Office of Clinical Pharmacology
351(k) Biosimilar Review

351(k) BLA Number	761080
Applicant	Hospira, a Pfizer company
Submission Date	09/21/2017
Submission Type	*Standard review*
Link to EDR	\\cdsesub1\evsprod\BLA761080\761080.env
Proprietary (Proposed) / Nonproprietary Name	NIVESTYM / PF-06881893[1]
Dosage Form and Strengths	Solution in single-dose vial (SDV) or pre-filled syringe (PFS): 300 mcg/0.5mL(in SDV) or 300 mcg/1.0 mL(in PFS); 480 mcg/0.8mL(in SDV) or 300 mcg/1.6 mL(in PFS)
Route of Administrations	SDV: Subcutaneous and intravenous injection PFS: Subcutaneous injection
Proposed Indication(s)	• *Reduce the time to neutrophil recovery and the duration of fever, following induction or consolidation chemotherapy treatment of patients with acute myeloid leukemia (AML)* • *Reduce the duration of neutropenia and neutropenia-related clinical sequelae, e.g., febrile neutropenia, in patients with nonmyeloid malignancies undergoing myeloablative chemotherapy followed by bone marrow transplantation (BMT)* • *Mobilize autologous hematopoietic progenitor cells into the peripheral blood for collection by leukapheresis* • *Reduce the incidence and duration of sequelae of severe neutropenia (e.g., fever, infections, oropharyngeal ulcers) in symptomatic patients with congenital neutropenia, cyclic neutropenia, or idiopathic neutropenia*
Associated IND	*IND-109991*
Reference Product Information (US-licensed)	
Proprietary/Nonproprietary Name	Neupogen®/ Filgrastim
Dosage Form and Strengths	SDV: 300 mcg/1.0 ml or 480 mcg/1.6 mL PFS: 300 mcg/0.5 ml or 480 mcg/0.8 mL
OCP Review Team Signers	
OCP Review Team	*Theingi Thway, Ph.D. and* *Olanrewaju Okusanya, Pharm.D., MS*
OCP Final Signatory	*Nam Atiqur Rahman, Ph.D.*

[1] In this document, we generally refer to the applicant's proposed product by the applicant-provided descriptor "PF-06881893", which was the name used to refer to this product during development

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1. EXECUTIVE SUMMARY

This Biologic License Application (BLA) for NIVESTYM, refered to as "PF-06881893" by the applicant during development and herein, has been submitted under Section 351(k) of the Public Health Service Act (42 U.S.C. 262(k)). The applicant is seeking approval for PF-06881893 as a proposed biosimilar to US-licensed Neupogen licensed under BLA 103353 by Amgen Inc. The applicant is requesting licensure for same indications as US-licensed Neupogen except for the indication of Hematopoietic Syndrome of Acute Radiation Syndrome, which is protected by orphan drug exclusivity.

The applicant submitted three clinical studies in healthy subjects to support a demonstration of no clinical meaningful difference between PF-06881893 and US-licensed Neupogen. Clinical studies included in this BLA are a single-dose PK/PD study (Study ZIN-FIL-1502) to assess PK and PD (absolute neutrophil count (ANC)) similarity in 24 healthy subjects, a multiple-dose PD study (Study ZIN-FIL-1501) to assess PD ($CD34^{+}$mobilization) similarity in 60 healthy subjects, and a multiple-dose study (Study C1121012) to assess comparative immunogenicity in 256 healthy subjects.

The 90% confidence intervals (CI) of the geometric mean ratio (GMR) for pre-defined PK (C_{max} and $AUC_{0\text{-}inf}$) and PD (ANC_{max} and $AUEC_{ANC}$) endpoints were within the limits of 0.8-1.25 in Study ZIN-FIL-1502 and within the limits of 0.8-1.25 for $CD34^{+}{}_{max}$ and $AUEC_{CD34+}$ in Study ZIN-FIL-1501. The immunogenicity results from Study C1121012 indicated that similar incidence of anti-drug antibody (ADAs) formation was observed between PF-06881893 and US-licensed Neupogen in healthy subjects indicating similar immunogenicity risk for PF-06881893 as compared to US-licensed Neupogen in healthy subjects.

In conclusion, the PK, PD, and immunogenicity results support a demonstration of no clinically meaningful difference between PF-06881893 and US-licensed Neupogen, and add to the totality of the evidence to support a demonstration of biosimilarity of PF-06881893 to US-licensed Neupogen.

