CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

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NON-CLINICAL REVIEW(S)

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY BLA REVIEW AND EVALUATION

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Supporting document/s:	1
Applicant's letter date:	September 21, 2017
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Product:	PF-06881893, a proposed biosimilar to filgrastim
Indication:	Decrease the incidence of infection as manifested by febrile neutropenia
Applicant:	Hospira, Inc.
Review Division:	Division of Hematology Oncology Toxicology (DHOT)
Reviewer:	Pedro L. Del Valle, PhD
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1 Executive Summary

1.1 Introduction

Pfizer (Hospira) is requesting marketing approval for PF-06881893, as a proposed biosimilar product to the reference product US-licensed Neupogen. Neupogen (BLA 103353 by Amgen Inc.) was approved in the US in 1991 and the current label includes several indications and usage information related to neutropenia. Pfizer proposes clinical populations and dosing regimens that are consistent with current US-licensed Neupogen labeling except for the indication of *Patients Acutely Exposed to Myelosuppressive Doses of Radiation (Hematopoietic Syndrome of Acute Radiation Syndrome)* which is under orphan drug exclusivity until March 2022.

PF-06881893 (Filgrastim Hospira) is a 175-amino acid, single-chain, non-glycosylated recombinant methionyl human granulocyte colony-stimulating factor (r-metHuG-CSF) produced in *Escherichia coli*. CSF are glycoproteins which act on hematopoietic cells by binding to specific cell surface receptors and stimulating proliferation, differentiation commitment, and some end-cell functional activation. Endogenous G-CSF is a lineage specific colony-stimulating factor which is produced by monocytes, fibroblasts, and endothelial cells. G-CSF regulates the production of neutrophils within the bone marrow and affects neutrophil progenitor proliferation, differentiation, and selected end-cell functional activation (including enhanced phagocytic ability, priming of the cellular metabolism associated with respiratory burst, antibody dependent killing, and the increased expression of some functions associated with cell surface antigens). G-CSF is not species-specific and has been shown to have minimal direct in vivo or in vitro effects on the production of hematopoietic cell types other than the neutrophil lineage.

Pfizer conducted a comparative nonclinical 4-week toxicology study in Sprague-Dawley rats that included a comparison of the potential toxicity, local tolerance, absolute neutrophil count as PD biomarker, PK/TK, and immunogenicity of PF-06881893 to US-licensed Neupogen. The lot used for nonclinical and clinical studies is the same; the batch was manufactured using the same equipment and at the same scale as that proposed for commercial manufacture. In addition, the (b) (4) Similar PK/PD effects and safety data was obtained from the 4-week toxicology study in rats. In addition, three orthogonal functional assays were utilized to assess filgrastim activity as part of the PF-06881893 analytical similarity assessment: an in vitro cell-based proliferation assay, a filgrastim receptor binding assay, and a surface plasmon resonance assay (see CMC review). From the perspective of nonclinical pharmacology and toxicology, there are no residual uncertainties regarding the similarity of PF-06881893 to US-Neupogen.

1.2 Brief Discussion of Nonclinical Findings

The nonclinical data submitted to the BLA provided evidence that PF-06881893 is similar to US-licensed Neupogen.

In the 4-week comparative toxicology study in Sprague-Dawley rats, comparative doses were 20 and 500 µg/kg. Both drugs had similar toxicity profiles. Dose-related increases in total leukocyte counts attributed to increases in neutrophil counts occurred progressively during the dosing phase to reach maximum around Day 22 or Day 29 and then progressively decrease until fully resolved by the end of the recovery phase on Day 43. This pharmacodynamic response corresponded with activation of hematopoiesis/granulocytopoiesis in the bone marrow, spleen, liver and lymph nodes with increased myeloid:erythroid ratios (M:E) on bone marrow. In general, filgrastim-related effects on total leukocyte and subtype counts were of similar magnitudes in rats administered equivalent doses of Filgrastim Hospira and Neupogen. Additionally, dose-related and similar incidence and severity were noted in clinical signs of hind limb swelling and/or impairment that occurred mostly in male rats given 500 µg/kg filgrastim Hospira (6 of 15 males) and Neupogen (4 of 15 males). These clinical signs occurred to a lesser extend in females (2 of 15 females) each given 500 µg/kg filgrastim Hospira or Neupogen. Swelling/thickening/ enlargement of the hind feet in one male each administered 500 µg/kg Filgrastim Hospira or 500 µg/kg Neupogen. Foot lesions corresponded microscopically with osteodystrophy of the bones and fibrosis noted within surrounding soft tissue.

Other noteworthy findings were similar between Filgrastim Hospira and Neupogen included dose-related increases in alkaline phosphatase activity (up to +3.5-fold), as well as enlarged spleen that corresponded with marked increase in spleen relative and absolute organ weight and microscopic findings of extramedullary hematopoiesis. Prolongations of small magnitude in prothrombin time and increases in fibrinogen likely related to an inflammatory response occurred at the end of the dosing phase at 500 µg/kg. All above mentioned treatment-related findings were not observed after the 6-week recovery period.

Systemic exposure to filgrastim was independent of sex with individual serum concentrations, AUC, and C_{max} values similar between males and females (female to male AUC ratios ranged from 0.744 to 1.18). In general, systemic exposure to filgrastim were similar in rats administered filgrastim Hospira and Neupogen with AUC ratios ranging from 0.892 to 1.06. Immunogenicity evaluations showed that nine rats starting on Day 15 tested positive for antibodies against filgrastim and occurred in both males and females given Filgrastim Hospira at either 20 µg/kg or 500 µg/kg and Neupogen at 500 µg/kg. No evidence that positive ADA responses had an impact on filgrastim systemic exposure. Local tolerance was assessed at the injection site following the Draize scale for scoring skin irritation. There were no observations of erythema, eschar, or edema.

1.3 Recommendations

1.3.1 Approvability

Approvable. From the Pharmacology/Toxicology perspective there are no residual uncertainties regarding the similarity of PF-06881893 (Filgrastim Hospira) to US-licensed Neupogen

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

The nonclinical sections of the label will be comparable to the label of the reference product US-licensed Neupogen.

