

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

761111Orig1s000

PRODUCT QUALITY REVIEW(S)

First Approval for Indication/First Biosimilar/Expedited or Breakthrough Review: No

Recommendation: Approval

BLA Number: 761111
Review Number: 1
Review Date: April 16, 2020

Drug Name/Dosage Form	Nyvepria- pegfilgrastim-apgf (PF-06881894); pre-filled syringe for single dose injection
Strength/Potency	6 mg/0.6 mL (10 mg/1 mL)
Route of Administration	Subcutaneous injection
Rx/OTC dispensed	RX
Indication	All indications for US-licensed Neulasta
Applicant/Sponsor	Hospira Inc., a Pfizer Company
US agent, if applicable	n/a

Product Overview: Nyvepria (PF-06881894; pegfilgrastim-apgf) is a covalent conjugate of recombinant methionyl human granulocyte-colony stimulating factor (G-CSF) (filgrastim) and a 20 kDa monomethoxypolyethylene glycol propionaldehyde (mPEG-p). PF-06881894 is a proposed biosimilar to the US-licensed Neulasta (pegfilgrastim). Endogenous G-CSF is the primary regulating factor for neutrophils. G-CSF binds to G-CSF receptors, which stimulates proliferation, differentiation, commitment, and target cell functional activation. Endogenous G-CSF is known to stimulate proliferation of mitotic cells, to reduce the maturation time of non-mitotic cells in the bone marrow, and to prolong the life span and enhance the function of mature neutrophils.

Quality Review Team:

Discipline	Reviewer	Branch/Division
Drug Substance	Xu (Michael) DI	OBP/Division of Biotechnology Review and Research (DBRR) III
Drug Product	Xu (Michael) Di	OBP/DBRRIII
Immunogenicity	Xu (Michael) Di; Susan Kirshner	OBP/DBRRIII
Labeling	Scott Dallas	OBP
Microbiology/Facility	Lindsey Brown; Scott Nichols; Ziyang Su	OPMA/DBM Branch 1
OPMA Team Lead	Thuy Thanh Nguyen	OPMA/DBM Branch 1
Device/Prefilled Syringe	Gang Peng; Rumi Young (TL)	CDRH
CMC Statistics	Xiaoyu Cai, Meiyu Shen (TL)	OB/DBVI
Application Team Lead	Ram Sihag	OBP/DBRRIII
RBPM	Grafton Adams	OPRO

Reviewers of Biosimilar Multi-Disciplinary Evaluation and Review

Regulatory Project Manager	Michael Gwathmey
Nonclinical Pharmacology/Toxicology Reviewer(s)	Emily Place
Nonclinical Pharmacology/Toxicology Team Leader(s)	Brenda Gehrke
Clinical Pharmacology Reviewer(s)	Xiling Jiang
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Clinical Reviewer(s)	Hyon-Zu Lee
Clinical Team Leader(s)	Kathy Robie Suh
Clinical Statistics Reviewer(s)	Wenjuan Gu
Clinical Statistics Team Leader(s)	Yeh-Fong Chen
Cross-Discipline Team Leader(s) (CDTL(s))	Kathy Robie Suh

Names:

- a. Proprietary Name: PF-06881894
- b. Trade Name: Nyvepria
- c. Non-Proprietary Name/USAN: pegfilgrastim-apgf
- d. CAS Name: 208265-92-3
- e. Common Name: pegfilgrastim
- f. INN Name: Not assigned
- g. Compendial Name: Not assigned
- h. OBP systematic name: CONJ: RPROT P09919 (CSF3_HUMAN); PEG [PF-06881894]

Submissions Assessed:

Submission(s) Assessed	Document Date
Response to Information Request #1	September 19, 2019
Response to Information Request #2	January 31, 2020, February 07 and March 27,2020
Response to Information Request #3	April 02, 2020

Quality Assessment Data Sheet

1. Legal Basis for Submission: 351(k)
2. Related/Supporting Documents:

A. DMFs:

DMF# and Type	DMF Holder	Item Referenced	Letter of Cross-Reference	Comments (status)
DMF (b) (4) Type II	(b) (4)	(b) (4)	Yes	Sufficient information provided in the BLA.
DMF (b) (4) Type III	(b) (4)	(b) (4)	Yes	Sufficient information provided in the BLA. Defer to CDRH reviewer.
DMF (b) (4) Type III	(b) (4)	(b) (4)	Yes	Sufficient information provided in the BLA.
DMF (b) (4) Type III	(b) (4)	(b) (4)	Yes	Sufficient information provided in the BLA.
DMF (b) (4) Type III	(b) (4)	(b) (4)	Yes	Sufficient information provided in the BLA.
DMF (b) (4) Type III	(b) (4)	(b) (4)	Yes	Sufficient information provided in the BLA.
510 (k) (b) (4)	(b) (4)	(b) (4)	Yes	Sufficient information provided in the BLA. Defer to CDRH reviewer.

Other documents: IND 124793

Consults:

- CDRH for pre-filled syringe assembly
- Office of Biostatistics VI for evaluation of equivalence testing in the comparative analytical assessment and release specifications.

4. Environmental Assessment of Claim of Categorical Exclusion: Hospira, Inc. claimed a categorical exclusion from the preparation of an environmental assessment for PF-06881894 in accordance with 21 CFR 25.31 (c). The claim is because PF-06881894 is considered “naturally occurring in the environment” and, when exposed to the environment, is not expected to significantly alter the concentration of the substance, its metabolites, or degradation products in the environment. No extraordinary circumstances exist.

The claim of a categorical exclusion is accepted.

Executive Summary:

I. Recommendations:

A. Recommendation and Conclusion on Approvability:

The Office of Pharmaceutical Quality, CDER, recommends approval of BLA 761111 for Nyvepria™ (PF-06881894, pegfilgrastim-apgf) manufactured by Hospira Inc., a Pfizer company. The data submitted in this application are adequate to support the conclusion that:

- The manufacture of PF-06881894 (Nyvepria™, pegfilgrastim-apgf) is well-controlled and leads to a product that is pure and potent
- PF-06881894 is highly similar to US-licensed Neulasta notwithstanding minor differences in clinically inactive components
- The strength, dosage form, and route of administration of PF-06881894, injection is the same as that of U.S. licensed-Neulasta
- The analytical component of the scientific bridge between PF-06881894, U.S.-licensed Neulasta, and E.U.-approved Neulasta was established to support the relevance of the data generated from studies using E.U. approved -Neulasta as a comparator product to the assessment of biosimilarity.

It is recommended that this product be approved for human use under conditions specified in the package insert.

C. Approval Action Letter Language:

- Manufacturing location:
 - Drug Substance:
Hospira Zagreb d.o.o., a Pfizer company
Prudnička cesta 60
10291 Prigorje Brdovečko Croatia
FEI: 3010630287

 - DS intermediate-
Hospira Adelaide Pty Ltd a, a Pfizer company
8 Dalglish Street,
Thebarton, Adelaide 5031
Australia
FEI: 3003961774

 - Drug Product:
Hospira Zagreb d.o.o a, a Pfizer Company
Prudnička cesta 60
10291 Prigorje Brdovečko

Zagreb, Croatia
FEI: 3010630287

- Fill size and dosage form

6 mg/0.6 mL solution for injection in a single-dose prefilled syringe

- Dating period:

- Drug Product: 36 months: 5 °C
- Drug Substance: (b) (4) months: (b) (4) °C
- Intermediate Substance: (b) (4) months: (b) (4) °C

- Stability Option:

- We have approved the stability protocol(s) in your license application for the purpose of extending the expiration dating of your DS intermediate (FI), drug substance, and drug product under 21 CFR 601.12.

- Exempt from lot release:

- Yes
- Rationale, if exempted: Nyvepria is exempted from lot release per FR 95-29960.

D. Summary Conclusion:

Nyvepria™ (PF-06881894; pegfilgrastim-apgf) is a proposed biosimilar to U.S.-licensed Neulasta and is proposed for use in all indications approved for U.S.-Neulasta.

The PF-06881894 manufacturing process and control strategy are sufficient and lead to a drug product of acceptable quality to ensure drug safety and effectiveness for patients. The data provided in the BLA support a determination that PF-06881894 is highly similar to U.S.-licensed Neulasta and that analytical component of scientific bridge was established. PF-06881894

Nyvepria™ is the same strength, dosage form, and route of administration as U.S.-approved Neulasta.

The technical assessments including the assessment of immunogenicity assays are located as separate documents in the Panorama informatics platform (see list at the end of this memo).

E. Recommendation on Phase 4 (Post-Marketing) Commitments, Requirements, Agreements, and/or Risk Management Steps, if approvable:

PMC 3825-2: To perform a simulated shipping validation study representing real world shipping conditions, such as temperature, mode of transport, shipping duration, and packaging configuration using PF-06881894 drug product representative of commercial drug

product to confirm that product quality is maintained. The simulated shipping validation data will be submitted in accordance with 21 CFR 601.12.

Final Report Submission: 08/31/2020

PMC 3825-3: To update the control strategy to include lot release testing for the safety activation force (also referred to as safety device trigger force) of the final finished combination product in order to demonstrate that the product is not more than (NMT) ^{(b) (4)} trigger force.

Final Report Submission: 11/30/2020

II. Comparative Analytical Assessment and Evaluation of the Analytical Component of the Scientific Bridge

I. Analytical Assessment Overview and Conclusions

The applicant provided results of the comparative analytical assessment to support:

- a demonstration that PF-06881894 is highly similar to U.S.-licensed Neulasta;
- the analytical component of the scientific bridge between PF-06881894, U.S.-licensed Neulasta, and E.U.-approved Neulasta that justifies the relevance of clinical data submitted in the application generated from studies using E.U.-approved Neulasta as a comparator product to the assessment of biosimilarity.

Critical quality attribute (CQA) assessment was used to identify appropriate analytical methods and statistical approaches for the comparative analytical studies. The applicant used Quality Risk Management principles, literature, and product knowledge to assess quality attribute criticality. Test results for the high risk CQA potency, measured using an in vitro cell-based assay, were evaluated using an equivalence testing approach. Moderate risk attributes and additional high risk attributes tested using quantitative assays were evaluated using a quality range approach. Low risk attributes and attributes assessed qualitatively were evaluated using visual display comparisons. The data analysis strategy used by the applicant is consistent with the recommendations FDA provided during product development, as well as those recommended in the draft guidance for industry, *Development of Therapeutic Protein Biosimilars: Comparative Analytical Assessment and Other Quality-Related Considerations* (May 2019). This guidance, when finalized, will represent the current thinking of the Agency on this topic.

The ten PF-06881894 DP lots used in the comparative analytical assessment are independent DP lots that were manufactured using the proposed commercial scale process. In addition, they were manufactured from different drug substance (DS) lots, with each DS lot manufactured using an independent filgrastim intermediate lot. The PF-06881894 DP lots used ranged in age from 0 – 51 months at time of testing, which allowed for meaningful comparisons to support the comparative analytical assessment. Lots of PF-06881894 used in the comparative analytical assessment were appropriately selected.

The 17 U.S.-licensed Neulasta lots and 17 E.U.-approved Neulasta lots used in the comparative analytical studies were purchased at regular intervals over a 5-year period from the regulated market without preselected purchasing criteria. At time of testing the lots ranged in age from 30 months prior to expiration to expiration. The age of the lots at time of testing was adequate to capture potential reference product differences over time and allowed for meaningful comparisons to support the comparative analytical assessment. The lots used in the comparative analytical assessment included lots used in the comparative nonclinical and clinical studies. The lots of U.S.-licensed Neulasta used in the comparative analytical assessment were appropriately selected.

The comparative analytical assessment included physicochemical and functional characterization studies of biological activity, primary and higher order structure, product-related substances and impurities, the stability profile of the product, and protein concentration. The comparative forced degradation studies were performed using an appropriate variety of forced degradation conditions, including peroxide, heat, light, and high pH. Stability studies were conducted to compare the rates and pathways of degradation for PF-06881894 and US-licensed Neulasta under intended, stressed, and accelerated storage conditions. The quality attributes and conditions tested were supported by the risk assessment and development studies and are appropriate.