1.1 Recommendations

The Office of Clinical Pharmacology recommends the approval of PF-06881893 based on a demonstration of PK and PD similarity and no increase in immunogenicity risk between PF-06881893 and US-licensed Neupogen.

Table 1. **Review summary**

Review Summary	Recommendations and Comments
Pivotal evidence of PK similarity	PK similarity was demonstrated between PF-06881893 and US-licensed Neupogen. The 90% CI of the GMR for the primary PK endpoints of C_{max} and $AUC_{0\text{-}inf}$ was within the pre-specified limits of 0.8-1.25.
Pivotal evidence of PD similarity	PD similarity was demonstrated between PF-06881893 and US-licensed Neupogen in single and multiple dose studies. The 90% CIs of the GMR for the primary PD endpoints of $AUEC_{ANC}$ and ANC_{max} in single dose study and $AUEC_{CD34+}$ and $CD34^{+}_{max}$ in multiple doses were within the pre-specified limits of 0.8-1.25.
Evidence of immunogenicity comparability	Similar incidence of ADA formation was observed for PF-06881893 and US-licensed Neupogen in healthy subjects . The upper bound of 90% CIs for risk difference was <10%, and met the pre-specified limit.

1.2 Post-Marketing Requirements and Commitments

None

2. SUMMARY OF CLINICAL PHARMACOLOGY ASSESSMENT

2.1 Clinical Pharmacology and Pharmacokinetics

PF-06881893 is a proposed biosimilar to US-licensed Neupogen, a 175 amino acid recombinant methionyl human G-CSF produced in *Escherichia coli*. This glycoprotein with approximate molecular weight of 19 kDa acts on hematopoietic cells by binding to G-CSF receptor and stimulating proliferation, and differentiation of neutrophil progenitors.

The applicant submitted three studies conducted in healthy subjects that evaluated 5 mcg/kg subcutaneous (SC) doses to demonstrate PK/PD similarity and comparative immunogenicity of PF-06881893 and US-licensed Neupogen. For PK and PD similarity assessment, ANC in a single-dose PK/PD study served as a PD maker while mobilization of $CD34^+$cells served as a PD marker in multiple-dose PD/PK study. Table 2 summarized the GMRs of PK and PD endpoints from these studies.

Table 2. Statistical analyses in PK/PD similarity assessment in studies ZIN-FIL-1502 and ZIN-FIL-1501 (FDA reviewer's analysis).

	GMR (90% CI)					
	Study ZIL-FIL 1502		Study ZIL-FIL 1502		Study ZIL-FIL 1501	
	PK Endpoints		PD Endpoints			
Product comparison	**C_{max}**	**$AUC_{0\text{-}inf}$**	**ANC_{max}**	**ANC $AUEC_{last}$**	**$CD34^+{}_{max}$**	**$AUEC_{CD34+last}$**
PF-06881893 and US-licensed Neupogen	1.11 (1.02 -1.21)	1.13 (1.05 -1.23)	1.02 (0.97 -1.07)	1.01 (0.97 -1.05)	1.05 (0.95 -1.17)	1.06 (0.99 -1.15)

The incidence of immunogenicity between PF-06881893 and US-licensed Neupogen was compared in multiple-dose, parallel arm study in 250 healthy subjects. The % risk difference (90% CI) was 2.56% (-2.72, 8.36), thus the upper bound was <10%. The result indicates similar immunogenicity risk between these two products.

In overall, the clinical studies adequately demonstrated the similarity of PK and PD and showed no increase in immunogenicity risk between PF-06881893 and US-licensed Neupogen. These results support a demonstration of no clinically meaningful differences between PF-06881893 and US-licensed Neupogen and add to the totality of the evidence to support the biosimilarity demonstration of PF-06881893 to US-licensed Neupogen.

2.2 Outstanding Issues

None

3. COMPREHENSIVE CLINICAL PHARMACOLOGY REVIEW

3.1 Regulatory Background

3.1.1 If applicable, describe relevant regulatory history for the review of this 351(k) BLA.

PF-06881893 is a proposed biosimilar to US-licensed Neupogen. The applicant is seeking licensure for these 4 indications as US-licensed Neupogen.