2 Drug Information

2.1 Drug

CAS Registry Number
121181-53-1

Generic Name
Nivestym

Code Name
PF-06881893, Filgrastim, Filgrastim DS, Filgrastim Hospira, Filgrastim Hospira DS

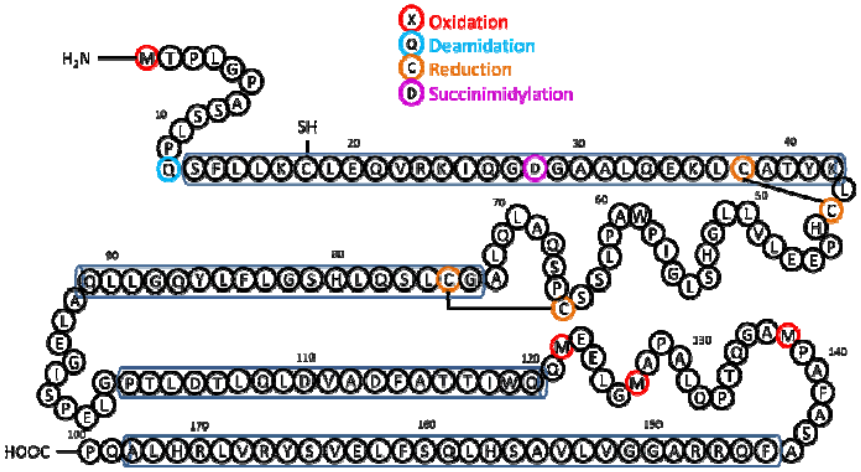
International Non-proprietary Name (INN)
Filgrastim

Chemical Name
Not Applicable

Molecular Formula
$C_{845}H_{1339}N_{223}O_{243}S_9$ (protein backbone)

Molecular Weight
18.8 kDa

Structure or Biochemical Description
PF-06881893 (filgrastim) is a 175-amino acid, single chain, α-helical protein that is produced by recombinant DNA technology in Escherichia coli. The filgrastim protein contains an N-terminal methionine residue and two intramolecular disulfide bonds at amino acid residues Cys 37 − Cys 43 and Cys 65 − Cys 75. Filgrastim also contains a free thiol group at Cys 18.



Pharmacologic Class
Recombinant granulocyte colony stimulating factor (G-CSF)

2.2 Relevant INDs, NDAs, BLAs and DMFs

None

2.3 Drug Formulation

(b) (4)

PF-06881893 DP is a sterile, colorless aqueous solution intended for subcutaneous injection. The DP contains PF-06881893 drug substance (DS) and the excipients sorbitol, (b) (4) polysorbate 80 and water for injection. (b) (4) The DP contains no preservatives and is for single dose only.

Table 1 Quantitative Composition of PF-06881893 DP

(Excerpted from Submission)

Name of the Component	Strength : 300 mcg/1.0 mL mg per 1.0 mL	Strength : 480 mcg/1.6 mL mg per 1.6 mL
PF-06881893 DS[b]	0.3	0.48
Sorbitol	50	80
Sodium[c]	0.035	0.056
Acetate[d]	0.59	0.94
Polysorbate 80	0.04	0.064
(b) (4)		
Water for injection	q.s. to 1 mL	q.s. to 1.6 mL

(b) (4)

b. drug substance

(b) (4)

q.s., quantum sufficit

The PF-06881893 DP prefilled syringe (PFS) presentation dose strengths were developed to match the originator [US-licensed NEUPOGEN (filgrastim)] PFS configurations of 300 mcg/0.5 mL and 480 mcg/0.8 mL, (b) (4) The PF-06881893 DP PFS presentation is supplied in a 1 mL long USP (b) (4) glass syringe with a 27 gauge ½ inch needle, sealed with (b) (4) stopper and rigid needle shield.

Table 2 Quantitative Composition of PF-06881893 DP-PFS

(Excerpted from Submission)

Name of the Component	Strength: 300 mcg/0.5 mL mg per 0.5 mL	Strength: 480 mcg/0.8 mL mg per 0.8 mL
PF-06881893 DS[a]	0.3	0.48
Sorbitol	25	40
Sodium[b]	0.0175	0.028
Acetate[c]	0.295	0.472
Polysorbate 80	0.02	0.032
(b) (4)		
Water for injection	q.s. to 0.5 mL	q.s. to 0.8 mL

a. drug substance

(b) (4)

q.s., quantum sufficit

2.4 Comments on Novel Excipients

None; the excipients used are all compendial.

2.5 Comments on Impurities/Degradants of Concern

None

2.6 Proposed Clinical Population and Dosing Regimen

Pfizer proposes clinical populations and dosing regimens that are consistent with current US-licensed Neupogen labeling except for the indication of *Patients Acutely Exposed to Myelosuppressive Doses of Radiation (Hematopoietic Syndrome of Acute Radiation Syndrome)* which is under orphan drug exclusivity until March 2022. See approved US-licensed Neupogen label for more detailed information.

2.7 Regulatory Background

BLA 761080 was submitted on September 21, 2017 for the biologic product PF-06881893 under Section 351(k) of the Public Health Service Act (42 U.S.C. 262(k)). A pre-IND meeting was held on March 11, 2011 to discuss the U.S. development program for PF-06881893 and IND 109991 was submitted for PF-06881893 ("Filgrastim Hospira") on September 4, 2015. A Biosimilar Product Development (BPD) Type 4 meeting was held on February 8, 2017 where the overall content and format of the planned 351(k) BLA for PF-06881893 was discussed.

Pfizer is seeking approval for PF-06881893 as a proposed biosimilar product to the single reference biologic product US-licensed Neupogen. To fulfill the nonclinical requirements for a biosimilar BLA, Hospira submitted a 4-week repeat-dose toxicity study in Sprague-Dawley rats comparing PF-06881893 to the US-licensed Neupogen. This toxicity study included an assessment of the potential toxicity, pharmacokinetics/ toxicokinetics (PK/TK), pharmacodynamics (PD), local tolerance, and potential associated immunogenicity of PF-06881893 compared to Neupogen.

3 Studies Submitted

3.1 Studies Reviewed

Study#	Title	Module
	General Toxicology	
1550-060	A 4-week Subcutaneous Comparative Repeat-Dose Toxicity Study with Filgrastim Hospira and Neupogen in Rats with a 6-week Recovery Period	4.2.3.2

3.2 Studies Not Reviewed

Study#	Title	Module
	Pharmacokinetics	
072115	Validation of an Immunoassay (ELISA) for the Quantitation of Granulocyte Colony Stimulating Factor (G-CSF) in Sprague Dawley Rat Serum	4.2.2.1
(b) (4)-2015-A-06-02	Partial Validation of a Homogeneous Bridging ECL Assay for the Detection of Antibodies against Filgrastim in Rat Serum	4.2.2.1

3.3 Previous Reviews Referenced

None

4 Pharmacology

4.1 Primary Pharmacology

The pharmacologic activity of PF-06881893 and Neupogen was evaluated by in vitro functional assays. Three orthogonal functional assays were utilized to assess filgrastim activity as part of the PF-06881893 analytical similarity assessment: an in vitro cell-based proliferation assay, a filgrastim receptor binding assay, and a surface plasmon resonance assay. These studies were submitted to Module 3, Section 3.2.R.5.5 Analytical Similarity Results and reviewed by the Quality Product team. The evaluation of a well-established pharmacodynamic (PD) marker (neutrophil count) for filgrastim was incorporated into the comparative 4-week toxicology study. No other studies were submitted for review.