The comparative analytical studies were performed using appropriate orthogonal analytical methods for each quality attribute. The methods were adequately validated or qualified to support that the methods were scientifically sound and suitable for their intended use.

Three functional activity assays that assess the mechanism of action of U.S.-licensed Neulasta were used. In vitro potency was determined using a cell-based assay that measures the induction of receptor-activated proliferation of hematopoietic cells. The applicant chose to evaluate results from the in-vitro cell-based assay with equivalence testing because of the high-risk level for this CQA. Other potency tests were evaluated using quality ranges that adequately reflected U.S.-licensed Neulasta manufacturing variability and assay variability. The binding of U.S.-licensed Neulasta to the G-CSF receptor was measured using the Competitive Receptor Binding Assay (CRBA), which measures the binding of biotin-labeled pegfilgrastim to an immobilized G-CSF receptor. The comparison of G-CSF receptor affinity (relative K_D and K_D), and on and off rates (K_{on} and K_{off}) support a determination that the higher order structure required for binding to the receptor and response binding kinetics are similar between PF-06881894 and U.S.-licensed Neulasta. A Surface Plasmon Resonance (SPR) Assay was used to evaluate the changes in receptor binding affinity parameters and binding rates as they are indicative of structural changes that may impact receptor binding. The three functional assays support the proposed mechanism of action of G-CSF.

In conclusion, the applicant used a comprehensive array of analytical and statistical methods that were suitable to evaluate critical quality attributes of PF-06881894, U.S.-licensed Neulasta and E.U.-approved Neulasta. The pair-wise analytical comparisons of PF-06881894, U.S.-licensed Neulasta and E.U.-approved Neulasta support a demonstration that PF-06881894 is highly similar to U.S.-licensed Neulasta notwithstanding minor differences in clinically inactive components. In addition, the applicant provided adequate data and information to establish the analytical component of the scientific bridge to support the relevance of clinical data using E.U.-approved Neulasta as a comparator product to the assessment of biosimilarity.

II. Results of the Comparative Analytical Assessment

The data submitted in this application to support a demonstration that PF-06881894 is highly similar to U.S.-licensed Neulasta are summarized in Table 1 below.

Table 1 Quality Attributes Analyzed to Support a Demonstration of Highly Similar

Parameter	Quality Attribute	Test Method	Supports a Demonstration of Highly Similar	
Biological Activity	Cell proliferation	In Vitro Cell-Based Bioassay (% Relative Potency)	Yes	
	Receptor binding	Competitive Receptor Binding Assay (% Relative potency)	Yes	
		Receptor Binding Affinity and Kinetics (Surface Plasmon Resonance Assay)	Binding Affinity: Relative K_D (%)	Yes
			Binding Affinity: $K_D \times 10^{-11}$ (M)	Yes
			Binding Kinetics: $K_{on} \times 10^6$ ($M^{-1}S^{-1}$)	Yes
Binding Kinetics: $K_{off} \times 10^{-5}$ (S^{-1})	Yes			
Primary Structure	Amino Acid Sequence	Glu-C Peptide Mapping (RP-UPLC-MS)	Yes	
	Pegylation Site and Linker Composition	Glu-C Peptide Mapping of Pegylated Peptide (RP-UPLC-MS)	Yes	
	Molecular Weight (including dispersity)	Intact Mass (RP-UPLC-MS)	Average Mass (483 EO units, Da)	Yes
			Mass-Averaged MW (kDa)	Yes*
			Molecular Weight Dispersity	Yes
	Free Thiol	Ellman's Assay (mol Thiol/mol pegfilgrastim)	Yes	
Isoelectric Point (pI)	cIEF	Yes		
Higher Order Structure	Secondary Structure	Far-UV circular dichroism (CD)	Alpha-helix (%)	Yes
			Beta-structure (%)	Yes
			Random Coil (%)	Yes

	Tertiary Structure (disulfide bond)	Disulfide Mapping	Free Thiol (peptide 16-21)	Yes
			Disulfide Cys37-Cys43 (peptide 33-47)	Yes
			Disulfide Cys65-Cys75 (peptide 51-76)	Yes
	Tertiary Structure (structure Dynamics)	Hydrogen- Deuterium Exchange (HDX): Deuterium uptake curves and Heat Maps		Yes
	Tertiary Structure (Sedimentation Coefficient)	Sedimentation Velocity-Analytical Ultracentrifugation (SV-AUC)	Sedimentation Coefficient (S)	Yes
			Monomer (%)	Yes
HMWS (%)			Yes*	
Tertiary Structure (protein structure)	Nuclear Magnetic Resonance Spectroscopy (NMR)		Yes	
Tertiary Structure (Melting Temperature (Tm))	Differential Scanning Calorimetry (°C)		Yes	
Product Related Substances and Impurities	Total Related Proteins	RP-HPLC (%)		Yes*
	Total Charge Variants (acid variants)	IC-HPLC (%)		Yes*
	Oxidation at Met127	RP-HPLC (%)		Yes*
	Total Size Variants: Dimer; Other HMWS; Des-pegylated Species	SEC	Dimer (%)	Yes*
			Other HMWs (%)	Yes
			Des-pegylated Species (%)	Yes
			Total size variants (%)	Yes
	Size Variants	Non-reducing SDS-PAGE (impurity bands are less than 1% standard solution)		Yes
	Residual PEG	RP-HPLC-ELSD (%)		Yes
	Oxidation	Glu-C peptide mapping	M122 (%)	Yes
			M127 (%)	Yes
M138 (%)			Yes	
Trp59 (%)			Yes	
Deamidation	RP-HPLC (Gln108)		Yes	
	IC-HPLC: LOQ=	RRT 0.85 (%):	Yes*	

		0.4%	Gln68 deamidation	
			RRT 0.89-0.90 (%): Gln71 and Gln174 deamidation	Yes*
		Glu-C Peptide Mapping: LOQ 0.5%	Gln21 (%)	Yes
			Gln91 (%)	Yes*
			Gln120 (%)	Yes
		Gln135 (%)	Yes	
	Reduced Species at RRT 1.05	RP-HPLC: (%) LOQ=0.3%		Yes
	Des-Pegylated Species at 1.04 (N- terminal des- pegylated, des-Met1 Species)	RP-HPLC: (%) LOQ=0.3%		Yes*
		SEC-HPLC (%)		Yes
	N-terminal Des- pegylated Species	Glu-C Peptide Mapping (%)		Yes
Drug Product- Related Attributes	Protein Concentration	UV-Visible Spectrometry (mg/mL)		Yes
	Deliverable Content	Protein Concentration x Deliverable Volume (mg)		Yes
	Deliverable Volume	USP <697> Ph. Eur. <2.9.17> (mL)		Yes
	Subvisible Particles	Micro Flow Imaging (MFI)	≥ 2 μm	Yes*
			≥ 5 μm	Yes
			≥ 10 μm	Yes
			≥ 25 μm	Yes
	pH	Ph. Eur. <2.2.3> and USP <791>		Yes*
	Osmolality	Ph. Eur. <2.2.35> and USP <785> (mOsmol/kg)		Yes
	Polysorbate 20	RP-HPLC (% w/v)		Yes
Appearance, Color, and Clarity	Ph. Eur. <2.2.2> and <2.2.1>		Yes	
Visible Particles	USP <790> Ph. Eur. <2.9.20>		Yes	

1. Yes = met acceptance criteria, Yes*= differences were noted but do not preclude a demonstration of highly similar and is further discussed below, No = did not meet acceptance criteria

The results of the comparative analytical assessment that are summarized above in Table 1, demonstrate that PF-06881894 and U.S.-licensed Neulasta are highly similar. Minor differences noted in Table 1 do not preclude a demonstration of highly similar and are discussed further in Section IV of this memo.

III. Comparative Analytical Studies to Support the Use of a Non-U.S.-Licensed Comparator Product

To support the relevance of clinical data generated using E.U.-approved Neulasta as a comparator product, the applicant performed a three-way comparative analytical assessment of PF-06881894, U.S.-licensed Neulasta, and E.U.-approved Neulasta using all the same tests listed in Table 1. The same minor differences for attributes noted in Table 1 were also identified for each of the pairwise comparisons described in this Section. In addition, there were minor differences in ranges observed between U.S.-Neulasta and E.U.-Neulasta and do not preclude a determination that the analytical component of the scientific bridge was established. These differences are discussed further in Section IV of this memo.

Based on our review of the data, we conclude that the applicant established the analytical portion of the scientific bridge between PF-06881894, US-licensed Neulasta, and E.U.-approved Neulasta.

IV. Assessment of Comparative Analytical Study Results

The observed levels of total related proteins, total size variants by SEC, total charge variants, deamidated glutamine, oxidized methionine, and depegylated species in PF-06881894 were lower than in both U.S.-licensed Neulasta and E.U.-approved Neulasta. The differences in these impurities between U.S.-licensed Neulasta and E.U.-approved Neulasta were minor and the highest content in each impurity was the same for both products. The lower levels of these impurities in the proposed biosimilar product do not preclude a demonstration of highly similar or the determination that the analytical portion of the scientific bridge was established as potency was not impacted and all of the impurities were present at less than 5%, levels the Agency considers to be low. MFI results showed broad ranges of sub-visible particles (SVP) ≥ 2 micron in PF-06881894, U.S.-licensed Neulasta, and E.U.-approved Neulasta, with PF-06991894 levels being below or at the lower end of the SVP ≥ 2 micron range of E.U.-approved Neulasta and below the range for U.S.-licensed Neulasta. E.U.-approved Neulasta had a broader range of SVP ≥ 2 micron than U.S.-licensed Neulasta, with the U.S.-licensed Neulasta range wholly contained within the E.U.-approved Neulasta range.

Analytical data showed that PF-06881894 exhibited a slightly higher MW than U.S.-licensed Neulasta and E.U.-approved Neulasta because PEG raw material with fewer ethylene oxide (EO) units was used to manufacture U.S.-licensed Neulasta and E.U.-approved Neulasta. This observed variability of EO units was determined to have no meaningful impact on potency, as demonstrated by the comparative analytical studies. In addition, data were provided showing there is variability in average mPEG MW used to manufacture U.S.-licensed Neulasta over time, but variability in average mPEG MW was not observed in PF-06881894.

Based on the above, there are no residual uncertainties regarding the comparative analytical assessment that would preclude a demonstration that PF-06881894 is highly similar to U.S.-licensed Neulasta or the determination that the analytical component of the scientific bridge was established.

A. Same Strength(s)

PF-06881894 has the same dosage form and route of administration as U.S.-licensed Neulasta. Hospira is seeking approval of 6 mg/0.6 mL PF-06881894 in a prefilled syringe. U.S.-licensed Neulasta is available at this strength 6.0 mg/0.6 mL in a prefilled syringe. Hospira is seeking approval of PF-06881894 for the same strength as U.S.-licensed Neulasta. Comparative protein concentration (mg/mL) was assessed as part of the comparative analytical assessment. The ^{(b) (4)} data were assessed as part of manufacturing process controls. The proposed presentation of PF-06881894 has the same total content of drug substance in units of mass in a container and the same concentration of drug substance in units of mass per unit volume as U.S.-licensed Neulasta (6 mg/0.6 mL). The strength of PF-06881894 prefilled syringe is the same as that of U.S.-licensed Neulasta and meets the statutory “same strength” requirement under section 351 (k)(2)(A)(i)(IV) of the PHS Act.

V. Summary of Quality Assessments:

A. CQA Identification, Risk and Lifecycle Knowledge Management

Table 1 below is a summary of critical quality attributes and the associated control strategies for attributes that are relevant to both Drug Substance and Drug Product. For additional information, see the primary reviews, including the Drug Substance Quality Review and Drug Product Quality Review by OBP/DBRRIII and the Drug Substance Microbiology Review and the Drug Product Microbiology Review by OPMA.