- Reduce the time to neutrophil recovery and the duration of fever, following induction or consolidation chemotherapy treatment of patients with acute myeloid leukemia (AML)
- Reduce the duration of neutropenia and neutropenia-related clinical sequelae, e.g., febrile neutropenia, in patients with nonmyeloid malignancies undergoing myeloablative chemotherapy followed by bone marrow transplantation (BMT)
- Mobilize autologous hematopoietic progenitor cells into the peripheral blood for collection by leukapheresis
- Reduce the incidence and duration of sequelae of severe neutropenia (e.g., fever, infections, oropharyngeal ulcers) in symptomatic patients with congenital neutropenia, cyclic neutropenia, or idiopathic neutropenia

The applicant is not seeking the approval for indication of Hematopoietic Syndrome of Acute Radiation Syndrome, which is protected by orphan drug exclusivity.

3.2 Clinical Pharmacology Review Questions

3.2.1 Are the design features of the clinical pharmacology and/or clinical studies to support biosimilarity acceptable?

The applicant conducted 2 pivotal clinical PK/PD similarity studies and 1 immunogenicity study in all healthy subjects as described in Table 3 below.

Table 3. Design features of clinical studies in the submission

Protocol	Title	Subjects	Objectives	Dose/ Route/Duration
PK/PD Similarity Study				
ZIN-FIL-1502	A randomized open-label, single-dose, cross-over study evaluating the pharmacokinetics and pharmacodynamics of PF-06881893 to US-approved Neupogen® following subcutaneous administration to healthy subjects	Healthy (N=24)	PK, PD (ANC), & safety	5 mcg/kg/day single SC dose of PF-06881893 versus US-licensed Neupogen with at least 28 days between treatments
ZIN-FIL-1501	A randomized open-label, multiple-dose, cross-over study evaluating the pharmacokinetics and pharmacodynamics of PF-06881893 to US-approved Neupogen® following subcutaneous administration to healthy subjects	Healthy (N=60)	PD ($CD34^+$), PK, & safety,	5 mcg/kg/day for 5 consecutive daily SC doses of PF-06881893 versus US-licensed Neupogen with at least 28 days between treatments
Immunogenicity Study				

C1121012	A randomized open-label, 2-period, parallel arm study to assess the immunogenicity of multiple subcutaneous (SC) doses of "Filgrastim Hospira" or US-approved Neupogen® reference product in healthy subjects	Healthy (N=250)	immunogenicity & safety,	5 mcg/kg/day for 5 consecutive daily SC doses in period 1 followed by a single SC dose in period 2 of PF-06881893 versus US-licensed Neupogen with at least 26 days between the periods

The study designs of ZIN-FIL-1502 and ZIN-FIL-1501 studies are considered adequate to demonstrate PK/PD similarity for the following reasons:

1. A study in healthy subjects is considered safe and more sensitive compared with that in patients with potentially confounding factors such as underlying disease, concomitant medications, and other factors.
2. Single or multiple SC doses of 5 mcg/kg/day of filgrastim are considered acceptable considering dose-exposure linearity and tolerability of filgrastim.
3. A cross-over study design is recommended for PK/PD similarity of products with short half-life and the rapid PD response (ANC) in study ZIN-FIL 1502.
4. The 4-week washout between each treatment period is deemed adequate in cross-over design. Serum filgrastim concentrations are near the lower limit of quantitation (LLOQ) by Day 2 post-dose after each treatment period, and ANC has returned to baseline by around Day 15 after each treatment as well.
5. Measurement of CD34+ cells in peripheral blood represents relevant PD marker to show mobilization of autologous hematopoietic progenitor cells in study ZIN-FIL 1501 and a cross-over study design is appropriate for PD similarity of products.

The study design of C1121012 is considered adequate to assess immunogenicity risk. It is a parallel arm study with a total of 125 healthy subjects per arm. Subjects in each arm received at least 5 daily doses in period 1 followed by a single SC dose in period 2 of PF-06881893 or US-licensed Neupogen with at least 26 days between the periods.

3.2.2 Are the endpoints in the clinical pharmacology and/or clinical studies to support biosimilarity acceptable?

Yes. In the study ZIN-FIL-1502, the pre-specified PK endpoints were C_{max} and $AUC_{0\text{-}inf}$ and the pre-specified PD endpoints were ANC_{max} and ANC $AUEC_{last}$. PK and PD similarity was concluded if the 90% CI of the GMRs between PF-06881893 and US-licensed Neupogen were within the pre-specified limits of 0.8 to 1.25. Sample collections were described below.