4.2 Secondary Pharmacology

No studies were submitted for review.

4.3 Safety Pharmacology

Comparative safety pharmacology studies with PF-06881893 and Neupogen were not performed.

5 Pharmacokinetics/ADME/Toxicokinetics

PK/TK assessment of PF-06881893 was included in the comparative 4-week repeat-dose toxicity study in rats

6 General Toxicology

6.1 Single-Dose Toxicity

Studies were not conducted

6.2 Repeat-Dose Toxicity

Study title: A 4-week Subcutaneous Comparative Repeat-Dose Toxicity Study with Filgrastim Hospira and Neupogen in Rats with a 6-week Recovery Period

Study no.:	1550-060
Study report location:	eCTD 4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	February 20, 2015
GLP compliance:	Yes, including Report Amendment
QA statement:	Yes
Drug, lot #, and % purity:	Filgrastim Hospira, 2075114, Protein concentration: (b) (4) mg/mL, Potency: (b) (4) IU/mg of protein. US-licensed Neupogen, 1047463.

Key Study Findings

Similar findings occurred in rats given filgrastim Hospira or Neupogen:

- Clinical signs of hind limb swelling and/or impairment and decreases in body weights occurred in rats given filgrastim Hospira or Neupogen
- Dose-related increases in total leukocyte counts attributed to increases in neutrophil counts that corresponded with increased M;E ratios on bone marrow smears, microscopic findings of granulocytic hyperplasia in the bone marrow and increased hematopoiesis in the spleen and other organs.
- Dose-related increases in alkaline phosphatase activity, prolongations of small magnitude in prothrombin time and increases in fibrinogen likely related to an inflammatory response occurred at the end of the dosing phase at 500 µg/kg.

- Spleen enlargement and dose-related increases in mean spleen spleen/body weight ratio that corresponded with microscopic findings of extramedullary hematopoiesis as well as granulocytic hyperplasia in bone marrow and bone osteodystrophy of similar incidence and magnitude.
- Systemic exposure to filgrastim and no evidence that positive ADA responses had an impact on filgrastim systemic exposure.

Methods

Doses:	Control 0 µ/kg Filgrastim Hospira: 20 or 500 µ/kg US-licensed Neupogen: 20 or 500 µ/kg
Frequency of dosing:	Daily x 29 days
Route of administration:	Subcutaneous
Dose volume:	2 mL/kg
Formulation/Vehicle:	(b) (4) diluent for PF-06881893 or Neupogen
Species/Strain:	Rats CD® [Crl:CD®(SD)]
Number/Sex/Group:	10 rats/sex/group + 5 rats/sex/group in control and HD for PF-06881893 or Neupogen
Age:	6 to 6.5 weeks at arrival
Weight:	M: 188 to 207 g; F: 172 to 189 g
Satellite groups:	TK Control 6 rat/sex/group; TK 12 rats/sex/group
Unique study design:	Include PK/TK, PD, local tolerance, and immunogenicity.
Deviation from study protocol:	

Observations

Table 3 Experimental Design of 4-Week Comparative Repeat-Dose Toxicity Study with Filgrastim Hospira and Neupogen in Rats

(Excerpted from Submission)

Group	Treatment	Daily Dose (µg/kg/day)	Number of Animals	
			Male	Female
1	Vehicle	0	10+5[a]	10+5[a]
2	PF-06881893	20	10	10
3	PF-06881893	500	10+5[a]	10+5[a]
4	Neupogen	20	10	10
5	Neupogen	500	10+5[a]	10+5[a]
6	Vehicle	0	6[b]	6[b]
7	PF-06881893	20	12[b]	12[b]
8	PF-06881893	500	12[b]	12[b]
9	Neupogen	20	12[b]	12[b]
10	Neupogen	500	12[b]	12[b]

a. The last surviving 5 rats/sex/ group remained in the study for a 6-week recovery period
b. The last surviving 6 rats/sex/group were assessed for immunogenicity.

Results

Mortality

None

Clinical Signs

Sporadic filgrastim-related clinical signs of hind limb swelling and/or impairment occurred mostly in male rats given 500 µg/kg filgrastim Hospira (6 of 15 males) and Neupogen (4 of 15 males). These clinical signs occurred to a lesser extend in females (2 of 15 females) each given 500 µg/kg filgrastim Hospira or Neupogen. A toxicokinetic male assigned to 20 µg/kg filgrastim Hospira presented clinical signs of hind limb swelling after low-dose groups 2 and 7 were given 500 µg/kg filgrastim Hospira by mistake. These clinical signs resolved spontaneously or after treatment with NSAID meloxicam. No erythema, eschar, or edema occurred in any animals.

Body Weights

Filgrastim-related reduce body weight gain occurred in 500 µg/kg filgrastim Hospira (↓22.7%), 20 µg/kg Neupogen (↓25.0%) and 500 µg/kg Neupogen (↓10.5%) compared to control male rats, see table below. Changes in body weight did not correspond with changes in food consumption. Body weight gain was similar during the recovery period for filgrastim Hospira or Neupogen 500 µg/kg male rats compared to controls. Similar body weight gain occurred in female rats during the dosing and recovery periods.

Table 4 Filgrastim-related Effects on Male Rats Body Weight

(Excerpted from Submission)

Study Interval	0 µg/kg			20 µg/kg (TA1)			500 µg/kg (TA1)			20 µg/kg (TA2)			500 µg/kg (TA2)		
(Day)	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
-1	244.7	6.63	15	246.5	8.97	10	244.6	7.17	15	243.3	9.15	10	245.9	9.58	15
7	293.6	11.85	15	295.2	9.19	10	291.9	9.54	15	288.1	14.44	10	292.5	12.57	15
14	332.3	13.89	15	332.0	16.01	10	324.3	14.22	15	324.0	19.96	10	330.5	18.47	15
21	365.0	15.96	15	367.3	17.62	10	352.4	26.21	15	352.7	23.78	10	361.2	25.36	15
28	387.6	24.56	15	396.9	18.36	10	365.9	26.51	15	363.6	45.40	10	382.1	33.38	15
30	400.8	21.27	10	400.3	18.13	10	365.3[a]	31.10	10	360.4	55.66	10	385.6	37.21	10

Feed Consumption

Similar food consumption occurred for all groups during the dosing and recovery periods.