Table 1: Active Pharmaceutical Ingredient CQA Identification, Risk and Lifecycle Knowledge Management

CQA (type)	Risk	Origin	Control Strategy (b) (4)	Other
Potency (In vitro cell-based bioassay)	Efficacy	Intrinsic to molecule		
Receptor Binding Affinity and Kinetics (Surface Plasmon Resonance, SPR)	Efficacy	Intrinsic to molecule		It was identified as a CQA and should be on the list of tests to do after certain major manufacturing changes
Competitive Receptor Binding (ELISA)	Efficacy	Intrinsic to molecule		
(b) (4)	Safety	Process related impurity		
	Safety	Process related impurity		
High-molecular weight species (product-related impurity)	Efficacy, pharmacokinetics, and immunogenicity	Manufacturing process & storage conditions. Can form due to agitation, temperature or light exposure.		

			(b) (4)	
Acidic and basic charge variants (product-related substances and impurities)	Efficacy, pharmacokinetics, and immunogenicity	Fermentation, purification, deamidation during storage	(b) (4)	
(b) (4)	Efficacy and safety	Manufacturing process and storage conditions		
	Potency and efficacy	Manufacturing process and storage conditions		
Identity	Safety, efficacy	Intrinsic to molecule		

B. Drug Substance Pegfilgrastim-apgf Quality Summary

CQA Identification, Risk, and Lifecycle Knowledge Management

Table 2: Drug Substance CQA Process Risk Identification and Lifecycle Knowledge Management. (see example in Attachment 2)

CQA (type)	Risk	Origin	Control Strategy	Other
Appearance (color and clarity)	Safety	Manufacturing process	(b) (4)	
Host Cell Proteins (Process-related impurity)	Safety and immunogenicity	Derived from host cell line	(b) (4)	(b) (4)
Host Cell DNA (process-related impurity)	Safety	Derived from host cell line		

(b) (4)	Safety, stability	Manufacturing process	(b) (4)
Endotoxin	Safety, Purity	Manufacturing process	
Bioburden	Safety, Purity and Efficacy due to degradation or modification of the product by microbial contamination	Raw materials and manufacturing process	

- Description: PF-06881894 is a covalent conjugate of recombinant methionyl human G-CSF (filgrastim) and monomethoxypolyethylene glycol. PF-06881894 contains 175 amino acids and shows a molecular weight of 37.0-42.5 kDa. G-CSF produced in E. coli by recombinant DNA technology, is not glycosylated, and contains an N-terminal methionine.
- Mechanism of Action (MoA): Endogenous G-CSF is the primary regulating factor for neutrophils. G-CSF binds to G-CSF receptors which stimulates proliferation, differentiation, lineage commitment, and target cell functional activation. Endogenous G-CSF is known to stimulate proliferation of mitotic cells, to reduce the maturation time of non-mitotic cells in the bone marrow, and to prolong the life span and enhance the function of mature neutrophils. Endogenous G-CSF is produced by different cell types including macrophages, monocytes, fibroblasts, stromal cells in bone marrow, and endothelial cells. Endogenous G-CSF is triggered by inflammatory signals as well as by lipopolysaccharide released from bacteria. Pegfilgrastim has the same MOA as endogenous G-CSF and filgrastim.
- Potency Assay: An in vitro cell-based assay that measures the induction of receptor-activated proliferation of hematopoietic cells is used to measure the potency of PF-06881894 DS and DP for release and stability testing, and comparative analytical assessments. PF-06881894 induces a dose-dependent proliferation of M-NFS-60 cells as a result of G-CSF receptor binding and subsequent signal transduction. The M-NFS-60 cells are seeded in a 96 well microtiter plate. PF-06881894 reference standard, assay control and samples are diluted to the same series of protein concentrations and incubated with the cells for 29-31 hours at 37°C. A fluorescent dye, CellTiter Blue, is then added to the cells and incubated for 19-23 hours at 37°C. Fluorescence intensity of the lysed cells is quantitated using a microplate reader at a wavelength of 560-590 nm.

Reference Materials: Two in-house reference materials are used in the testing of drug substance intermediate (DSI), DS and DP: filgrastim and pegfilgrastim reference materials. An in-house two-tier reference material system for filgrastim and pegfilgrastim

was implemented in March 2019 and February 2019, respectively. (b) (4)

Acceptance criteria for qualification and requalification of the reference materials are the same as or tighter than those for filgrastim or pegfilgrastim release and stability testing to ensure that the reference materials were suitable for their intended use.

- Critical starting materials or intermediates: (b) (4)

(b) (4)

No material of animal origin is used in the manufactured of DS intermediate, DS, and DP.

- Manufacturing process summary: (b) (4)

(b) (4)

- Container closure: The PF-06881894 DSI and DS container closure system is (b) (4)

(b) (4)

- Dating period and storage conditions: The dating period of DS intermediate at (b) (4) is (b) (4) months. (b) (4)

The date period of DS at (b) (4) months. (b) (4)

C. Drug Product Pegfilgrastim-apgf Quality Summary:

Table 3 provides a summary of the identification, risk, and lifecycle knowledge management for drug product COAs that derive from the drug product manufacturing process and general drug product attributes.

Table 3: Drug Product CQA Identification, Risk, and Lifecycle Management

CQA (Type)	Risk	Origin	Control Strategy	Other
Sterility	Safety, product stability	Manufacturing process during manufacture or container closure integrity failure	(b) (4)	n/a
Endotoxin (contaminant)	Safety	Manufacturing process, contamination		n/a
Appearance (color and clarity)	General CQA	Formulation		n/a
Protein Concentration (DP composition)	General CQA	Formulation		
Osmolarity (DP composition)	Product stability, patient discomfort	Formulation		n/a
pH (DP composition)	Stability	Formulation		n/a
Polysorbate 80 (excipient)	Safety, stability	Manufacturing process, Formulation		
Visible Particles (Impurity)	Immunogenicity, patient safety	Stability, accidental throughout process,		n/a
Subvisible particles (general)	Safety	Manufacturing process, product degradation		
Container closure (Process related)	Negative impact of leachables on product quality. Introduction of container related impurities (b) (4)	Filling/storage		n/a
Volume	Efficacy	Manufacturing process		n/a

			(b) (4)
Syringe function (Break loose force; maximum extrusion force)	Safety	Manufacturing process	
Container closure Integrity	Safety (maintenance of sterility during shelf life)	Container closure breaches during storage. May be impacted by storage conditions.	

- **Potency and Strength:**

The in vitro cell-based assay is used to measure the potency of PF-06881894 DS and DP for release and stability testing, and analytical similarity assessment. The potency assay is discussed above in DS section. The strength of the Drug Product is 6 mg/ 0.6mL.

- **Summary of Product Design:**

PF-06881894 drug product (DP) is a sterile, clear, preservative-free, free from visible particle, colorless solution, developed as a proposed biosimilar to the U.S.-licensed Neulasta. The PF-06881894 DP container closure system is a single-dose prefilled syringe (PFS) consisting of a 1 mL (b) (4) glass syringe barrel with 27-gauge ½ inch needle, and a rigid need shield, sealed with gray (b) (4) elastomeric stopper. Each prefilled syringe contains 6 mg of pegfilgrastim, 30.0 mg sorbitol, (b) (4) mg sodium, 0.35 mg acetate, and (b) (4) mg polysorbate 20 with a nominal fill volume of 0.6 mL for subcutaneous injection.

- **List of Excipients:** Excipients include sorbitol, (b) (4), polysorbate 20, and water for injection.

- **Reference Materials:** Reference material used for DP is the same as for DS and is described above in the DS section.

- **Manufacturing process summary:** The DP manufacturing process consists of (b) (4)

(b) (4)

The DP commercial manufacturing process is adequately controlled.

- **Container closure:** The PF-06881894 DP container closure system is a single-dose prefilled syringe (PFS) consisting of a 1 mL Long (b) (4) glass syringe barrel with 27-gauge ½ inch needle, and a rigid need shield, sealed with gray (b) (4) elastomeric stopper.

- Dating period and storage conditions: The date period of DP at 5°C is 36 months. DP may be stored at room temperature for up to 15 days. Protect DP from light.

- List of co-package components, if applicable: n/a

D. Novel Approaches/Precedents: No.

E. Any Special Product Quality Labeling Recommendations:

Protect from light. Drug product can be kept at room temperature for 15 days after which it should be discarded.

F. Establishment Information:

Overall Recommendation:					
Drug Substance Intermediate					
Function	Site Information	DUNS/FEI Number	Preliminary Assessment	Inspectional Observations	Final Recommendation
1. Preparation and storage of cell banks 2. Manufacture, release and stability testing of PF-06881894 IB, and FI.	Hospira Adelaide Pty Ltd a, a Pfizer company 8 Dalglish Street Thebarton Adelaide 5031 South Australia Australia	FEI: 3003961774 DUNS: 756118717	None	None	Acceptable
DRUG SUBSTANCE					
Function	Site Information	DUNS/FEI Number	Preliminary Assessment	Inspectional Observations	Final Recommendation
1. Manufacture, release and stability of DS 2. Release and stability testing of DSI (only for host cell DNA and potency)	Hospira Zagreb d.o.o. a, a Pfizer company Prudnička cesta 60 10291 Prigorje Brdovečko Croatia	FEI: 3010630287 DUNS: 500625201	None	None	Acceptable
1. Preparation, storage, testing and release of cell banks	Pfizer St. Louis 875 Chesterfield Parkway West Chesterfield, MO 63017 USA	FEI: 1940118 DUNS: 004954111	None	None	Acceptable
1. Storage of cell banks	Pfizer Ireland Pharmaceuticals Grange Castle Business Park Clondalkin Dublin 22 Ireland	FEI: 3004145594 DUNS: 985586408	None	None	Acceptable
DRUG PRODUCT					

Function	Site Information	DUNS/FEI Number	Preliminary Assessment	Inspectional Observations	Final Recommendation
1. Manufacture 2. In-process control testing 3. Release testing 4. Stability testing 5. Stability storage 6. Primary packaging 7. Secondary packaging and labeling 8. Quality release of final combination product 9. Storage of final combination product 10. Receipt and inspection of incoming material used for manufacturing	Hospira Zagreb d.o.o, a Pfizer Company Prudnička cesta 60 10291 Prigorje Brdovečko Croatia	FEI: 3010630287 DUNS: 500625201	None	None	Acceptable

G. Facilities:

Hospira Zagreb, Croatia, a Division of Pfizer, is responsible for drug substance (DS) and drug product (DP) manufacturing (FEI 3010630287). A pre-approval inspection (PAI) for PF-06881894 DS and DP was conducted November 14-22, 2019. The Hospira Zagreb site is also responsible for packaging and labeling, and DS and DP release and stability testing. The inspection was system based and covered Quality, Facilities and Equipment, Production, Laboratory Control and Materials. A 2-item FDA Form 483 was issued at the conclusion of the inspection. (Refer to the FDA Form 483 for a list of the 483 observations). The inspection was classified as voluntary action indicated (VAI).

A PAI for PF-06881894 filgrastim intermediate manufacturing site at Thebarton, Australia (FEI 3003961774) was conducted on January 23-31, 2020. The site is responsible for manufacture of DS filgrastim intermediate (FI), and FI release and stability testing. The inspection was system based and covered Quality, Facilities and Equipment, Production, Laboratory Control and Materials. The inspection was classified as no action indicated (NAI). The details of the inspection are covered in the EIR.

H. Lifecycle Knowledge Management:

a. Drug Substance:

- i. Protocols approved: Stability protocol for the extension of shelf life, annual stability protocol, qualification of WCB, qualification of reference standards, annual stability testing for primary and working reference standards.

