulative lifetime exposure to doxorubicin greater than 240 mg/m^2 or epirubicin greater than 600 mg/m^2
- prior bone marrow or stem cell transplant
- active infection
- prior radiation therapy within 4 weeks of enrollment
- any premalignant myeloid condition
- a history of prior malignancy other than breast cancer or surgically cured malignancies
- clinically symptomatic brain metastases
- known hypersensitivity to *E coli*-derived products
- previously received Pegfilgrastim
- any evidence of pregnancy
- received systemic anti-infectives within 72 hours of chemotherapy

In addition, subjects were to be excluded if they gave concerns for compliance with the protocol, or were enrolled in another investigational trial,

Randomization

Randomization of subjects was to occur after calling a central interactive voice response system (IVRS) randomization center Subjects at each center were to be randomized separately and assigned a unique 7-digit number. Each subject was to be assigned specific blinded study drug pack numbers through the IVRS that were to be subsequently confirmed by facsimile. Subjects were to be assigned in a 1:1 ratio to the study-appropriate dose Pegfilgrastim treatment group or to the Filgrastim 5 µg/kg/day treatment groups using a stratified permuted-block randomization schedule with center and previous chemotherapy as the stratification variables. In Study 990479, the 6 mg fixed dose study,

subjects were to be assigned to a treatment group using a stratified randomization with weight (less than 50 kg, greater than or equal to 50 kg and less than 80 kg, or greater than or equal to 80 kg), prior chemotherapy exposure, and location (US or non-US) as the stratification variables. In both studies, the randomization list was to be generated by a CRO.

The investigator, pharmacist, or designee in both studies was to complete the subject randomization log included with the individual subject dispensing records. The randomized treatment assignment was to remain blinded to subjects, center personnel, and all Amgen personnel, with the exception of Amgen personnel involved in study drug packaging, sample management, and pharmacokinetic analyses.

Treatments

Chemotherapy (doxorubicin 60 mg/m^2 and docetaxel 75 mg/m^2) was to be administered on day 1 of each cycle for a maximum of 4 cycles. To begin full-dose chemotherapy on day 1 of the next cycle (day 22 of the previous cycle), a subject would have to have ANC > 1 x 10^9/L and platelet count > 100 x 10^9/L. Once recovery to these levels had occurred, treatment could be resumed at the full dose given in the previous cycle. Chemotherapy dose reductions of 25% (resultant doses: doxorubicin 50 mg/m^2 and docetaxel 60 mg/m^2) could be made if subjects experienced specific chemotherapy related adverse events.

In both studies, subjects were to receive study drug as follows:

Pegfilgrastim: On day 2 of each cycle, subjects were to receive 2 SC injections, one injection of placebo and one of the study-appropriate dose of Pegfilgrastim. On subsequent days, subjects were to receive a SC injection of placebo, continuing until either they achieved an ANC greater than or equal to 10 x 10^9/L after the expected nadir or for up to 14 days, whichever came first.

Filgrastim: On day 2 of each cycle, subjects would receive 2 SC injections, one injection of placebo and one of Filgrastim 5 µg/kg. On subsequent days, subjects would receive a SC injection of Filgrastim, 5 µg/kg/day continuing until either they achieved an ANC greater than or equal to 10 x 10^9/L after the expected nadir or for up to 14 days, whichever came first.

Because rapidly dividing myeloid cells are sensitive to myelosuppressive chemotherapy, the first dose of study drug was to be administered approximately 24 hours after completion of chemotherapy as per the licensed prescribing information for Filgrastim. Subsequent study drug and placebo injections were also to be administered according to the licensed prescribing information for Filgrastim, specifically with regard to frequency and threshold for cessation of dosing. Timing of injections with respect to time of day was not specified.

The subjects were required to return used and unused study drug and were to be interviewed by the investigator or designee to assess compliance.

In both studies, blood samples were to be collected in each cycle for complete blood counts (CBCs) with differential on cycle day 1, day 3, and days 5 through 9 continuing daily until ANC greater than or equal to 2 x 10^9/L after the expected nadir, then twice weekly, and at the end of treatment.

In Study 980226, serum samples for pharmacokinetic analyses were to be collected predose and then concurrently with CBC samples on day 7 of each cycle. In Study 990749, serum samples for pharmacokinetic analyses were to be collected concurrently with the CBC samples in cycle 1 only. In both trials, serum samples for a clinical chemistry panel and antibody analysis were to be collected before premedication in each chemotherapy cycle and at the end of cycle 4. Daily temperature logs were to be maintained throughout each study.

In Study 980226, short-term follow-up visits were to be scheduled at 1 month and at 3 months after the end of treatment period for collection of blood for CBC and of serum for antibody analyses. Long-term follow-up visits were to be scheduled quarterly during the first year and then twice yearly for the second year for collection of data on disease progression and survival. In Study 990749, follow-up visits were to be scheduled at 1 month and at 3 months after the end of treatment for collection of blood samples for CBCs and serum antibody analysis. In both studies, the end of study for a subject was to be considered the final study evaluation visit for that subject. An external Safety Data Monitoring Committee (SDMC) was to periodically review the safety data and recommended whether to continue the study at periodic meetings.