Ophthalmoscopy

No filgrastim-related findings.

ECG

Not evaluated

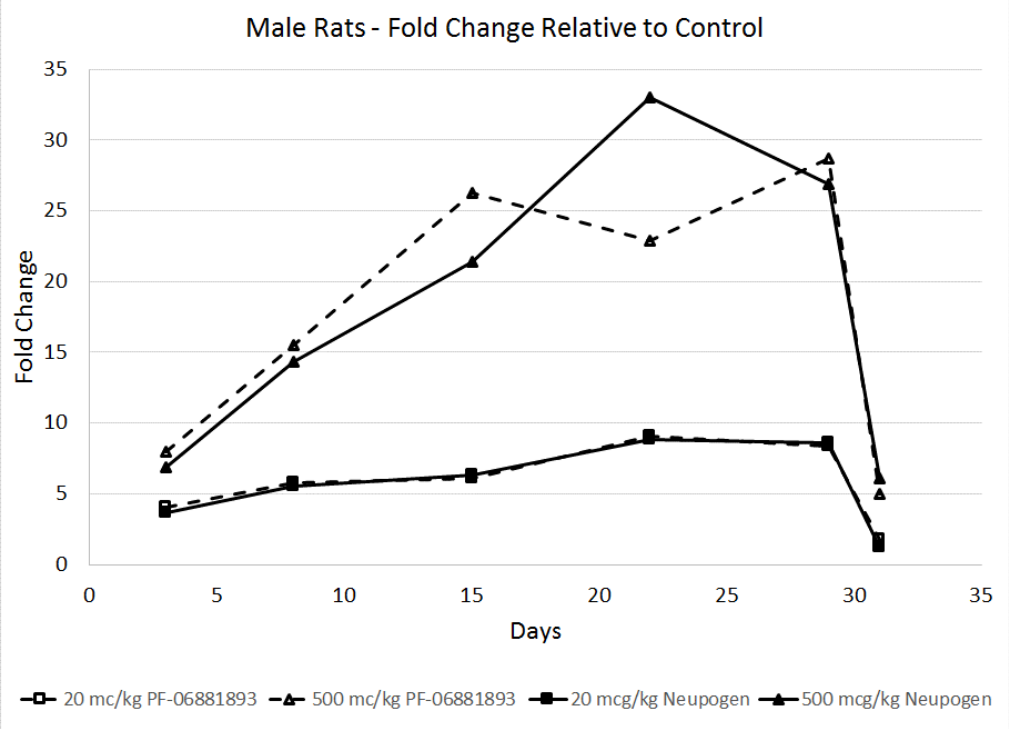
Hematology

Consistent with the expected pharmacological action, filgrastim- and dose-related increases in total leukocyte counts occurred during the dosing phase and were mostly

attributed to increases in neutrophil counts. Increases in other type of leukocyte cells also contributed to increases in total leukocyte counts particularly in rats at 500 µg/kg Filgrastim Hospira and Neupogen, compared to controls; monocyte counts (up to +12.1-fold), lymphocyte (up to +2.3-fold), eosinophil (up to +2.6-fold), basophil (up to +2.5-fold) and "other cell" counts such as large unstained cells and typically reactive lymphocytes (up to +7.4-fold). The increase in neutrophils corresponded with increased M:E ratios on bone marrow smears, microscopic findings of granulocytic hyperplasia in the bone marrow and increased hematopoiesis in the spleen and other organs.

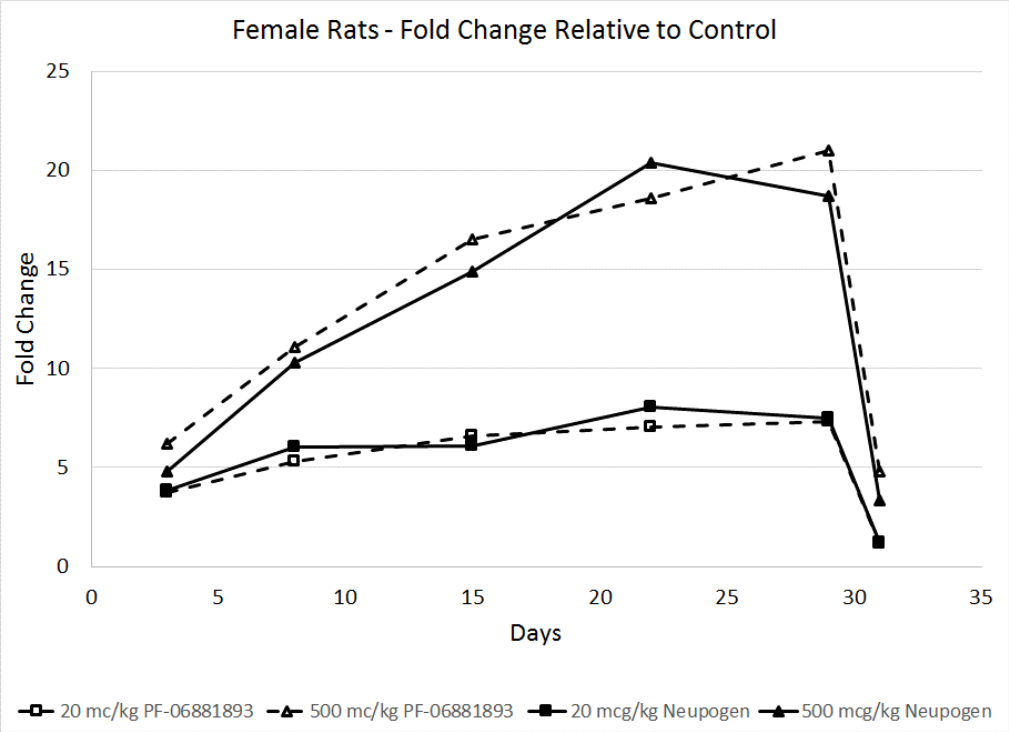
Neutrophil counts increased progressively during the dosing phase to reach maximum around Day 22 or Day 29 and then progressively decrease until fully resolved by the end of the recovery phase on Day 43 (Figure 1 and Figure 2). In general, filgrastim-related effects on total leukocyte and subtype counts were of similar magnitudes in rats administered equivalent doses of Filgrastim Hospira and Neupogen.