- PK serum samples were collected 1 hour prior to dose administration on Day 1 and 0.25, 0.5, 0.75, 1, 2, 3, 4, 6, 8, 10, 12, 16, 24, and 48 hours postdose of each period.
- Whole blood samples for ANC were collected 1 hour prior to dose administration on Day 1 and 0.5, 1, 2, 4, 6, 8, 24, 48, 72, 96, and 120 hours postdose of each period.
- Serum for ADA testings were collected at baseline (Day 1 predose prior to treatment), and Days 12, and 28 postdose of each period.

In the study ZIN-FIL-1501, the pre-specified PD endpoints were $CD34+_{max}$ and $AUEC_{CD34+}$. PD similarities were concluded if the 90% CI of the GMRs between PF-06881893 and US-licensed Neupogen were within the pre-specified limits of 0.8 to 1.25. Sample collections were described below.

- Whole blood samples for CD34+ cell count were collected prior to dose administration on Days 1, 2, 3, 4 and 5 and 0.5, 1, 2, 3, 4, 6, 9, 12, 16, and 24 hours postdose on Day 5 of each period.
- PK serum samples were collected 1 hour prior to dose administration on Days 1, 2, 3, 4, and 5 and 0.5, 1, 2, 3, 4, 6, 9, 12, 16, and 24 hours postdose on Day 5 of each period.
- Serum for ADA testings was collected at baseline, and Days 12, and 33 postdose of each period.

In the study C1121012, non-inferiority in immunogenicity between PF-06881893 and US-licensed Neupogen was determined if the 90% CI of the upper bound in risk difference was <10 %.

- Serum for ADA testings was collected at 3 time points per period; Day 1 predose, and Days 12, and 28 (for treatment period 1 only) or 31 (for treatment period 2 only).

3.2.3 Are the pharmacogically active moieties of the proposed biosimilar and the reference product in biological matrix appropriately identified and measured to assess the PK parameters?

Yes. See Appendix 4.1.1 for details.

3.2.4 Is PK similarity met?

Yes. PK similarity between PF-06881893 and US-licensed Neupogen was demonstrated in single-dose PK study ZIN-FIL-1502. Geometric mean serum concentration-time profiles and a summary of PK parameters are also shown in Figure 1 and Table 4 respectively.

Figure 1. Geometric mean (+SD) serum concentration (ng/mL) vs. time (hrs) in linear scale from a single-dose study ZIL-FIL 1502 (FDA reviewer's analysis).

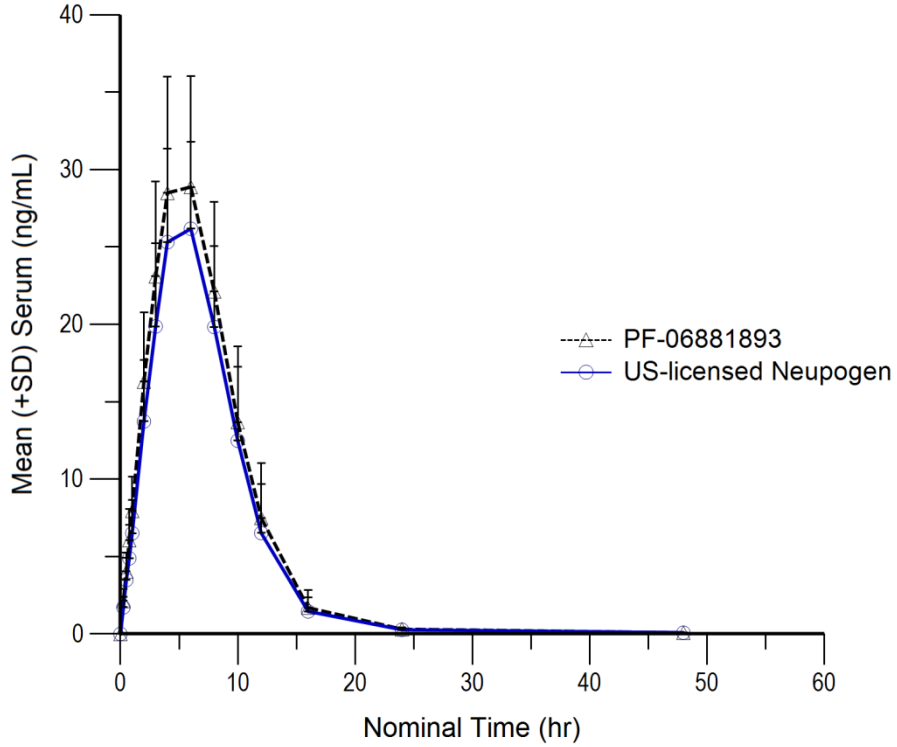

Table 4. Summary of PK parameters from study ZIN-FIL-1502 (FDA reviewer's analysis).