In accordance with the current revision of the Declaration of Helsinki and other applicable regulations, subjects had the right to withdraw from the study at any time and for any reason without prejudice to his or her future medical care by the physician or at the institution.

In both studies, subjects were to be removed from study if any one or more of the following events occurred:

- Significant protocol deviation or noncompliance, on the part of either the subject or investigator
- Refusal of the subject to continue treatment and/or observations
- Unacceptable toxicity to the chemotherapy regimen, Filgrastim, or Pegfilgrastim
- Decision by the investigator or Amgen that termination was in the subject's best medical interest
- Unrelated medical illness or complication
- Lost to follow-up
- Disease progression

- Death of subject
- Withdrawal of consent
- Pregnancy

End-of-treatment tests and evaluation were to be completed at the time of the subject's withdrawal, and if applicable, all subsequent protocol follow-up evaluations were to be performed as specified in the protocol. Subjects who were withdrawn were not to be replaced.

Efficacy Endpoints

In both studies, DSN was used as the primary endpoint because of the documented relationship between DSN and incidence of FN. The primary efficacy endpoint in both studies was the DSN in cycle 1. The common secondary efficacy endpoints were the DSN in each of cycles 2 through 4 , the depth of ANC nadir in each of cycles 1 through 4, rates of FN by cycle and across all cycles, and time to ANC recovery by cycle and across all cycles. In Study 980226, an additional secondary endpoint was the ANC-time profile in each cycle.

Pharmacokinetic Endpoints

The pharmacokinetic endpoint in Study 980226 was characterization of the serum cytokine concentrations on cycle day 7.

The pharmacokinetic endpoints for Study 990749 were the maximum observed serum concentration, the time to maximum observed serum concentration, the area under the serum concentration-time curve (AUC) from time zero to the time of the last measurable concentration, the AUC from time zero to infinity, the terminal half-life, and the relative clearance after SC administration.

Safety Endpoints

The primary safety endpoint in both studies was the subject incidence of adverse events (all, severe, and serious) classified by treatment group, body system, preferred term, frequency, and relationship to study drug. Vital signs, the presence of antibodies, and clinical laboratory results also were monitored.

Statistical Methods

Sample Size Considerations

Sample size estimates were calculated using a non-inferiority design and the normal approximation for 2 independent groups as implemented in the computer software package, nQuery 1.0.

For Study 980226, assumptions regarding the mean DSN in the absence of hematopoietic growth factor support were based on literature reports on dose-intensive doxorubicin and docetaxel chemotherapy regimens (Dieras et al, 1998; Dieras, 1997; Kennedy et al, 1997). The predicted mean DSN of 6 to 7 days with the proposed dose-intensive regimen in the absence of growth factor support was similar to that observed in the control arm of Amgen Filgrastim Study 8801, the pivotal study for the approval of NEUPOGEN® (Filgrastim) in the setting of chemotherapy-induced neutropenia. At the time of the design of this study, limited data were available for patients with breast cancer treated with dose-intensive doxorubicin and docetaxel regimens with concurrent growth factor support. The predicted the mean duration and standard deviation of SN for the use of Filgrastim with the proposed dose-intensive regimen, therefore, were based on the treatment arm of Amgen Filgrastim Study 8801. Using these data, the predicted treatment effect of Filgrastim was estimated to be a reduction in mean DSN of 4 to 5 days (from mean DSN of 6 to 7 days without growth factor to mean DSN of 2 days with Filgrastim. The planned sample size of 150 subjects in each treatment arm would, under the assumption of no difference between the treatment groups, yield a 95% probability that a one-sided upper 97.5% confidence limit would exclude the possibility that the mean DSN observed with Pegfilgrastim would exceed the mean DSN observed with Filgrastim by greater than 1 day. The 1-day non-inferiority margin was chosen to ensure that 75% to 80% of the Filgrastim reduction in DSN was retained. This sample size calculation assumed a 10 to 20% dropout rate.

For Study 990749, the mean DSN in the absence of growth factor support was assumed to be 6 to 7 days based on the literature references cited above. The mean DSN predicted for the proposed dose-intensive doxorubicin and docetaxel chemotherapy regimen with concurrent Filgrastim support was based on the mean DSN of 2.2 days observed in Amgen Study 980147. Using this estimate and a conservative estimate for the standard deviation of DSN of 1.5 days, a planned sample size of 75 subjects in each treatment group would, under the assumption of no difference between the treatments, yield a 95% probability that a one-sided upper 97.5% confidence limit would exclude the possibility that the mean DSN observed with Pegfilgrastim would exceed the mean DSN observed with Filgrastim by greater than 1 day. The 1-day non-inferiority margin was chosen to ensure that 75% of the Filgrastim reduction in DSN was retained. This sample size calculation assumed a 20% dropout rate during the first cycle, which was higher than observed in Amgen Study 980147.