Figure 1 Filgrastim Effects on Neutrophils in Male Rats



Additional filgrastim-related effects on hematological parameters on most Filgrastim Hospira and Neupogen treatment groups (excluding males at 20 µg/kg Filgrastim Hospira) included transient decreases in red cell mass (erythrocytes, hemoglobin and hematocrit; up to -14%) with associated mild increases in reticulocyte counts (up to +65%) at the Day 15, Day 22 and/or Day 29 collections, compared to controls. Increases in reticulocyte counts is an indicator of increased erythropoiesis, and were considered in the report as a compensatory response to the decreases in red cell mass. All hematological findings were not present at the end of the recovery period.

Figure 2 Filgrastim Effects on Neutrophils in Female Rats



Coagulation

Filgrastim-related, although of small magnitude, prolongations in prothrombin time occurred at the end of the dosing phase in males at 500 µg/kg Filgrastim Hospira (+8%) and 500 µg/kg Neupogen (+9%), compared to controls. Prothrombin time changes did not occur in female rats. All findings were not present at the end of the recovery period.

Increases in fibrinogen concentration that occurred in individual rats at both doses of Filgrastim Hospira and Neupogen lacked a dose response, and were generally of similar incidence and magnitudes in rats administered equivalent doses of Filgrastim Hospira and Neupogen. Changes were considered secondary to inflammation that corresponded with hind limb swelling present in rats at 500 µg/kg Filgrastim Hospira and Neupogen. All findings were not present at the end of the recovery period.

Clinical Chemistry

Filgrastim- and dose-related increases in alkaline phosphatase activity (up to +3.5-fold) occurred in rats at 20 µg/kg and 500 µg/kg Filgrastim Hospira and Neupogen, compared to controls, at the end of the dosing phase. All findings were not present at the end of the recovery period. Increases in ALP activity may be secondary to bony changes associated with bone marrow expansion/increased activity, as well as bony changes in the femur, tibia and foot/feet.

Bone Marrow

Filgrastim-related increases in M:E compared to controls were primarily the result of granulocytic hyperplasia which were predominantly segmented neutrophils. These changes reflected hematologic alterations in the blood (increases in segmented neutrophils), and correlated with the microscopic findings of granulocytic hyperplasia in the bone marrow via histopathology and were of comparable magnitudes in rats administered 500 µg/kg Filgrastim Hospira and Neupogen.

Urinalysis

No filgrastim-related findings occurred.

Gross Pathology

Filgrastim-related findings at the terminal necropsy included:

- Spleen enlargement in three males administered 500 µg/kg Filgrastim Hospira and one female administered 500 µg/kg Neupogen that corresponded with microscopic findings of extramedullary hematopoiesis.
- Bilateral swelling/thickening/ enlargement of the hind feet in one male each administered 500 µg/kg Filgrastim Hospira or 500 µg/kg Neupogen. Foot lesions corresponded microscopically with osteodystrophy of the bones and fibrosis noted within surrounding soft tissue.
- All macroscopic findings were resolved or not observed at the recovery necropsy.

Organ Weights

Filgrastim- and dose-related increases in mean spleen weight (absolute and relative to body and brain weight) occurred in rats dosed with Filgrastim Hospira or Neupogen. The increases in mean spleen/body weight ratio corresponded with microscopic findings of extramedullary hematopoiesis. Differences in organ weight were resolved or not observed at the recovery necropsy.

Table 5 Filgrastim-related Changes in Organ Weight in Rats

(Excerpted from Submission)

Test Article-related Organ Weight Changes - Terminal Male and Female (Percent change relative to control)								
Dose level: µg/kg	20[c]		500[c]		20[d]		500[d]	
Sex	M	F	M	F	M	F	M	F
Number Examined	10	10	10	10	10	10	10	10
Mean Body Weight (g)	↑0.27	↓3.72	↓8.87	↑0.83	↓8.06	↑1.65	↓4.03	↑0.41
Spleen (g)	↑53.35[b]	↑20.26	↑127.72[b]	↑105.82[b]	↑20.72	↑31.14[a]	↑158.72[b]	↑119.32[b]
Spleen/BWt%	↑52.52[b]	↑23.77[a]	↑149.53[b]	↑104.11[b]	↑30.19[a]	↑27.88[a]	↑169.53[b]	↑118.17[b]
Spleen/BrWt ratio	↑50.60[b]	↑22.28[a]	↑123.98[b]	↑102.93[b]	↑16.82	↑33.19[a]	↑147.27[b]	↑119.80[b]
[a]Significantly different from control; ($p<0.05$)				↑ - Increased				
[b]Significantly different from control; ($p<0.01$)				↓ - Decreased				
[c]Dosed with Filgrastim Hospira				M - Male				
[d]Dosed with Neupogen®				F - Female				
BWt - Body Weight								
BrWt - Brain Weight								

Histopathology

Adequate Battery: Yes
Peer Review: No
Histological Findings:
Filgrastim- and dose-related findings at the terminal necropsy included:

- Extramedullary hematopoiesis in spleen, lymph nodes and liver
- Granulocytic hyperplasia in bone marrow and bone osteodystrophy of similar incidence and magnitude in rats dosed with Filgrastim Hospira or Neupogen.

Table 6 Filgrastim-Related Microscopic Findings after Daily Subcutaneous Repeat-Dose for 29-Days in Rats

(Excerpted from Submission)

Test Article-related Microscopic Observations - Terminal										
Dose level: µg/kg	0		20[a]		500[a]		20[b]		500[b]	
Sex	M	F	M	F	M	F	M	F	M	F
Number Examined	10	10	10	10	10	10	10	10	10	10
Bone marrow, femur										
Hyperplasia, granulocytic	0	0	10	10	10	10	10	10	10	10
-mild	0	0	3	1	0	0	2	2	0	0
-moderate	0	0	7	9	0	0	6	5	0	0
-severe	0	0	0	0	10	10	2	3	10	10
Bone marrow, sternum										
Hyperplasia, granulocytic	0	0	10	10	10	10	10	10	10	10
-minimal	0	0	0	1	0	0	0	0	0	0
-mild	0	0	3	7	0	0	4	2	0	0
-moderate	0	0	7	2	0	0	4	8	0	0
-severe	0	0	0	0	10	10	2	0	10	10
Spleen										
Hematopoiesis, extramedullary, increased	0	1	10	5	10	10	8	9	10	10
-minimal	0	1	3	2	0	0	3	6	0	0
-mild	0	0	7	3	0	2	5	3	0	0
-moderate	0	0	0	0	10	8	0	0	10	10
Liver										
Hematopoiesis, extramedullary	0	0	3	5	10	7	7	6	10	8
-minimal	0	0	3	5	7	7	7	6	8	6
-mild	0	0	0	0	3	0	0	0	2	2
Lymph node, mandibular										
Hematopoiesis, extramedullary										
-minimal	0	0	0	0	3	0	0	0	3	1
Lymph node, mediastinal										
Hematopoiesis, extramedullary										
-minimal	0	0	0	0	1	1	0	0	2	0
Bone, femur										
Osteodystrophy	0	0	3	0	8	1	2	1	7	4
-minimal	0	0	1	0	3	0	0	0	3	1
-mild	0	0	1	0	3	1	2	0	2	2
-moderate	0	0	1	0	1	0	0	1	2	1
-severe	0	0	0	0	1	0	0	0	0	0
Bone, tibia										
Osteodystrophy	0	0	0	0	5	0	0	1	1	2
-minimal	0	0	0	0	4	0	0	0	0	0
-mild	0	0	0	0	1	0	0	0	1	2
-moderate	0	0	0	0	0	0	0	1	0	0
M - Male F - Female	[a]Dosed with Filgrastim Hospira [b]Dosed with Neupogen®									