Study	Products	Geometric mean values (%CV)	
		C_{max} (ng/mL)	$AUC_{0\text{-}inf}$ (hr*ng/mL)
ZIN-FIL-1502	PF-06881893	29.6 (23%)	251 (23%)
	US-licensed Neupogen	26.6 (21%)	221 (22%)

PK data between PF-06881893 and US-licensed Neupogen in study ZIN-FIL-1501 was also reviewed and the results consistent with that observed in study ZIN-FIL-1502.

3.2.5 Is PD similarity met?

Yes. PD similarity between PF-06881893 and US-licensed Neupogen was demonstrated in single-dose study ZIN-FIL-1502. As shown in Figure 2 and Figure 3, the mean ANC and CD34+ count data over time overlaid each other. A summary of the PD parameters for ANC and CD34+ are shown in Table 5.

Figure 2. Mean (+SD) ANC count versus time profile in a single-dose study ZIL-FIL 1502 (FDA reviewer's analysis).

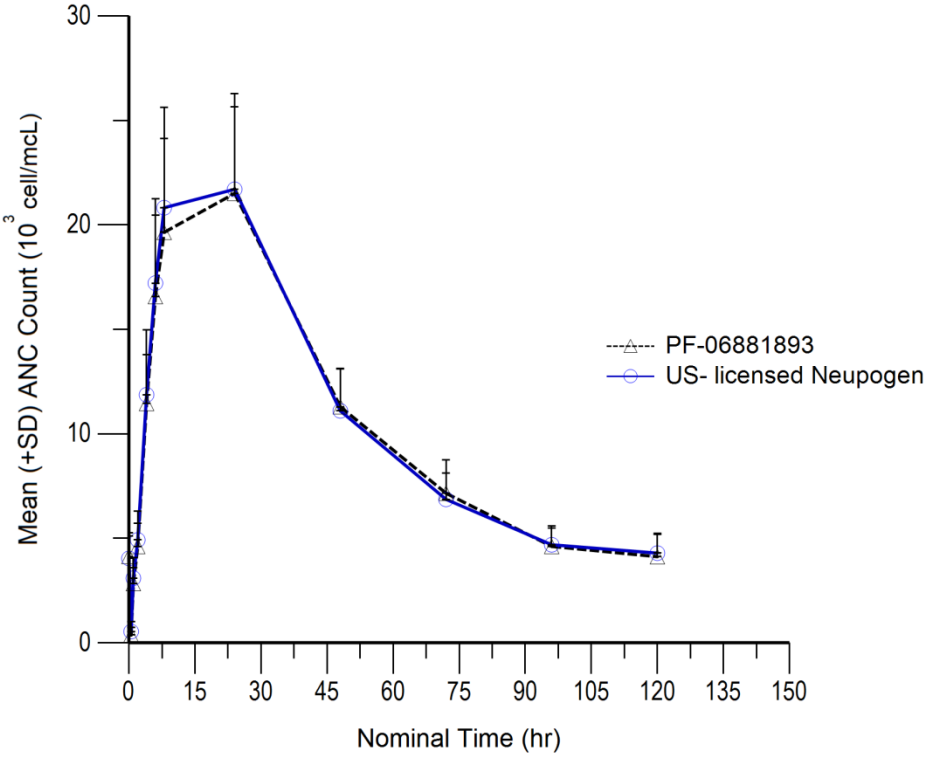

Figure 3. Mean (+SD) $CD34^{+}$count versus time profile in a multiple-dose study ZIL-FIL 1501 (FDA reviewer's analysis).