- ii. Outstanding review issues/residual risk: None
- iii. Future inspection points to consider: None

b. Drug Product

- i. Protocols approved: Stability protocol for the extension of shelf life, annual stability protocol.
- ii. Outstanding review issues/residual risk: There is one PMC on shipping validation.
- iii. Future inspection points to consider: None

VI. Review documents related to this Executive Summary:

- Drug substance quality review by Xu Di, PhD (OPQ/OBP/DBRR III)
- Drug product quality review by Xu Di, PhD (OPQ/OBP/DBRR III)
- Analytical similarity review by Xu Di, PhD (OPQ/OBP/DBRR III)
- Drug substance and Drug product microbiology review by Lindsey Brown, PhD (OPQ/OPMA/DBM)
- Facility review by Ziyang Su, PhD (OPQ/OPMA/DBM)
- Immunogenicity assay review by Xu Di, PhD and Susan Kirshner, PhD (OPQ/OBP/DBRR III)
- Labeling review by Scott Dallas, PhD (OPQ/OBP)
- Establishment inspection report for drug substance intermediate by Xu Di, PhD and Scott Nichols, PhD (OPQ/OPMA/DBM)
- Establishment inspection report for drug substance and drug product by Lindsey Brown, PhD (OPQ/OPMA/DBM)

This is a representation of an electronic record that was signed electronically. Following this are manifestations of any and all electronic signatures for this electronic record.

/s/

RAM K SIHAG
05/28/2020 12:09:31 PM

SUSAN L KIRSHNER
05/28/2020 12:44:17 PM



Center for Drug Evaluation and Research
Office of Pharmaceutical Quality
Office of Biotechnology Products

LABELS AND LABELING ASSESSMENT

Date of Assessment:	April 28, 2020
Assessor:	Scott Dallas, RPh, Labeling Assessor Office of Biotechnology Products (OBP)
Through:	Xu (Michael) Di, PhD, Product Quality Reviewer OBP/Division of Biotechnology Review and Research III
Application:	BLA 761111
Applicant:	Hospira, Inc., a Pfizer Company
Submission Dates:	June 10, 2019; and January 17, February 18, March 13 and April 27, 2020
Product:	NYVEPRIA (pegfilgrastim-apgf)
Dosage form:	injection
Strength and Container-Closure:	6 mg/ 0.6 mL Single-Dose Prefilled Syringe
Purpose of assessment:	The Applicant submitted a biologics license application seeking approval for a proposed biosimilar to and for the same indications as Neulasta (pegfilgrastim) Injection, 6 mg/ 0.6 mL.
Recommendation:	The prescribing information, patient labeling, instructions for use, container labels, and carton labeling are acceptable from an OBP labeling perspective.

Materials Considered for this Label and Labeling Assessment	
Materials Assessed	Appendix Section
Proposed Labels and Labeling	A
Evaluation Tables	B
Acceptable Labels and Labeling	C

DISCUSSION

We assessed the proposed labels and labeling for compliance with applicable requirements in the Code of Federal Regulations. Also, we assessed the proposed labels and labeling for consistency with recommended labeling practices. (see Appendix B)

CONCLUSION

The prescribing information, patient labeling, instructions for use, submitted on April 27, 2020 container labels and carton labeling submitted on March 13, 2020 were assessed and found to be acceptable (see Appendix C) from an OBP labeling perspective.

APPENDICES

Appendix A: Proposed Labeling

- Prescribing Information, Patient Information and Instructions for Use (submitted on June 10, 2019)
<\\cdsesub1\evsprod\bla761111\0001\m1\us\lab-1186-0-1-uspi-clean.doc>

- Syringe Container Label (submitted on June 10, 2019)



Container⁴ Label Evaluation

Proper Name (container label)	Acceptable
Regulations: 21 CFR 610.60(a)(1), 21 CFR 201.10(g)(2), 21 CFR 610.62(a), 21 CFR 610.62(b), 21 CFR 610.62(c), 21 CFR 610.60(c), 21 CFR 201.50(b), 21 CFR 201.10(a), 21 CFR 201.10(h)(2)(i)(1)(i)	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A

¹ Per 21 CFR 1.3(b) *Label* means any display of written, printed, or graphic matter on the immediate container of any article, or any such matter affixed to any consumer commodity or affixed to or appearing upon a package containing any consumer commodity.

² Per CFR 600.3(dd) *Label* means any written, printed, or graphic matter on the container or package or any such matter clearly visible through the immediate carton, receptacle, or wrapper.

³ Per 21 CFR 1.3(a) *Labeling* includes all written, printed, or graphic matter accompanying an article at any time while such article is in interstate commerce or held for sale after shipment or delivery in interstate commerce.

⁴ Per 21 CFR 600.3(bb) *Container* (referred to also as "final container") is the immediate unit, bottle, vial, ampule, tube, or other receptacle containing the product as distributed for sale, barter, or exchange.

<i>Recommended labeling practices (placement of dosage form below the proper name)</i>	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
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Comment/Recommendation:
The dosage form is to the right of the proper name, but this is acceptable because the label is small.
March 13, 2020: The applicant submitted revised labels to include the suffix apgf.
FDA Response: The applicant's revision is acceptable.

Manufacturer name, address, and license number (container label)	Acceptable
Regulations: 21 CFR 610.60(a)(2), 21 CFR 201.1(a), 21 CFR 610.60(c), 21 CFR 201.10(h)(2)(i)(1)(iv), 21 CFR 201.100(e)	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
<i>Recommended labeling practices (using the qualifying phrase "Manufactured by:")</i> (see comment below)	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
<i>Recommended labeling practices (U.S license number for container bearing a partial label⁵)</i>	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A

Comment/Recommendation:
The syringe label contains the abbreviation "Mfd by" to represent the phrase "Manufactured by". Inclusion on the abbreviation is acceptable.

Lot number or other lot identification (container label)	Acceptable
Regulations: 21 CFR 610.60(a)(3), 21 CFR 610.60(c), 21 CFR 201.18, 21 CFR 201.100(b)(6), 21 CFR 201.10(h)(2)(i)(1)(iii)	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A

Comment/Recommendation:

Expiration date (container label)	Acceptable
Regulations: 21 CFR 610.60(a)(4), 21 CFR 201.17	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
<i>Recommended labeling practices references: USP General Chapters <7> Labeling, Draft Guidance Safety Considerations for Container Labels and</i>	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No

⁵ Per 21 CFR 610.60 (c) *Partial label*. If the container is capable of bearing only a partial label, the container shall show as a minimum the name (expressed either as the proper or common name), the lot number or other lot identification and the name of the manufacturer; in addition, for multiple dose containers, the recommended individual dose. Containers bearing partial labels shall be placed in a package which bears all the items required for a package label.

<i>Carton Labeling Design to Minimize Medication Errors, April 2013 lines 178-184, which, when finalized, will represent FDA's current thinking on topic</i>	<input type="checkbox"/> N/A
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Comment/Recommendation:

Beyond Use Date (Multiple-dose containers) (container label)	Acceptable
<i>Recommended labeling practices: USP General Chapters: <659> Packaging and Storage Requirements and <7> Labeling</i>	<input type="checkbox"/> Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> N/A

Comment/Recommendation:

Product Strength (container label)	Acceptable
Regulations: 21 CFR 201.10(d)(1), 21 CFR 201.100(b)(4)	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
<i>Recommended labeling practices (expression of strength for injectable drugs) references: Draft Guidance Safety Considerations for Container Labels and Carton Labeling Design to Minimize Medication Errors, April 2013 line 176, which, when finalized, will represent FDA's current thinking on topic USP General Chapters: <7> Labeling</i>	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A

Comment/Recommendation:

Multiple-dose containers (container label)	Acceptable
Regulations: 21 CFR 610.60(a)(5), 21 CFR 201.55 <i>(recommended individual dose)</i>	<input type="checkbox"/> Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> N/A

Comment/Recommendation:

Statement: "Rx only" (container label)	Acceptable
Regulations: 21 CFR 610.60(a)(6), 21 CFR 201.100(b)(1)	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
<i>Recommended labeling practices (prominence of Rx Only statement) reference: Draft Guidance Safety Considerations for Container Labels and Carton Labeling Design to Minimize Medication Errors, April 2013 line 147, which, when finalized, will represent FDA's current thinking on topic</i>	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A

Comment/Recommendation:

Medication Guide (container label)	Acceptable
Regulations: 21 CFR 610.60(a)(7), 21 CFR 208.24(d)	<input type="checkbox"/> Yes

	<input checked="" type="checkbox"/> No <input checked="" type="checkbox"/> N/A
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Comment/Recommendation: This product does not require a Medication Guide.

No Package for container (container label)	Acceptable
Regulation: 21 CFR 610.60(b)	<input type="checkbox"/> Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> N/A

Comment/Recommendation:

No container label (container label)	Acceptable
Regulation: 21 CFR 610.60(d)	<input type="checkbox"/> Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> N/A

Comment/Recommendation:

Ferrule and cap overseal (for vials only)	Acceptable
<i>Recommended labeling practices references: United States Pharmacopeia (USP) General Chapters: <7> Labeling (Ferrules and Cap Overseals)</i>	<input type="checkbox"/> Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> N/A

Comment/Recommendation:

Visual inspection	Acceptable
Regulation: 21 CFR 610.60(e)	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A

Comment/Recommendation:
 To Applicant: Please confirm that sufficient area on the prefilled syringe remains uncovered for its full length or circumference to allow for visual inspection when the label is affixed to the prefilled syringe and indicate where the visual area of inspection is located per 21 CFR 610.60(e).

 January 17, 2020: The applicant confirmed that there is sufficient area on the prefilled syringe that remains uncovered to allow for visual inspection when the label is affixed to the single-dose prefilled syringe. The applicant also provided a figure identifying a portion of the label that is transparent, and thus permits visual inspection of the syringe contents.

 FDA Response: The applicant's response is acceptable.

Route of administration (container label)	Acceptable
Regulations: 21 CFR 201.5(f), 21 CFR 201.100(b)(3), 21 CFR 201.100(d)(1)	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
<i>Recommended labeling practices (route of administration statement to appear after the strength statement on the principal display panel)</i>	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A

Comment/Recommendation:

To Applicant: Consider relocating the route of administration statement, "Subcutaneous Use Only" to appear directly below the expression of strength.

January 17, 2020: The applicant relocated the route of administration statement directly below the expression of strength. In addition, the applicant has revised the route of administration statement to read "For Subcutaneous Use Only" to align with the unit carton and shelf carton labeling.

FDA Response: The applicant's revision is acceptable.

NDC numbers (container label)	Acceptable
Regulations: 21 CFR 201.2, 21 CFR 207.35	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A

Comment/Recommendation:

Preparation instructions (container label)	Acceptable
Regulation: 21 CFR 201.5(g)	<input type="checkbox"/> Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> N/A
<i>Recommended labeling practices: Draft Guidance Safety Considerations for Container Labels and Carton Labeling Design to Minimize Medication Errors, April 2013 (lines 426-430), which, when finalized, will represent FDA's current thinking on topic</i>	<input type="checkbox"/> Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> N/A

Comment/Recommendation:

Package type term (container label)	Acceptable
<i>Recommended labeling practices: Guidance for Industry: Selection of the Appropriate Package Type Terms and Recommendations for Labeling Injectable Medical Products Packaged in Multiple-Dose, Single-Dose, and Single-Patient-Use Containers for Human Use (October 2018) USP chapter <659> Packaging and Storage Requirements</i>	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A

Comment/Recommendation:

Misleading statements (container label)	Acceptable
Regulation: 21 CFR 201.6	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A

Comment/Recommendation: There are no misleading statements.