Special Evaluation: Immunogenicity

Anti-drug antibodies (ADA) to filgrastim were measured in 480 serum samples from 60 treated rats using a validated homogeneous bridging ADA assay based on the electrochemiluminescence (ECL) technology for the detection of anti-filgrastim antibodies. Positive ADA responses were observed in nine rats starting on Day 15 and occurred in both males and females given Filgrastim Hospira at either 20 µg/kg or 500 µg/kg and Neupogen at 500 µg/kg.

Table 7 Anti-drug antibodies to Filgrastim in Rats

(Excerpted from Submission)

Effects of Subcutaneous Administration of Filgrastim Hospira or Neupogen® on an Anti-Drug Antibody Response in Rats										
	Number of Rats with Positive ADA Response/Total Number Evaluated									
	Vehicle		TA1[a] 20 µg/kg		TA1[a] 500 µg/kg		TA2[b] 20 µg/kg		TA2[b] 500 µg/kg	
Interval	M	F	M	F	M	F	M	F	M	F
Pretest	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
Day 8	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
Day 15	0/6	0/6	1/6	0/6	1/6	0/6	0/6	0/6	0/6	1/6
Day 22	0/6	0/6	1/6	0/6	1/6	1/6	0/6	0/6	2/6	2/6
Day 29	0/6	0/6	2/6	1/6	1/6	1/6	0/6	0/6	0/6	2/6
Day 43	0/6	0/6	2/6	0/6	1/6	1/6	0/6	0/6	1/6	1/6
Day 57	0/6	0/6	2/6	1/6	1/6	1/6	0/6	0/6	0/6	1/6
Day 71	0/6	0/6	1/6	0/6	1/6	1/6	0/6	0/6	0/6	1/6
[a] Test Article 1 = Filgrastim Hospira [b] Test Article 2 = Filgrastim Innovator (Neupogen®)										

An increase in antibody titer over time was observed for only 4 rats (Table 7). On Day 29, positive ADA responses occurred in two 500 µg/kg females; however, filgrastim serum concentrations in ADA positive female were similar to ADA negative females suggesting that the presence of ADA to filgrastim had no impact on systemic exposure to filgrastim during the interval of TK assessments Day 1 through Day 29.

Special Evaluation: Local Tolerance

The injection sites of main study animals were scored for erythema/eschar and edema weekly following the Draize scale for scoring skin irritation. There were no observations of erythema, eschar, or edema in any animals during the course of the study.

Table 8 ADA Positive Samples with an Increase in Antibody Titer over Time

(Excerpted from Submission)

Filgrastim Hospira 20 µg/mL | Filgrastim Hospira 500 µg/mL | Neupogen 500 µg/mL

Group 7	Animal 7007 (Sex: M)		Group 8	Animal 8012 (Sex: M)		Animal 8511 (Sex: F)		Group 10	Animal 10507 (Sex: F)	
Interval	Result	ADA Titer	Interval	Result	ADA Titer	Result	ADA Titer	Interval	Result	ADA Titer
pretest (Day -6)	negative	-	pretest (Day -6)	negative	-	negative	-	pretest (Day -6)	negative	-
Day 8	negative	-	Day 8	negative	-	negative	-	Day 8	negative	-
Day 15	positive	1 : 4	Day 15	positive	1 : 8	negative	-	Day 15	positive	1 : 1
Day 22	positive	1 : 64	Day 22	positive	1 : 16	positive	1 : 4	Day 22	positive	1 : 8
Day 29	positive	1 : 64	Day 29	positive	1 : 128	positive	1 : 8	Day 29	positive	1 : 32
Day 43	positive	1 : 256	Day 43	positive	1 : 256	positive	1 : 16	Day 43	positive	1 : 64
Day 57	positive	1 : 256	Day 57	positive	1 : 128	positive	1 : 8	Day 57	positive	1 : 32
Day 71	positive	1 : 256	Day 71	positive	1 : 128	positive	1 : 8	Day 71	positive	1 : 32

Toxicokinetics

Filgrastim concentrations were below the bioanalytical limit of quantitation (< 50.0 pg/mL) in all serum samples obtained from control rats on Days 1 and 29 and all predose samples obtained from treated rats on Day 1. Systemic exposure to filgrastim was independent of sex with individual serum concentrations, AUC, and C_{max} values similar between males and females (female to male AUC ratios ranged from 0.744 to 1.18). No evidence that positive ADA responses had an impact on filgrastim systemic exposure. In general, and taking into consideration an n=3 per time point assessment, systemic exposure to filgrastim were similar in rats administered filgrastim Hospira and Neupogen.

Table 9 Summary of TK Parameters of Filgrastim in Rats

(Excerpted from Submission)

Filgrastim TK Parameters on Days 1 and 29 Following Subcutaneous Injection of Filgrastim Hospira or Neupogen® to Rats (Males and Females Combined)

Group	Dose (ug/kg)	Day	C_{max} (pg/mL)	T_{max} (hr)	T_{last} (hr)	AUC_{Tlast} (hr*pg/mL)	$AUC_{0\text{-}24hr}$ (hr*pg/mL)	R[a]	Biosimilar Ratio[b]
7[c]	20	1	48400	2	12	269000	270000	NA	1.00
		29	64700	2	24	273000	273000	1.01	0.892
8[c]	500	1	1680000	2	24	8960000	8960000	NA	0.902
		29	2260000	2	24	12400000	12400000	1.38	1.06
9[d]	20	1	49200	2	12	268000	269000	NA	NA
		29	68200	2	24	306000	306000	1.14	NA
10[d]	500	1	1850000	2	24	9930000	9930000	NA	NA
		29	2250000	1	24	11700000	11700000	1.18	NA