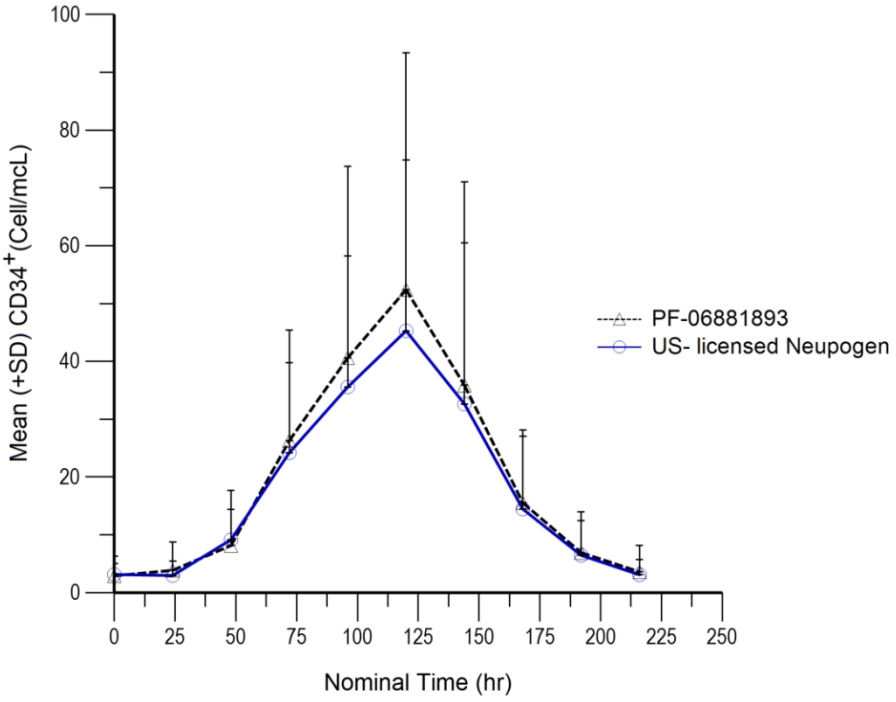

Table 5. Summary of PD primary parameters in studies ZIN-FIL-1502 and ZIN-FIL-1501 (FDA reviewer's analysis).

Product	Geometric Mean values (% CV)			
	Study ZIL-FIL 1502		Study ZIL-FIL 1501	
	PD Endpoints			
	ANC_{max} (10^3 /mL)	ANC $AUEC_{last}$ (10^3*hr/mL)	$CD34+_{max}$ (cell /mL)	$AUEC_{CD34+last}$ (cell*hr/mL)
PF-06881893	21.9 (21%)	1280 (18%)	43.7 (88%)	3590 (82%)
US-licensed Neupogen	22.3 (21%)	1290 (17%)	41.4 (75%)	3370 (74%)

Immunogenicity

3.2.6 What is the ability of the immunogenicity assay to detect antidrug antibodies (ADA) in the presence of concentration of product in the study samples?

Serum ADAs were detected using a homogenous ECL-based bridging format method with biotinylated PF-06881893 as capture reagent and ruthenylated PF-06881893 as detection reagent (without an acid dissociation procedure). The sensitivity for screening assay was 2.2 ng/mL with affinity purified goat anti-human G-CSF polyclonal antibody (R&D System) as positive control (PC). The screening assay had a drug tolerance limit of up to 0.5 mcg/mL for both PF-06881893 and US-licensed Neupogen when tested with 6 ng/mL of PCs. The sensitivity for confirmatory assay was 4.04- 8.11 ng/mL of ADAs against PF-06881893 and 3.85 ng/mL of ADAs against US-licensed Neupogen. The confirmatory assay had a drug

tolerance limit of up to 0.5mcg/mL when tested with 8 ng/mL of PCs. Mean serum G-CSF concentration are expected to be < 9 pg/mL on 48 hours post dose, well below the assay drug tolerance levels. Thus, both screening and confirmatory assays are drug-tolerant for the sampling schedules listed in section 3.2.2.

Neutralizing ADAs in serum were determined using a cell-based receptor signaling method with GloResponseTM SIE-luc2P stable U937 cells (Promega). The sensitivity was 448.03 ng/mL and 409.84 ng/mL for detection of ADAs against PF-06881893 and US-licensed Neupogen respectively. This method has a drug tolerance limit of 6.2 ng/mL when tested with 3.5 mcg/mL of PCs. It is drug-tolerant for the sampling schedules listed in section 3.2.2.

Refer to the immunogenicity review by the Office of Biotechnology Products for details regarding the ADA assay methods.

3.2.7 Is the sampling plan adequate to capture baseline, and anti-drug antibodies (ADA) formation?

See section 3.2.2 for the sampling schedules. Sampling plans in the studies were appropriate to minimize interference from the presence of filgrastim in the samples.