Prominence of required label statements (container label)	Acceptable
Regulation: 21 CFR 201.15	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A

Comment/Recommendation:

Spanish-language (Drugs) (container label)	Acceptable
Regulation: 21 CFR 201.16	<input type="checkbox"/> Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> N/A

Comment/Recommendation:

FD&C Yellow No. 5 and/or FD&C Yellow No. 6 (container label)	Acceptable
Regulation: 21 CFR 201.20	<input type="checkbox"/> Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> N/A

Comment/Recommendation:

Bar code label requirements (container label)	Acceptable
Regulations: 21 CFR 201.25, 21 CFR 610.67	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
<i>Recommended labeling practices references: Guidance for Industry: Bar Code Label Requirements Questions and Answers, August 2011</i> <i>Draft Guidance for Industry: Safety Considerations for Container Labels and Carton Labeling Design to Minimize Medication Errors, April 2013 (lines 511-512), lines 780-786), which, when finalized, will represent FDA's current thinking on topic</i>	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A

Comment/Recommendation:

Strategic National Stockpile (exceptions or alternatives to labeling requirements for human drug products) (container label)	Acceptable
Regulations: 21 CFR 610.68, 21 CFR 201.26	<input type="checkbox"/> Yes <input type="checkbox"/> No

	<input checked="" type="checkbox"/> N/A
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Comment/Recommendation:

Net quantity (container label)	Acceptable
Regulation: 21 CFR 201.51	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
<i>Recommended labeling practices references: Draft Guidance for Industry: Safety Considerations for Container Labels and Carton Labeling Design to Minimize Medication Errors (line 461- 463) which, when finalized, will represent FDA's current thinking on topic Allowable Excess Volume and Labeled Vial Fill Size in Injectable Drug and Biological Products Guidance for Industry, June 2015 (line 68, 93-99) USP General Chapters <1151> Pharmaceutical Dosage Forms (Excess volume in injections).</i>	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A

Comment/Recommendation:

Statement of Dosage (container label)	Acceptable
Regulations: 21 CFR 610.60(a)(5), 21 CFR 610.60(c), 21 CFR 201.55, 21 CFR 201.100(b)(2)	<input type="checkbox"/> Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> N/A

Comment/Recommendation:
Partial label

Inactive ingredients (container label)	Acceptable
Regulation: 21 CFR 201.100	<input type="checkbox"/> Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> N/A
<i>Recommended labeling practices reference: USP General Chapters <1091> Labeling of Inactive Ingredients and USP General Chapters <7> Labeling</i>	<input type="checkbox"/> Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> N/A

Comment/Recommendation:
Partial label

Storage requirements (container label)	Acceptable
<i>Recommended labeling practices references: USP General Chapters <7> Labeling, USP General Chapters <659> Packaging and Storage Requirements</i>	<input type="checkbox"/> Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> N/A

Comment/Recommendation:
Partial Label

Dispensing container (container label)	Acceptable
Regulation: 21 CFR 201.100(b)(7)	<input type="checkbox"/> Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> N/A

Comment/Recommendation:
The applicant's original package is designed and intended to be dispensed to patients without repackaging.

Package⁶ Labeling Evaluation
(Unit and Shelf Carton Labeling)

Proper name (package labeling)	Acceptable
Regulations: 21 CFR 610.61(a), 21 CFR 201.50(b), 21 CFR 201.10(g)(2)	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A

Comment/Recommendation:
March 13, 2020: The applicant submitted revised labeling to include the suffix apgf.
FDA Response: The applicant's revision is acceptable.

Manufacturer name, address, and license number (package labeling)	Acceptable
Regulations: 21 CFR 610.61(b), 21 CFR 201.1(a), 21 CFR 201.1(i), 21 CFR 201.100(e)	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
<i>Recommended labeling practices (using the qualifying phrase "Manufactured by:")</i>	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A

Comment/Recommendation:
Shelf Carton:
To Applicant: Consider revising your Country of Origin statement from "Made in Croatia" to read "Product of Croatia". Please also refer to U.S. Customs Border and Protection regulations 19 CFR 134.11.
January 17, 2020: The Applicant appreciated the Agency's comment and guidance regarding the Country of Origin (CoO) regulations (19 CFR 134.11 and 19 CFR 134.22) enforced by U.S. Customs and Border Protection (CBP), which require every product imported into the United States to display an accurate CoO statement. In line with these

⁶ Per 21 CFR 600.3(cc) *Package* means the immediate carton, receptacle, or wrapper, including all labeling matter therein and thereon, and the contents of the one or more enclosed containers. If no package, as defined in the preceding sentence, is used, the container shall be deemed to be the package. Thus, this includes the carton, prescribing information, and patient labeling.

regulations, as well as CBP's Informed Compliance Publication (<https://www.cbp.gov/trade/rulings/informed-compliance-publications/markings-countryorigin-us-imports>), the Applicant's established policies and procedures (a) set forth "Made in [applicable country]" as the language to be displayed on all of its products; and (b) require the CoO statement to be uniform across all products. Recognizing the applicable CFR and United States Code (19 USC 1304) sections allow for flexibility in the wordings of such a CoO, we acknowledge that both "Product of Croatia" and "Made in Croatia" are appropriate terms. In order, however, for the Applicant to follow its current labeling practices and maintain consistency with its existing marketed product line, we respectfully request leeway to maintain the current CoO language ("Made in Croatia") for the pegfilgrastim-xxxx carton labeling.

FDA Response: The applicant's response is acceptable.

Lot number or other lot identification (package labeling)	Acceptable
Regulation: 21 CFR 610.61(c), 21 CFR 201.18	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A

Comment/Recommendation:

Expiration date (package labeling)	Acceptable
Regulations: 21 CFR 610.61(d), 21 CFR 201.17	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A

Comment/Recommendation:

Beyond Use Date (Multiple-dose containers) (package labeling)	Acceptable
<i>Recommended labeling practices: USP General Chapters: <659> Packaging and Storage Requirements and <7> Labeling</i>	<input type="checkbox"/> Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> N/A

Comment/Recommendation:

Preservative (package labeling)	Acceptable
Regulation: 21 CFR 610.61(e)	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A

Comment/Recommendation:

Number of containers (package labeling)	Acceptable
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Regulation: 21 CFR 610.61(f)	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
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Comment/Recommendation:

Product Strength (package labeling)	Acceptable
Regulations: 21 CFR 610.61(g), 21 CFR 201.10(d)(1), 21 CFR 201.100(b)(4)	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
<i>Recommended labeling practices references: Draft Guidance Safety Considerations for Container Labels and Carton Labeling Design to Minimize Medication Errors, April 2013 (line 176), which, when finalized, will represent FDA's current thinking on topic</i> <i>USP General Chapters: <7> Labeling</i>	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A

Comment/Recommendation:
Shelf and Unit Carton: (DMEPA comment)
 To Applicant: As currently presented, the strength is located at the bottom of the principal display panel. We recommend relocating the strength so that it appears directly below the proper name to ensure it is not missed.

 January 17, 2020: The applicant relocated the expression of strength statement to appear directly below the proper name and dosage formulation.

 FDA Response: The applicant's revision is acceptable.

Storage temperature/requirements (package labeling)	Acceptable
Regulation: 21 CFR 610.61(h)	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
<i>Recommended labeling practices reference: USP General Chapters: <7> Labeling, USP General Chapters <659> Packaging and Storage Requirements</i>	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A

Comment/Recommendation:

Handling: "Do Not Shake", "Do not Freeze" or equivalent (package labeling)	Acceptable
Regulation: 21 CFR 610.61(i)	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A

Comment/Recommendation:

Multiple dose containers (recommended individual dose) (package labeling)	Acceptable
Regulation: 21 CFR 610.61(j)	<input type="checkbox"/> Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> N/A

Comment/Recommendation:

Route of administration (package labeling)	Acceptable
Regulations: 21 CFR 610.61(k), 21 CFR 201.5(f), 21 CFR 201.100(d)(1)	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
<i>Recommended labeling practices (route of administration statement to appear after the strength statement on the principal display panel)</i>	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A

Comment/Recommendation:
Shelf and Unit Carton:
 To Applicant: Consider rephrasing and relocating the route of administration, "Subcutaneous Use Only" to read "For Subcutaneous Use Only" and relocating the statement to appear directly below the expression of strength statement, 6 mg/0.6 mL.

 January 17, 2020: The applicant revised the route of administration statement to read "For Subcutaneous Use Only" and relocated the statement to appear directly below the expression of strength statement.

 FDA Response: The applicant's revision is acceptable.

Known sensitizing substances (package labeling)	Acceptable
Regulations: 21 CFR 610.61(l), 21 CFR 801.437 (User labeling for devices that contain natural rubber)	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A

Comment/Recommendation:
 The carton contains the statement "The syringe plunger stopper and needle cover are not made with natural rubber latex", which is accurate and acceptable.

Inactive ingredients (package labeling)	Acceptable
Regulations: 21 CFR 610.61, 21 CFR 201.100	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
<i>Recommended labeling practices references: USP General Chapters <1091> Labeling of Inactive Ingredients, USP General Chapters <7> Labeling</i>	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No

	<input type="checkbox"/> N/A
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Comment/Recommendation:

Shelf and Unit Carton:

To Applicant: We recommend revising the phrase "(based on protein content)" to read "(based on protein weight)" to be consistent with the prescribing information.

January 17, 2020: The applicant revised the phrase "(based on protein content)" to read "(based on protein weight)" to be consistent with the prescribing information.

FDA Response: The applicant's revision is acceptable.

To Applicant: We recommend removing the trailing zeros that appear on the back panel (i.e., 4.0 and 30.0 mg) to avoid misinterpretation of the numbers (i.e., 4 versus 40 and 30 versus 300).

January 17, 2020: The applicant deleted the trailing zeros.

FDA Response: The applicant's revisions are acceptable.

Source of the product (package labeling)	Acceptable
Regulation: 21 CFR 610.61(p)	<input checked="" type="checkbox"/> Yes <input checked="" type="checkbox"/> No <input type="checkbox"/> N/A

Comment/Recommendation:

The carton indicates the product was derived from E.coli, and this reference is acceptable.

Minimum potency of product (package labeling)	Acceptable
Regulation: 21 CFR 610.61(r)	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A

Comment/Recommendation:

Rx only (package labeling)	Acceptable
Regulations: 21 CFR 610.61(s), 21 CFR 201.100(b)(1)	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
<i>Recommended labeling practices references: Draft Guidance Safety Considerations for Container Labels and Carton Labeling Design to Minimize Medication Errors, April 2013 (line 147-149), which, when finalized, will represent FDA's current thinking on topic</i>	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A

Comment/Recommendation:

Shelf and Unit Carton: (DMEPA comment)

To Applicant: We recommend de-bolding the "Rx Only" statement as this information appears with equal prominence to critical information on the principal display panel.

January 17, 2020: The applicant debolded the "Rx Only" statement.

FDA Response: The applicant's revision is acceptable.

Divided manufacturing (package labeling)	Acceptable
Regulation: 21 CFR 610.63 (Divided manufacturing responsibility to be shown)	<input type="checkbox"/> Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> N/A

Comment/Recommendation:

Distributor (package labeling)	Acceptable
Regulation: 21 CFR 610.64, 21 CFR 201.1(h)(5)	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A

Comment/Recommendation:

Bar code (package labeling)	Acceptable
Regulations: 21 CFR 610.67, 21 CFR 201.25	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
Recommended labeling practices references: <i>Guidance for Industry: Bar Code Label Requirements Questions and Answers, August 2011</i> <i>Draft Guidance for Industry: Safety Considerations for Container Labels and Carton Labeling Design to Minimize Medication Errors, April 2013 (lines 511-512), lines 780-786)</i>	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A

Comment/Recommendation:

Strategic National Stockpile (exceptions or alternatives to labeling requirements for human drug products) (package labeling)	Acceptable
Regulations: 21 CFR 610.68, 21 CFR 201.26	<input type="checkbox"/> Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> N/A

Comment/Recommendation:

NDC numbers (package labeling)	Acceptable
Regulations: 21 CFR 201.2, 21 CFR 207.35	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A

Comment/Recommendation:

Preparation instructions (package labeling)	Acceptable
Regulation: 21 CFR 201.5(g)	<input type="checkbox"/> Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> N/A
<i>Recommended labeling practices references: Draft Guidance Safety Considerations for Container Labels and Carton Labeling Design to Minimize Medication Errors, April 2013 (lines 426-430), which, when finalized, will represent FDA's current thinking on topic USP General Chapters <7> Labeling</i>	<input type="checkbox"/> Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> N/A

Comment/Recommendation:

Package type term (package labeling)	Acceptable
<i>Recommended labeling practices: Guidance for Industry: Selection of the Appropriate Package Type Terms and Recommendations for Labeling Injectable Medical Products Packaged in Multiple-Dose, Single-Dose, and Single-Patient-Use Containers for Human Use (October 2018) USP chapter <659> Packaging and Storage Requirements</i>	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A

Comment/Recommendation:

Misleading statements (package labeling)	Acceptable
Regulation: 21 CFR 201.6	<input type="checkbox"/> Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> N/A

Comment/Recommendation:

Prominence of required label statements (package labeling)	Acceptable
Regulation: 21 CFR 201.15	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A

Comment/Recommendation:

Spanish-language (Drugs) (package labeling)	Acceptable
Regulation: 21 CFR 201.16	<input type="checkbox"/> Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> N/A

Comment/Recommendation:

FD&C Yellow No. 5 and/or FD&C Yellow No. 6 (package labeling)	Acceptable
Regulation: 21 CFR 201.20	<input type="checkbox"/> Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> N/A

Comment/Recommendation:

Phenylalanine as a component of aspartame (package labeling)	Acceptable
Regulation: 21 CFR 201.21(c)	<input type="checkbox"/> Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> N/A

Comment/Recommendation:

Sulfites; required warning statements (package labeling)	Acceptable
Regulation: 21 CFR 201.22(b)	<input type="checkbox"/> Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> N/A

Comment/Recommendation:

Net quantity (package labeling)	Acceptable
Regulation: 21 CFR 201.51	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
<i>Recommended labeling practices references: Draft Guidance for Industry: Safety Considerations for Container Labels and Carton Labeling Design to Minimize Medication Errors (line 461- 463) which, when finalized, will represent FDA's current thinking on topic</i> <i>Allowable Excess Volume and Labeled Vial Fill Size in Injectable Drug and Biological Products Guidance for Industry, June 2015 (line 68, 93-99)</i> <i>USP General Chapters <1151> Pharmaceutical Dosage Forms (Excess volume in injections).</i>	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A

Comment/Recommendation:

Statement of Dosage (package labeling)	Acceptable
Regulations: 21 CFR 201.55, 21 CFR 201.100(b)(2)	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A

Comment/Recommendation:
Shelf and Unit Carton:

To Applicant: Revise "Usual Dosage: see prescribing information for dosage and instructions for use" to read "Dosage: See Prescribing Information" to ensure consistency with all doses described in the prescribing information.

January 17, 2020: The applicant revised the Dosage statement to read "Dosage: See Prescribing Information."

FDA Response: The applicant's revision is acceptable.

Dispensing container (package labeling)	Acceptable
Regulation: 21 CFR 201.100(b)(7)	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A

Comment/Recommendation:

Medication Guide (package labeling)	Acceptable
Regulations: 21 CFR 610.60(a)(7), 21 CFR 208.24(d)	<input type="checkbox"/> Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> N/A

Comment/Recommendation: This product does not require a Medication Guide.

Other (package labeling)	Acceptable
	<input type="checkbox"/> Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> N/A

Comment/Recommendation:

Prescribing Information Evaluation

PRESCRIBING INFORMATION

Highlights of Prescribing Information	
PRODUCT TITLE	Acceptable
Regulation: 21 CFR 201.57(a)(2)	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
<i>Recommended labeling practices reference: Draft Guidance for Industry on Product Title and Initial U.S. Approval in the Highlights of Prescribing Information for Human Prescription Drug and Biological Products - Content and Format (January 2018), which, when finalized, will represent FDA's current thinking on topic</i>	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A

Comment/Recommendation:

Highlights of Prescribing Information	
DOSAGE AND ADMINISTRATION	Acceptable
<i>Recommended labeling practices reference: USP nomenclature for diluents and intravenous solutions</i>	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A

Comment/Recommendation:

Highlights of Prescribing Information	
DOSAGE FORMS AND STRENGTHS	Acceptable
Regulations: 21 CFR 201.57(a)(8), 21 CFR 201.10, 21 CFR 201.100	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
<i>Recommended labeling practices references: Guidance for Industry: Selection of the Appropriate Package Type Terms and Recommendations for Labeling Injectable Medical Products Packaged in Multiple-Dose, Single-Dose, and Single-Patient-Use Containers for Human Use (October 2018) USP chapter <659> Packaging and Storage Requirements USP General Chapters: <7> Labeling</i>	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A

Comment/Recommendation:
Dr. Di confirmed the product strength, 6 mg/0.6 mL, is accurate for this prefilled syringe.

Full Prescribing Information	
2 DOSAGE AND ADMINISTRATION	Acceptable
Regulation: 21 CFR 201.57(c)(3)(iv)	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
<i>Recommended labeling practices reference: USP nomenclature for diluents and intravenous solutions and storage instructions for reconstituted and diluted products</i>	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A

Comment/Recommendation:

Dr. Di confirmed the syringe plunger stopper and needle cover are not made with natural rubber latex.

Section 2.2 Administration
Dr. Di and Dr. Sihag confirmed the drug product can be kept at room temperature for 15 days.

Section 2.2 Administration

To Applicant: Revised to provide the verbatim statement per 21 CFR 201.57(c)(3)(iv), and revised the second sentence to provide additional clarity.

Revised the third paragraph to read: "Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. NYVEPRIA is supplied as a clear and colorless solution. Do not administer NYVEPRIA if discoloration or particulates are observed."

February 18, 2020: The applicant accepted FDA's recommendations.

FDA Response: The applicant's revisions are acceptable.

Full Prescribing Information	
3 DOSAGE FORMS AND STRENGTHS	Acceptable
Regulation: 21 CFR 201.57(c)(4)	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
<i>Recommended labeling practices references: Guidance for Industry: Selection of the Appropriate Package Type Terms and Recommendations for Labeling Injectable Medical Products Packaged in Multiple-Dose, Single-Dose, and Single-Patient-Use Containers for Human Use (October 2018)</i> <i>USP chapter <659> Packaging and Storage Requirements</i> <i>USP General Chapters: <7> Labeling</i>	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A

Comment/Recommendation:

Dr. Di confirmed the identifying characteristics, clear and colorless, for this product are accurate.

To Applicant: Revised the text, because the word "sterile" should appear in Section 11 and the needle guard description should appear in section 16.

February 18, 2020: The applicant accepted FDA's recommendation to delete the word sterile and delete the needle guard information from Section 3.

FDA Response: The applicant's revisions are acceptable.

Full Prescribing Information	
11 DESCRIPTION	Acceptable
Regulations: 21 CFR 201.57(c)(12), 21 CFR 610.61 (m), 21 CFR 610.61(o), 21 CFR 610.61 (p), 21 CFR 610.61 (q)	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A

<i>Recommended labeling practices references: USP General Chapters <1091>, USP General Chapters <7></i>	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
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<p>Comment/Recommendation: Dr. Di confirmed the drug substance information presented in the first paragraph was accurate. Dr. Di confirmed the drug product is sterile, preservative-free and the qualitative and quantitative information was accurate.</p> <p>First paragraph of Section 11: To Applicant: The reference to the name filgrastim was removed from this paragraph for clarity. (The name "filgrastim" was replaced with the name "recombinant methionyl human G-CSF".)</p> <p>February 18, 2020: The applicant accepted FDA's recommendation to replace the name "filgrastim" with the name "recombinant methionyl human G-CSF".</p> <p>FDA Response: The applicant's revisions are acceptable.</p> <hr/> <p>Second paragraph of Section 11: To Applicant: Information concerning the type of needle guard is more appropriate for Section 16, thus the information was deleted from this section.</p> <p>February 18, 2020: The applicant accepted FDA's deletion of the needle guard information.</p> <p>FDA Response: The applicant's revision is acceptable.</p>
--

Full Prescribing Information	
15 Cytotoxic Drug reference	Acceptable
Regulation: 21 CFR 201.57(c)(17)(iv) xxxx is a cytotoxic drug. Follow applicable special handling and disposal procedures.1 1.OSHA Hazardous Drugs. OSHA. [Accessed on June 9, 2017, from http://www.osha.gov/SLTC/hazardousdrugs/index.html	<input type="checkbox"/> Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> N/A

<p>Comment/Recommendation: This drug product is not a cytotoxic agent.</p>
--

Full Prescribing Information	
16 HOW SUPPLIED/ STORAGE AND HANDLING	Acceptable
Regulation: 21 CFR 201.57(c)(17)	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A

<i>Recommended labeling practices: to ensure placement of detailed storage conditions for reconstituted and diluted products</i>	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
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Comment/Recommendation:
Dr. Di confirmed the storage and handling conditions for the drug product were accurate. (refrigerate, 1 freeze thaw cycle as proposed is acceptable, protect from light and do not shake). Dr. Di and Dr. Sihag confirmed the drug product can be kept at room temperature for 15 days.

OBP Labeling: To Applicant: The attributes sterile and preservative-free were deleted, because they are not required for Section 16 and are presented in Section 11.

February 18, 2020: The applicant accepted FDA’s recommendation to delete the attributes sterile and preservative-free information from Section 11.

FDA Response: The applicant’s revisions are acceptable.

Full Prescribing Information	
MANUFACTURER INFORMATION	Acceptable
Regulations: 21 CFR 201.100(e), 21 CFR 201.1	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
<i>Recommended labeling practices references: 21 CFR 610.61(b) (add the US license number for consistency with the carton labeling), and 21 CFR 610.64 (Name and address of distributor may appear and use a qualifying phrase for consistency with the carton labeling, when applicable)</i>	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A

Comment/Recommendation:

Instructions for Use Evaluation

INSTRUCTIONS FOR USE	
TITLE (NAMES AND DOSAGE FORM)	
<i>Recommended Labeling Practices references: To ensure consistency with the product title in the Highlights of Prescribing Information (see Draft Product Title and Initial U.S. Approval in the Highlights of Prescribing Information for Human Prescription Drug and Biological Products - Content and Format Guidance for Industry (January 2018). For the recommended dosage form (see USP General Chapters: <1> Injections, Nomenclature and Definitions, Nomenclature form).</i>	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A

Comment/Recommendation:

INSTRUCTIONS FOR USE	
STORAGE AND HANDLING	Acceptable
<i>Recommended labeling practices for Patient Labeling or IFU: To ensure that applicable storage and handling requirements are consistent with the information provided in the PI (Reference: Section 2 (Dosage and Administration) and Section 16 (How Supplied Storage and Handling) of the PI)</i>	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A

Comment/Recommendation:

INSTRUCTIONS FOR USE	
INGREDIENTS	Acceptable
<i>Recommended labeling practice: To ensure labeling of inactive ingredients are in alphabetical order (see USP General Chapters <1091>)</i>	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A

Comment/Recommendation: There is no list of ingredients in the Instructions for Use and this omission is acceptable.

INSTRUCTIONS FOR USE	
MANUFACTURER INFORMATION	Acceptable
21 CFR 201.1, 19 CFR 134.11	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
<i>21 CFR 610.61 (add the US license number for consistency with the carton labeling), 21 CFR 610.64 (Name and address of distributor may appear and use a qualifying phrase for consistency with the carton labeling, when applicable)</i>	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A

Comment/Recommendation:

Patient Information Labeling Evaluation

PATIENT INFORMATION LABELING	
TITLE (NAMES AND DOSAGE FORM)	Acceptable
<i>Recommended Labeling Practices references: To ensure consistency with the product title in the Highlights of Prescribing Information (see Draft Product Title and Initial U.S. Approval in the Highlights of Prescribing Information for Human Prescription Drug and Biological Products - Content and Format Guidance for Industry (January 2018). For the recommended dosage form (see USP General Chapters: <1> Injections, Nomenclature and Definitions, Nomenclature form).</i>	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A

Comment/Recommendation:

PATIENT INFORMATION LABELING	
STORAGE AND HANDLING	Acceptable
<i>Recommended labeling practices for Patient Labeling or IFU: To ensure that applicable storage and handling requirements are consistent with the information provided in the PI (Reference: Section 2 (Dosage and Administration) and Section 16 (How Supplied Storage and Handling) of the PI)</i>	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A

Comment/Recommendation:

PATIENT INFORMATION LABELING	
INGREDIENTS	Acceptable
<i>Recommended labeling practice: To ensure labeling of inactive ingredients are in alphabetical order (see USP General Chapters <1091>)</i>	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A

Comment/Recommendation:

PATIENT INFORMATION LABELING	
MANUFACTURER INFORMATION	Acceptable
21 CFR 201.1, 19 CFR 134.11	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
<i>21 CFR 610.61 (add the US license number for consistency with the carton labeling), 21 CFR 610.64 (Name and address of distributor may appear and use a qualifying phrase for consistency with the carton labeling, when applicable)</i>	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A