NA - Not applicable
a: $R = AUC_{0\text{-}24hr\ Day\ 29}/AUC_{0\text{-}24hr\ Day\ 1}$
b: Biosimilar Ratio = $AUC_{0\text{-}24hr\ Filgrastim\ Hospira\ (TA1)}/AUC_{0\text{-}24hr\ Neupogen®\ (TA2)}$
c: Groups 7 and 8 were given Filgrastim Hospira (TA1)
d: Groups 9 and 10 were given Neupogen® (TA2)

Figure 3 Comparison of Filgrastim Hospira and Neupogen Serum Concentration-Time Profiles on Days 1 and 29

(Excerpted from Submission)

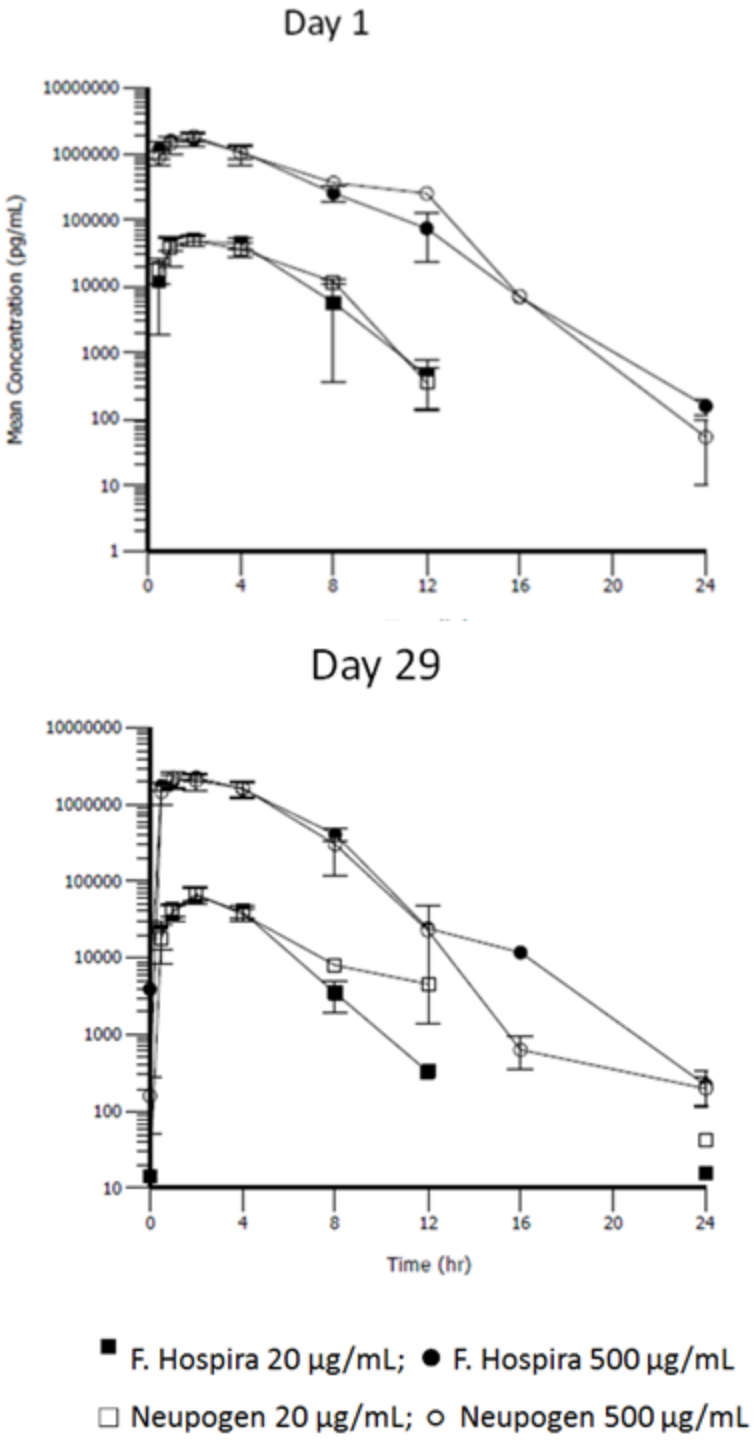


Table 10 Additional Toxicokinetic Data of Filgrastim in Rats

	Days / Units	Filgrastim Hospira	Neupogen
Filgrastim Mean Concentration Variability (CV%)	Day 1	175 to 178	7.73 to 140
	Day 29	10 to 245	17.3 to 245
Day 1: a 25-fold increase in dose represents	C_{max}	↑ 34.7	↑ 37.6
	AUC	↑ 33.2	↑ 36.9
Day 29: a 25-fold increase in dose represents	C_{max}	↑ 34.9	↑ 33.0
	AUC	↑ 45.4	↑ 38.2
Accumulation ratios	20 µg/mL	1.01	1.14
	500 µg/mL	1.38	1.18
Lambda z (elimination constant)	L/h	0.377 to 0.574	0.336 to 0.693
ADA effect	Day 1 – Day 29	None	None

Dosing Solution Analysis

Results for homogeneity and concentration of dosing formulations were within an acceptable range, see tabulated results below.

Table 11 Dose Formulation Analysis

(Excerpted from Submission)

Homogeneity				
Dose Level (µg/kg)	Nominal Concentration (µg/mL)	Average Calculated Concentration (µg/mL)	Average %Recovery[a]	%Relative Standard Deviation
TA1[b]				
20	10	9.9168	99.2	1.370
500	250	236.1104	94.4	1.023
TA2[c]				
20	10	9.3274	93.3	0.717
500	250	226.8021	90.7	0.568

[a] Average %recovery was calculated from the nominal concentration.
[b] TA1 = Test Article 1 = Filgrastim Hospira
[c] TA2 = Test Article 2 = Filgrastim Innovator (Neupogen®)

Concentration				
Dose Level (µg/kg)	Nominal Concentration (µg/mL)	Average Calculated Concentration[a] (µg/mL)	Average %Recovery[a]	%Relative Standard Deviation
Day 1				
TA1[b]				
0	0	BLQ	NA	NA
20	10	9.9168	99.2	1.370
500	250	236.1104	94.4	1.023
TA2[c]				
20	10	9.3274	93.3	0.717
500	250	226.8021	90.7	0.568
Day 15				
TA1[b]				
0	0	BLQ	NA	NA
20	10	9.3045	93.0	3.102
500	250	241.8645	96.7	0.103
TA2[c]				
20	10	9.1938	91.9	1.585
500	250	236.5541	94.6	0.724
Day 29				
TA1[b]				
0	0	BLQ	NA	NA
20	10	9.3081	93.1	1.363
500	250	233.5975	93.4	5.076
TA2[c]				
20	10	9.4000	94.0	0.838
500	250	243.1803	97.3	1.305

[a] Average %recovery was calculated from the nominal concentration.
[b] TA1 = Test Article 1 = Filgrastim Hospira
[c] TA2 = Test Article 2 = Filgrastim Innovator (Neupogen®)
BLQ – Below the Limit of Quantitation (<5.0000 µg/mL)
NA – Not Applicable

7 Genetic Toxicology

Genetic toxicology studies are not required for biotechnology-derived pharmaceuticals per ICH S6 Guidance.