3.2.8 What is the incidence of anti-drug antibodies (ADA)?

The baseline and treatment-induced binding ADA incidence rate observed in Study C1121012 is shown in Table 6. The % risk difference (90% CI) between PF-06881893 and US-licensed Neupogen was 2.56% (-2.72, 8.36). Refer to the Statistical Review Memorandum. These data indicate that there is no increase in immunogenicity risk for PF-06881893 compared to US-licensed Neupogen, and supports the demonstration that there are no clinically meaningful differences between PF-06881893 and US-licensed Neupogen.

Table 6. Incidence of binding ADA formation in study C1121012 (Applicant data from CSR C1121012)

Study	Product	N[a]	Incidence of anti-filgrastim antibodies	
			Baseline	Treatment-induced
C1121012	PF-06881893	128	4/128 (3.1%)	9/121 (7.4%)
	US-licensed Neupogen	127	4/127 (3.1%)	6/123 (4.9%)

[a]Number of subject with predose data

Treatment-induced binding ADA incidence rates for PF-06881893 and US-licensed Neupogen were 0.0% and 4.3% for study ZIN-FIL-1502 and 1.7% and 0.0% for study ZIN-FIL-1501 respectively.

3.2.9 Do the anti-drug antibodies (ADA) have neutralizing activity?

None of the subjects tested positive for neutralizing antibodies in all 3 studies (Studies C1121012, ZIN-FIL-1502, ZIN-FIL-1501).

3.2.10 What is the impact of anti-drug antibodies (ADA) on the PK, PD, efficacy and safety of the therapeutic protein?

The formation of binding ADAs had no observable impact on the PK, PD (ANC or CD34+), or safety of PF-06881893 and US-licensed Neupogen.

4. APPENDICES

4.1 Summary of Bioanalytical Method Validation and Performance

4.1.1 Pharmacokinetics

4.1.1.1 How are the concentrations of the pharmacologically active moieties (parent and/or any relevant catabolites) measured in the biological matrices in the clinical pharmacology studies?

Serum PF-06881893, or US-licensed Neupogen concentrations were determined using a validated Enzyme-Linked Immunosorbent Assay (ELISA). Method validation and sample analyses for the quantification of recombinant G-CSF (rG-CSF) in human serum for the study ZIN-FIL-1502 were performed at (b) (4). Serum rG-CSF was measured using a sandwich ELISA in which mouse monoclonal anti-human G-CSF antibody was used as capture reagent and biotinylated polyclonal anti-human G-CSF antibodies, and streptavidin HRP were used as detection reagents (Validation reports 6849.03311.1 and 10542.053016). Standard calibrators were prepared by spiking WHO G-CSF in 100% human serum. The validation reports and bioanalytical study report for the study ZIN-FIL-1502 were reviewed. A summary of the ELISA method validation and in-study performance for measurement of filgrastim are show in Table 7 below.

Table 7. Summary of the ELISA method validation and in-study performance for measurement of filgrastim

Bioanalytical review summary	Method validation was adequate to support the study ZIN-FIL-1502		
Material for calibration curve, lot & Concentration	Human recombinant DNA derived WHO international standard G-CSF (National Institute for Biological Standard and Control, UK, Lot 09/136, 1 mcg/mL)		
Validated assay range	70 (LLOQ) – 5000 (ULOQ) pg/mL in human serum		
Source & reagents	Mouse monoclonal anti-human G-CSF antibody and biotinylated polyclonal anti-human G-CSF antibody in DuoSet kits from R&D Systems part DY214E		
Material for QC, lot & Concentration	PF-06881893 (Lot# 2075114, 480 mg/0.8mL) US-licensed Neupogen (Lot# 1050859, 480 mg/0.8mL)		
Minimum required dilution	1/5 in assay diluent		
Regression model & weighting	5 PL & $1/Y^2$		
Validation parameters	**Method validation summary**		**Acceptability**
Standard curve (WHO G-CSF) performance during accuracy & precision	No of standard calibrators from LLOQ to ULOQ	7	Yes
	Cumulative accuracy (%bias) in standard calibrators	-1.6 to 1.3%	Yes
	Cumulative precision (%CV) in 7 levels within LLOQ to ULOQ	≤ 5.2%	Yes
QCs performance during accuracy & precision	**Cumulative accuracy (%bias) in 5 QCs** PF-06881893 QCs: US-licensed Neupogen QCs:	 -5.8 to 3.0% -15.9 to -3.7%	Yes