Comment/Recommendation:

APPENDIX C. Acceptable Labels and Labeling

- Prescribing Information, Patient Information and Instructions for Use (submitted on April 27, 2020)
<\\cdsesub1\evsprod\bla761111\0020\m1\us\lab-1186-0-2-lab-1187-0-2-lab-1188-0-3-combined-clean.doc>

- Svrinœ Container Label (submitted on March 13, 2020)





Scott
Dallas

Digitally signed by Scott Dallas
Date: 4/28/2020 09:33:24AM
GUID: 508da712000294048aa136a18a6af06a



Xu
Di

Digitally signed by Xu Di
Date: 4/28/2020 10:11:01AM
GUID: 543400e20010de74ddcb797efe7f3df8

BLA 761111

Nyvepria™ [pegfilgrastim-apgf, pegylated G-CSF, PF-06881894]

A proposed biosimilar to Neulasta

Hospira, Inc., a Pfizer Company

Comparative Analytical Assessment

Xu Di, Ph.D., Product Quality Reviewer

Ram Sihag, Ph.D., Team Leader

Susan Kirshner, Ph.D., Review Chief

Division of Biotechnology Research and Review III

Office of Biotechnology Products (OBP)

Office of Pharmaceutical Quality (OPQ)

Center for Drug Evaluation and Research (CDER)

3.2.R Comparative Analytical Assessment

The data provided in Section 3.2R support the following conclusions:

1. *Pair-wise analytical comparisons of PF-06881894, U.S.-licensed Neulasta, and E.U.-approved Neulasta support a determination that PF-06881894 is highly similar to U.S.-licensed Neulasta and that the analytical component of a scientific bridge between the three products was established. Hereafter, U.S.-licensed Neulasta will be referred to as pegfilgrastim-U.S. and E.U.-approved Neulasta will be referred as pegfilgrastim-E.U.*
2. *The PF-06881894 DP lots used in the comparative analytical studies are independent DP lots that were manufactured from different drug substance (DS) lots with each DS lot manufactured using an independent filgrastim intermediate lot. The 10 PF-06881894 DP lots used in the comparative analytical assessment were manufactured using the proposed commercial scale process, including the development lots, clinical lots, process validation lots, and stability lots. Seventeen pegfilgrastim-U.S. lots and 17 pegfilgrastim-E.U. lots were used in the comparative analytical studies, and appropriately included lots used in the comparative nonclinical and clinical studies.*
3. *The age of lots at time of testing allows for a meaningful comparison to support the comparative analytical assessment. Pegfilgrastim-U.S. and pegfilgrastim-E.U. lots were tested prior to their expiration and ranged at time of testing from 30 months prior to expiration to expiration. Most of PF-06881894 lots selected for the comparative analytical assessment are within and span across 36 months, which the applicant assumes is the shelf-life of pegfilgrastim-U.S. and pegfilgrastim-E.U. However, several PF-06881894 lots were older than 36 months at the time of testing, which is acceptable because products under IND do not have an expiration date. The impact of product age at time of testing is discussed as appropriate below.*
4. *The comparative analytical studies were performed using appropriate orthogonal analytical methods for each quality attribute, which included testing for functional activities, product-related substances and impurities, primary and higher order structure, and drug product specific attributes. The methods were adequately validated or qualified to support that the methods were scientifically sound and suitable for their intended use.*
5. *Comparative forced degradation studies were performed using an appropriate variety of forced degradation conditions, including peroxide, heat, photo, and high pH stress. Stability studies were conducted to compare the rates and pathways of degradation for PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U. under long-term, accelerated, and stress stability storage conditions.*
6. *The applicant used appropriately justified quality ranges based on a 3x standard deviation as acceptance criteria for quality range attributes. Attributes assessed by visual comparison of results were appropriately justified.*

7. *The comparison of process-related impurities, such as host cell protein (HCP) and host cell DNA (HCD), appropriately were not included as part of the comparative analytical assessment. However, the manufacturing process of PF-06881894 was demonstrated to have a robust capacity to consistently remove process-related impurities to acceptable ranges.*
8. *The strength of pegfilgrastim-U.S. is labeled in mass per unit volume (6 mg/0.6mL) and filled into a single-use prefilled glass syringe. PF-06881894 is seeking approval for the same strength and presentation as pegfilgrastim-U.S. PF-06881894 has the same formulation, route of administration, and frequency and duration of dosing as pegfilgrastim-U.S., and pegfilgrastim-E.U. Comparative protein concentration was evaluated as part of the comparative analytical assessment and [REDACTED] (b) (4) [REDACTED] data were evaluated as part of manufacturing process controls. The results from deliverable volume and filling weight tests support a determination that PF-06991984 had the same strength and presentation as pegfilgrastim-U.S. The presentation meets the statutory “same strength” requirement under section 351(k)(2)(A)(i)(IV) of the PHS Act.*

Overall Strategy

PF-06881894 drug product (DP) and pegfilgrastim-U.S. were compared in analytical studies to support a determination that they are highly similar. Analytical studies for making pairwise comparisons between PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U. were performed to establish the analytical component of a scientific bridge between the three products. Pegfilgrastim-U.S. and pegfilgrastim-E.U. lots were purchased without preselected purchasing criteria at regular intervals from the regulated market to establish the originator product profile.

The comparative physicochemical and functional assessments include:

1. Characterization studies of the following quality attributes (QA)
 - Biological activity
 - Primary structure, post-translational modifications, and sequence variants
 - Product related substances and impurities
 - Higher order structure
 - Drug product attributes
2. Forced degradation studies, including peroxide, heat, light, and high pH stress conditions.
3. Stability studies at the intended storage condition of 5°C for up to 36 months, the accelerated storage condition of 25°C/60% RH for up to 6 months, and the stress storage condition of 40°C/75% RH for up to 3 months.

A total of 10 PF-06881894 DP lots, 17 pegfilgrastim-U.S. lots, and 17 pegfilgrastim-E.U. lots were used in the comparative analytical assessments. The 10 PF-06881894 DP lots were manufactured from different DS lots. If a DP lot was manufactured using pooled DS lots, none of the individual DS lots pooled were used in the manufacture of any other DP lot. Additionally, each DS lot was produced from a unique filgrastim intermediate DS lot. A summary of PF-

06881894 DP lots, pegfilgrastim-U.S. lots, and pegfilgrastim-E.U. lots used in the comparative analytical assessments are provided below in Tables 2.3.R.5.3-1 and 3.2.R.5.3-2.

Table 3.2.R.5.3-1. Summary of PF-06881894 DP Lots Used in the Analytical Similarity Assessment

DP Lot Number	DS Batch Number	DP Date of Manufacture	Lot Use (In Addition to Similarity Testing)
2056034	PF S01P/14	Mar 2014	Stability
2078064	PF S03P/14	Jun 2014	Stability
2082094	1803074 and 1805074	Oct 2014	Stability
2051124	1803094 and 1806084	Dec 2014	Clinical (C1221001 and C1221002), Stability
2573125	1459065	Dec 2015	Stability
2459066	1551036	Jun 2016	Clinical (C1221002 and C1221005), Stability
213047	1037	Apr 2017	Engineering
3058V	1048V	May 2018	PPQ, Stability
4058V	2048V	Jun 2018	PPQ, Stability
2068V	1058V and 2058	Jun 2018	PPQ, Stability

DP, drug product; DS, drug substance; PPQ, process performance qualification

Table 3.2.R.5.3-2. Summary of Pegfilgrastim-US and Pegfilgrastim-EU Lots Used in the Analytical Similarity Assessment

Product	Lot Number	Expiration Date	Lot Use (In Addition to Similarity Testing)
Pegfilgrastim-US	1035686	Sep-15	--
	1036285	Oct-15	--
	1057096	Nov-17	Stability
	1057097	Feb-18	--
	1057133	Oct-17	Clinical (C1221001), Stability
	1057373	Jan-18	Clinical (C1221001), Nonclinical
	1057416	Mar-18	--
	1060058	Jun-18	--
	1064191	Sep-18	--
	1071087	Jan-19	Clinical (C1221005)
	1072044	Jul-19	Clinical (C1221005)
	1078875	May-19	--
	1083446	Apr-20	--
	1084476	Nov-19	--
	1085896	Apr-20	--
1089511	Nov-19	--	
1094104	Jun-20	--	
Pegfilgrastim-EU	1039830D	Oct-15	--
	1041021D	Nov-15	--
	1058436B	Aug-17	Nonclinical, Stability
	1060064C	Oct-17	Clinical (C1221001), Stability
	1061466C	Oct-17	Clinical (C1221001), Stability
	1061815D	Nov-17	--
	1065041B	Apr-18	--
	1066011C	Sep-18	--
	1069490C	Dec-18	--
	1079877A	Oct-19	--
1085288A	Jan-20	--	
Pegfilgrastim-EU	1087927A	Jun-20	--
	1088493A	Jun-20	--
	1088500D	Nov-20	--
	1090139B	Nov-20	--
	1093686K	Dec-20	--
	1094582	Feb-21	--

Assessor's Comment:

The PF-06881894 DP lots used in the comparative analytical assessment are appropriate. The lots are considered independent because they were manufactured using different DS lots. PF-06881894 and reference product lots used in the comparative non-clinical and clinical studies were included in the comparative analytical assessment. PF-06881894 DP lots also included lots used for stability and process validation studies. This approach is consistent with FDA Draft Guidance for Industry Development of Therapeutic Protein Biosimilars: Comparative Analytical Assessment and Other Quality-Related Considerations (2019) and allows for a meaningful comparison to support the comparative analytical assessment.

Product ages at the time of test for primary structure, higher order structure, product-related substance and impurities, and DP attributes are summarized in the applicant's Tables provided in Appendix 1 of this review. The ages of product lots used for functional activity, comparative forced degradation study, and comparative stability study are summarized and discussed in corresponding sections.

Assessor's Comment:

Pegfilgrastim-U.S. and pegfilgrastim-E.U. lots were all within their dating period at the time of testing, ranged from ~30 - 0 months from their expiration date, and were acquired over a period of several years, which helps ensure that reference product variability was captured by the samples. For some tests, at time of testing some lots of PF-06881894 were older than the currently proposed dating period, which is acceptable because products under IND do not have an expiration date. Potential lot age-related effects were assessed and are discussed in the assessments for those tests. Overall, I concluded that lot age did not impact the validity of the comparative analytical studies.

Identification of Critical Quality Attributes

The applicant assessed quality attribute criticality by evaluating attribute impact on clinical performance and patient safety. The critical quality attribute (CQA) assessment was the foundation for the comparative analytical assessment and was used to identify appropriate analytical methods for the studies. The applicant used Quality Risk Management principles, literature, and product knowledge for the CQA assessment. The CQA assessment for PF-06881894 DS and DP is summarized in Scientific Report LF-235-R-208-18. The attributes selected for comparative testing are provided in Table 3.2.R.5.2-1.

Assessor's Comment:

The applicant is using a standard approach to risk assessment which is acceptable. More detailed analyses are provided below.

Table 3.2.R.5.2-1. Summary of PF-06881894 DS and DP CQAs		
Attribute	CQA for DS/DP	Comparative Testing in Support of Analytical Similarity
Identity	DS and DP	Yes
Primary Structure	DS and DP	Yes
Higher Order Structure (including disulfide linkages)	DS and DP	Yes
Potency	DS and DP	Yes
Appearance, Color, Clarity	DS and DP	Yes
pH	DS and DP	Yes
Molecular Weight Variants and High Molecular Weight Species	DS and DP	Yes
Oxidation (Met122, Met127, Met138)	DS and DP	Yes
Deamidation (Gln21)	DS and DP	Yes
Reduced Species	DS and DP	Yes
Endotoxin	DS and DP	No
Bioburden	DS	No
Sterility	DP	No
Polysorbate 20	DS and DP	Yes
Protein Concentration	DS and DP	Yes
Des-pegylated species	DS and DP	Yes
Deliverable Volume	DP	Yes
Visible Particles	DP	Yes
Subvisible Particles	DP	Yes
Osmolality	DP	Yes
Container Closure Integrity	DP	No
Syringe Function	DP	No
Leachables	DP	No
Residual Heavy Metals	DP	No
Residual PEG	Non-CQA	Yes
Isoelectric Point (pI)	Non-CQA	Yes
Oxidation (Trp59)	Non-CQA	Yes
Deamidation (Gln91, Gln120, Gln135)	Non-CQA	Yes
Other Low Abundant Pegfilgrastim Related Species	Non-CQA	Yes

CQA, critical quality attribute; DP, drug product; DS, drug substance, PEG, polyethylene glycol

Comparisons between PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U. were conducted either by direct comparative testing or indirectly by meeting compendial requirements. Some CQAs, such as sterility and endotoxin, are evaluated directly against compendial limits without measuring reference product ranges.