8 Carcinogenicity

Carcinogenicity studies are not required for biotechnology-derived pharmaceuticals per ICH S6 Guidance.

9 Reproductive and Developmental Toxicology

Reproductive and developmental toxicity studies to assess the similarity of PF-06881893 to Neupogen were not conducted.

11 Integrated Summary and Safety Evaluation

The Applicant conducted limited nonclinical testing to support the 351(k) BLA for PF-06881893 based on the premises that structural and functional characterization of PF-06881893 was shown to be similar to Neupogen and therefore all the data generated by the originator for Neupogen can be extrapolated to PF-06881893.

The toxicity of Neupogen was assessed in a comprehensive toxicology program sponsored by Amgen. This program evaluated the effects of Neupogen in single-dose (mouse, rat, hamster, and monkey) and repeat-dose (rat, hamster, dog, and monkey) toxicity studies [Neupogen US Summary Basis of Approval, 1991; Neulasta European Public Assessment Report, 2004]. The Applicant included a summary of these studies, along with the routes of administration, study duration, and species in Section 2.4.4.1.

The Applicant conducted a 4-week comparative toxicology study in Sprague-Dawley rats that included a comparison of the potential toxicity, local tolerance, absolute neutrophil count as PD biomarker, PK/TK, and immunogenicity of PF-06881893 to US-licensed Neupogen. PF-06881893 DP, Batch (Lot) 2075114 was used for the comparative 4-week repeat dose toxicity study. This lot was manufactured in Zagreb, Croatia by Hospira Zagreb at the Savski-Marof site and released for nonclinical use according to development acceptance criteria. The lot used for nonclinical and clinical studies is the same; the batch was manufactured using the same equipment and at the same scale as that proposed for commercial manufacture.

Similar clinical signs of hind limb swelling and/or impairment and decreases in body weights occurred mostly in male rats given 500 µg/kg filgrastim Hospira or Neupogen. Dose-related increases in total leukocyte counts attributed to increases in neutrophil counts (Table 12, Figure 1 and Figure 2) that corresponded with increased M:E ratios on bone marrow smears, microscopic findings of granulocytic hyperplasia in the bone marrow and increased hematopoiesis in the spleen and other organs.

Table 12 Fold-Increase in Leukocytes and Neutrophil Counts in Rats

(Excerpted from Submission)

Summary of Effects on Leukocytes and Neutrophils[a]									
		Filgrastim Hospira				Neupogen®			
		20 µg/kg		500 µg/kg		20 µg/kg		500 µg/kg	
	Interval	M	F	M	F	M	F	M	F
Total Leukocytes	Day 3	+1.5x[b]	+1.6x[b]	+2.3x[b]	+2.1x[b]	+1.4x[b]	+1.5x[b]	+2.0x[b]	+1.7x[b]
	Day 8	+2.0x[b]	+1.7x[b]	+3.9x[b]	+2.8x[b]	+1.9x[b]	+1.9x[b]	+3.6x[b]	+2.5x[b]
	Day 15	+1.9x[b]	+1.7x[b]	+5.1x[b]	+3.0x[b]	+1.7x[b]	+1.5x[b]	+3.9x[b]	+2.8x[b]
	Day 22	+2.4x[b]	+1.6x[b]	+4.4x[b]	+3.2x[b]	+2.2x[b]	+1.7x[b]	+5.5x[b]	+2.9x[b]
	Day 29	+2.1x[b]	+1.8x[b]	+4.7x[b]	+3.3x[b]	+2.1x[b]	+1.6x[b]	+5.1x[b]	+3.0x[b]
	Day 31 (Terminal)	--	--	+1.5x[b]	+1.4x	--	--	+1.5x[b]	+1.2x
Neutrophils	Day 3	+4.1x[b]	+3.7x[b]	+8.0x[b]	+6.2x[b]	+3.6x[b]	+3.8x[b]	+6.8x[b]	+4.8x[b]
	Day 8	+5.8x[b]	+5.3x[b]	+15.5x[b]	+11.1x[b]	+5.6x[b]	+6.0x[b]	+14.3x[b]	+10.3x[b]
	Day 15	+6.1x[b]	+6.6x[b]	+26.3x[b]	+16.5x[b]	+6.3x[b]	+6.1x[b]	+21.4x[b]	+14.9x[b]
	Day 22	+9.1x[b]	+7.1x[b]	+22.9x[b]	+18.6x[b]	+8.8x[b]	+8.0x[b]	+33.0x[b]	+20.4x[b]
	Day 29	+8.4x[b]	+7.3x[b]	+28.7x[b]	+21.0x[b]	+8.6x[b]	+7.5x[b]	+26.9x[b]	+18.7x[b]
	Day 31 (Terminal)	+1.8x	+1.2x	+5.0x[b]	+4.8x[b]	+1.2x	+1.2x	+6.1x[b]	+3.4x[b]

M – Male; F – Female
X – Fold-change relative to controls
[a] – Changes relative to controls
[b] – Mean value statistically different from controls
-- – No meaningful change relative to controls

Similar dose-related increases in alkaline phosphatase activity, prolongations of small magnitude in prothrombin time and increases in fibrinogen likely related to an inflammatory response occurred at the end of the dosing phase at 500 µg/kg filgrastim Hospira or Neupogen. Filgrastim- and dose-related increases in mean spleen weight (absolute and relative to body and brain weight) occurred in rats dosed with Filgrastim Hospira or Neupogen and corresponded with microscopic findings of extramedullary hematopoiesis. Similar incidence and severity in microscopic findings occurred in rats dosed with Filgrastim Hospira or Neupogen and all findings during the dosing phase were resolved or were not present at the recovery sacrificed.

Positive ADA responses were observed in nine rats starting on Day 15 and occurred in both males and females given Filgrastim Hospira at either 20 µg/kg or 500 µg/kg and Neupogen at 500 µg/kg. No evidence that positive ADA responses had an impact on filgrastim systemic exposure. In general, and taking into consideration an n=3 per time point assessment, systemic exposure to filgrastim were similar in rats administered filgrastim Hospira and Neupogen.

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