	Interbatch %CV PF-06881893 QCs: US-licensed Neupogen QCs:	 ≤ 5.2% ≤ 7.9%	Yes
	Total error (TE) PF-06881893 QCs: US-licensed Neupogen QCs:	 ≤ 16% ≤ 24%	Yes
Selectivity & matrix effect	10 serum lots tested. At least 80% of the lots within 20 %bias		Yes
Hemolysis effect	Total of 11 serum lots tested. % bias ranged from -42 to 32%		No[a]
Lipemic effect	5 serum lots tested. All lots within 20 %bias		Yes
Dilution linearity & hook effect	Linear within 10 to 200 fold dilutions. Tested at 60 ng/mL No hook effect		Yes
Bench-top stability	Stable at room temperature for 25 hrs in serum		Yes
Freeze-Thaw stability	Up to 5 cycles		Yes
Long-term storage	At nominal -80°C for 71 and 72 days (PF-06881893 and US-licensed Neupogen respectively)		Yes
Method performance in study ZIN-FIL- 1502			
Assay passing rate	• 29 out of 30 runs (including incurred sample reanalysis (ISR)) met the method acceptance criteria.		Yes
Standard curve performance	• Cumulative bias range: -5.3 to 1.0% • Cumulative precision: ≤ 6%CV		Yes
QC performance	• Cumulative bias range: -0.5 to 4.3% • Cumulative precision: ≤ 7%CV • TE: ≤ 11%		Yes
Method reproducibility	• Incurred sample reanalysis was performed in 10% of study samples and 100% of samples met the pre-specified criteria		Yes
Study sample stability	Analyzed within 68 days from collection (within established stability)		

[a]Concentration data from impacted samples were removed for PK analysis

4.1.2 Pharmacodynamics

4.1.2.1 What bioanalytical methods were used to assess the pharmacodynamic (PD) biomarker(s) and/or the PD effect(s) of the biologic?

For study ZIN-FIL-1502, ANC in whole blood samples was determined with flow cytometry method using an automated Beckman-Coulter LH750 Hematology analyzer in clinical laboratories at SeaView Labs, LLC (Miami, FL). Coulter LH750 Hematology analyzer is a validated leucocyte differential counter capable of measuring white blood cell (WBC) accurately. ANC is calculated as the total leukocyte count (WBC) x (percent neutrophils)/100. Method performance and study reports were reviewed.

Review summary	Method validation was adequate to support the study ZIN-FIL-1502		
	Method performance summary		
Functional sensitivity	0.01 ($x10^3$ cell/µL) (for WBC)	Matrix	K_2EDTA whole blood
Linear range	0.01 to 367.4 ($x10^3$ cell/µL) for WBC		
Percent bias	±4% for WBC		
Intra-batch CV%	≤ 2% for WBC		
Inter-batch CV%	≤ 2% for WBC		
Inter-lab %CV	≤ 23% for WBC		
	Performance in study ZIN-FIL- 1502		
Inter-batch CV%	≤ 3% in 3 QCs		
Sample analysis	Whole blood samples were collected and analyzed on same day (within 24 hrs) based on the pre-established sample stability duration at ambient room temperature. ANC was determined using 3 levels QCs (Normal, Abnormal I and II).		

For study ZIN-FIL-1501, mobilized CD34+ stem cell in peripheral whole blood was determined with a 3-color lyse/no-wash flow cytometry method using Becton Dickinson FACSCantoTM II at (b) (4) . The protocol utilized the maximum information available from the intensity of CD34 and CD45 staining combined with a cell viability marker (7-AAD). Anti-human CD34 antibodies used in this method detects class II epitopes. Whole blood samples were collected at SeaView (Miami, FL) and shipped to (b) (4) at ambient room temperature (18°-25°C). Method performance and study reports were reviewed.

Review summary	Method validation was adequate to support the study ZIN-FIL-1501		
	Method performance summary		
Functional sensitivity	0.5 cells/µL	Matrix	Whole blood
Linear range	1.143 to 292.110 cells/µL		
Intra-batch CV%	≤ 19% (for absolute count)		
Inter-batch CV%	≤ 10% (for absolute count)		
	Performance in study ZIN-FIL- 1501		
Inter-assay CV%	≤ 26% (in 3 runs with N=3 from 2 spiked with Stem-Trol)		
Sample analysis	Samples with acceptable condition were analyzed within 48 hrs using 2 levels QC.		

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/s/

THEINGI M THWAY
06/01/2018

OLANREWAJU OKUSANYA
06/08/2018

NAM ATIQUR RAHMAN
06/12/2018
I agree with the recommendation.