Efficacy Analyses

All efficacy analyses were performed on cycle-specific per-protocol (pP) and modified intent to treat (mITT) subsets as defined below. The pP analyses were considered primary because of the non-inferiority approach used to evaluate efficacy.

The following subsets of subjects were defined for analysis:

- Per-protocol (pP) subset (defined for each cycle): subjects who were randomized, who were exposed to assigned study drug within a cycle, who had at least one post-baseline ANC, who signed the informed consent at the time study procedures began, who received the correct chemotherapy medication and study drug, and who had no protocol deviations likely to interfere with the ability of the analysis to detect a difference

- Modified intent-to-treat (mITT) subset (defined for each cycle): subjects who were randomized, who were exposed to the assigned study drug within a given cycle, who had at least one post-baseline ANC, who signed the informed consent at the time study procedures began, and who received the correct chemotherapy medication.

- Safety subset: subjects who received at least one dose of Filgrastim or Pegfilgrastim during this study. These subjects were analyzed according to the treatment received irrespective of the treatment assignment.

- Pharmacokinetics subset: subjects who had pharmacokinetic data for analysis as defined in the pharmacokinetics report.

In both studies, treatment differences in DSN were analyzed for each cycle using confidence intervals calculated based upon 10,000 bootstrap samples. For each sample, the difference between treatment arms was calculated, and the distribution of differences was tabulated. Both an upper 97.5% one-sided confidence interval and a 95% two-sided confidence interval were evaluated with respect to a non-inferiority margin of 1 day. In Study 990749, additional exploratory analyses were performed to examine the relationship between DSN and subject body weight.

Treatment differences in depth of the ANC nadir in each of cycles 1 through 4 was assessed by calculating a 90% two-sided confidence interval to determine the lower 95% one-sided confidence limit for the difference between treatment groups in the means of the log-transformed nadirs. The logarithmic transformation was used in calculating the summary statistics for ANC nadirs because the long right tail of the distribution of values suggested that a logarithmic transformation would be appropriate. Therefore, the summary statistics for ANC nadirs include geometric means, which are the inverse-transformed means of the logarithm values.

For the purposes of all analyses, if one or more ANC values were missing, the values were estimated using linear interpolation. The endpoints were then based on these imputed values. The 2 exceptions to this rule were the following:

- Any missing ANC values within the expected period of severe neutropenia that were part of a consecutive series of missing values were replaced by values indicating severe neutropenia

- Two or more consecutive missing ANC values bounded by an ANC $< 0.5 \times 10^9$/L and another ANC $< 0.5 \times 10^9$/L were replaced by values indicating severe neutropenia.

In both studies, if a subject terminated the study early, every attempt was made to collect study data for the balance of the cycle; however, if a subject was lost to follow-up during a cycle, the following rules were used to estimate the endpoints:

- If a subject was lost to analysis before experiencing any neutropenia for that cycle, the endpoints were considered unknown.

- If a subject was lost to analysis during the period of severe neutropenia, the duration was considered equal to the greatest duration actually observed in that cycle.

- If a subject was lost to analysis after the period of severe neutropenia, the endpoints were coded as they were observed.

The incidence of FN was included as a secondary endpoint. The definition of FN used in the efficacy analyses was an observed or imputed ANC $< 0.5 \times 10^9$/L and concurrent oral-equivalent temperature $\geq$38.2°C. Treatment differences in FN during the study and for each cycle between the Pegfilgrastim and Filgrastim groups were assessed using an upper 97.5% one-sided confidence interval (95% two-sided) using normal-based methods. FN was analyzed both by treatment group alone and by treatment group and cycle. The incidence of FN as defined in this manner for the efficacy analyses was not expected to correspond to that

observed in the safety analyses because the latter relied exclusively on adverse event reports. In both studies, the original statistical analysis plan listed a safety analysis using a definition of FN that also required hospitalization or the use of intravenous antibiotics, the definition commonly used in the literature. This was designed to served in comparing the results of this study with those of previously published studies. This definition is essentially the same as the one defining a serious adverse event, because in both studies, all subjects receiving intravenous antibiotics for FN were hospitalized.

Safety Analyses

Adverse events were tabulated by body system, severity, relationship to study drug, and treatment group. Changes in laboratory variables were depicted using shift tables and through the tabulation of summary statistics for each variable. Serum samples from all subjects who received study drug were assayed for evidence of anti-drug antibodies. Dose reductions and toxicity-related dose delays were tabulated by cycle and by treatment group. No interim efficacy analyses were conducted. An external safety data monitoring committee periodically reviewed aggregate blinded safety data.