Assessor's Comment:

Some CQAs, such as host cell proteins (HCP), host cell DNA (HCD) and leachables, are not appropriate for direct comparison due to differences in the manufacturing processes and product packaging. These appropriately were assessed as part of the commercial control strategies of the PF-06881894 DS and DP, e.g., process-related impurities and container closure-specific attributes. This approach is consistent with recommendations in the FDA Draft Guidance for Industry Development of Therapeutic Protein Biosimilars: Comparative Analytical Assessment and Other Quality-Related Considerations (2019) and is acceptable. The validated HCP and HCD methods demonstrated that the process effectively and consistently removes these process-related impurities through testing of multiple commercial-scale FI lots. Residual levels of HCD (N=29 lots) and HCP (N=30 lots) were all below 1 ng/mg.

3.2.R.2 Comparative Analytical Assessment Results

A summary of the analytical similarity results prepared by the reviewer are provided in the following table.

Parameter	Quality Attribute	Test Method	Number of Batches (PF-06881894: U.S.-licensed Neulasta: E.U.-approved Neulasta)	U.S.-licensed Neulasta Range or US Quality Range (QR: Mean $\pm 3SD$)	PF-06881894 Min-Max Range	E.U.-approved Neulasta Range	PF vs. U.S.-licensed Neulasta/ PF vs. E.U.-approved Neulasta/ U.S.-licensed Neulasta vs E.U.-approved Neulasta ¹	
Biological Activity	Binding Pegfilgrastim	In Vitro Cell-Based Bioassay (% Relative Potency)	10:14:14	90-115	88-103	89-110	Yes/yes/yes	
		Competitive Receptor Binding Assay (% Relative potency)	10:13:14	92-108; 89-112(US QR)	95-107	94-110	Yes/yes/yes	
		Receptor Binding Affinity and Kinetics (Surface Plasmon Resonance Assay)	Binding Affinity: Relative K_D (%)	10:13:14	81-116; 74-128(US QR)	82-104	92-110	Yes/yes/yes
			Binding Affinity: $K_D \times 10^{-11}$ (M)	10:13:14	2.8-4.5	2.6-4.1	2.5-4.1	Yes/yes/yes
			Binding Kinetics: $K_{on} \times 10^6$ ($M^{-1}S^{-1}$)	10:13:14	2.3-3.3	2.3-2.9	2.3-3.4	Yes/yes/yes
			Binding Kinetics: $K_{off} \times 10^{-5}$ (S^{-1})	10:13:14	6.8-11	6.5-12	6.1-12	Yes/yes/yes
Primary Structure	Amino Acid Sequence	Glu-C Peptide Mapping (RP-UPLC-MS)	7:6:6	Peak pattern of chromatograms is visually superimposable	Peak pattern of chromatograms is visually superimposable	Peak pattern of chromatograms is visually superimposable	Yes/yes/yes	

	Pegylation Site and Linker Composition	Glu-C Peptide Mapping of Pegylated Peptide (RP-UPLC-MS)		2:1:1	Pegylation occurred at the N-terminal Met1; the mass is consistent with the expected mass of the -CH ₂ CH ₂ CH ₂ linker	Pegylation occurred at the N-terminal Met1; the mass is consistent with the expected mass of the -CH ₂ CH ₂ CH ₂ linker	Pegylation occurred at the N-terminal Met1; the mass is consistent with the expected mass of the -CH ₂ CH ₂ CH ₂ linker	Yes/yes/yes
	Molecular Weight (including dispersity)	Intact Mass (RP-UPLC-MS)	Average Mass (483 EO units, Da)	7:6:6	40148.4 – 40148.5	40148.4 – 40148.5	40148.3 – 40148.5	Yes/yes/yes
			Mass-Averaged MW (kDa)	7:6:6	39.77 - 39.93	40.15 - 40.21	39.77 - 39.92	Yes/yes/yes (Note: The PEG raw material with lower number of ethylene oxide (EO) units was used to manufacture the pegfilgrastim-U.S. and pegfilgrastim-E.U. lots, which resulted in a lower MW. However, it doesn't preclude a determination of highly similar because data were provided showing there is variability in average mPEG MW used to manufacture pegfilgrastim-U.S. and pegfilgrastim-E.U. over time.)
			Molecular Weight Dispersity	7:6:6	1.001	1.001	1.001	Yes/yes/yes
	Free Thiol	Ellman's Assay (mol Thiol/mol pegfilgrastim)		10:9:9	1.01-1.15	1.01-1.07	1.01-1.15	Yes/yes/yes
	Isoelectric Point (pI)	cIEF		10:6:6	6.14-6.22	6.14-6.22	6.14-6.22	Yes/yes/yes

Higher Order Structure	Secondary Structure	Far-UV circular dichroism (CD)	Alpha-helix (%)	9:6:6	73-74	72-74	72-75	Yes/yes/yes
			Beta-structure (%)	9:6:6	15-16	15-16	15-16	Yes/yes/yes
			Random Coil (%)	9:6:6	13-14	13-14	13-14	Yes/yes/yes
	Tertiary Structure (disulfide bond)	Disulfide Mapping	Free Thiol (peptide 16-21): theoretical mass: 732.384 Da	3:3:3	732.383-732.385	732.384-732.385	732.383-732.385	Yes/yes/yes
			Disulfide Cys37-Cys43 (peptide 33-47): theoretical mass: 1788.817 Da	3:3:3	1788.816-1788.823	1788.817-1788.820	1788.817-1788.820	Yes/yes/yes
			Disulfide Cys65-Cys75 (peptide 51-76): theoretical mass: 2616.319 Da	3:3:3	2616.18-2616.26	2616.19-2616.23	2616.320-2616.327	Yes/yes/yes
	Tertiary Structure (structure Dynamics)	Hydrogen-Deuterium Exchange (HDX): Deuterium uptake curves and Heat Maps		2:2:2	Deuterium uptake rate	Similar deuterium uptake rate to US-licensed Neulasta	Similar Deuterium uptake rate to US-licensed Neulasta	Yes/yes/yes
	Tertiary Structure (Sedimentation Coefficient)	Sedimentation Velocity-Analytical Ultracentrifugation (SV-AUC)	Sedimentation Coefficient (S)	7:6:6	1.0-1.1	1.0-1.1	1.0-1.1	Yes/yes/yes
			Monomer (%)	7:6:6	98.3-100.0	99.1-100.0	98.3-100.0	Yes/yes/yes
			HMWS (%)	7:6:6	0.0-1.7	0.0-0.9	0.0-1.7	Yes*/yes/yes

	Tertiary Structure (protein structure)	Nuclear Magnetic Resonance Spectroscopy (NMR)		4:2:2	NMR 1D and 2D spectra	Similar NMR 1D and 2D spectra to US-licensed Neulasta	Similar NMR 1D and 2D spectra to US-licensed Neulasta	Yes/yes/yes
	Tertiary Structure (Melting Temperature, T _m)	Differential Scanning Calorimetry (°C)		10:10:10	69.5-69.7; 69.3-69.9 (US QR)	69.4-69.8	69.4-69.7	Yes/yes/yes
Product Related Substances and Impurities	Total Related Proteins	RP-HPLC (%)		10:10:10	2.4-2.8; 2.2-3.1 (US QR)	0.6-1.4	2.4-2.8	Yes*/yes/yes
	Total Charge Variants (acid variants)	IC-HPLC (%)		10:10:10	2.6-4.6; 1.4-6.1 (US QR)	0.2-3.0	2.3-4.2	Yes*/yes/yes
	Total Size Variants: Dimer; Other HMWS; Des-pegylated Species	SEC	Dimer (%)	10:10:10	1.6-1.7	0.3-0.6	1.4-1.7	Yes*/yes/yes
			Other HMWS (%)	10:10:10	0.2-0.4	<0.1-0.5	0.2-0.3	Yes/yes/yes
			Des-pegylated Species (%)	10:10:10	0.1	<0.1-0.2	0.1	Yes/yes/yes
			Total size variants (%)	10:10:10	1.9-2.1	0.3-1.3	1.7-2.1	Yes*/yes/yes
	Size Variants	Non-reducing SDS-PAGE (impurity bands are less than 1% standard solution)		9:6:6	<0.1%	<0.1%	<0.1%	Yes/yes/yes
	Residual PEG	RP-HPLC-ELSD (%)		7:10:10	0.019-0.033	0.01-0.038	0.018-0.034	Yes/yes/yes
	Oxidation	RP-HPLC (%)	Met127 (%)	10:10:10	1.5-1.7; 1.4-1.9 (US QR)	0.6-0.8	1.4-1.7	Yes*/yes/yes
		Glu-C peptide mapping	M122 (%)	7:6:6	<0.5	<0.5	<0.5	Yes/yes/yes
M127 (%)			7:6:6	<0.5	<0.5	<0.5	Yes/yes/yes	
M138 (%)			7:6:6	<0.5	<0.5	<0.5	Yes/yes/yes	
	Trp59 (%)	7:6:6	<0.5-0.9	<0.5-1.0	<0.5-0.9	Yes/yes/yes		
Deamidation	RP-HPLC (Gln108): (%) LOQ=0.3%		10:10:10	0.4-0.6	0.3-0.5%	0.4-0.5	Yes/yes/yes	

		IC-HPLC: LOQ=0.4%	RRT 0.85 (%): Gln68 deamidation	10:10:10	0.6-1.0	<0.4%	0.7-0.9	Yes*/yes/yes	
			RRT 0.89-0.90 (%): Gln71 and Gln174 deamidation	10:10:10	0.7-1.0	0.4-0.5	0.6-1.0	Yes*/yes/yes	
		Glu-C Peptide Mapping: LOQ 0.5%	Gln21 (%)	7:6:6	0.6-0.9	<0.5-0.7	0.5-0.8	Yes/yes/yes	
			Gln91 (%)	7:6:6	0.8-1.5	<0.5-1.0	0.7-1.2	Yes*/yes/yes	
			Gln120 (%)	7:6:6	<0.5	<0.5	<0.5	Yes/yes/yes	
			Gln135 (%)	7:6:6	<0.5	<0.5	<0.5	Yes/yes/yes	
	Reduced Species at RRT 1.05	RP-HPLC: (%) LOQ=0.3%		10:10:10	<0.3	<0.3	<0.3	Yes/yes/yes	
	Des-Pegylated Species at 1.04 (N-terminal des-pegylated, des-Met1 Species)	RP-HPLC: (%) LOQ=0.3%		10:10:10	0.4-0.8	<LOQ	0.4-0.8	Yes*/yes/yes	
		SEC-HPLC (%)		10:10:10	0.1	<0.1%-0.2	0.1	Yes/yes/yes	
	N-terminal Des-pegylated Species	Glu-C Peptide Mapping (%)		7:6:6	0.5	<0.5-0.5	0.5	Yes/yes/yes	
Drug Product-Related Attributes	Protein Concentration	UV-Visible Spectrometry (mg/mL)		5:15:15	9.87-10.1; 9.7-10.3 (US QR)	9.8-9.9	9.79-10.1	Yes/yes/yes	
	Deliverable Content	Protein Concentration x Deliverable Volume (mg)		5:10:10	6.1-6.4; 5.9-6.5 (US QR)	6.1-6.2	6.2-6.3	Yes/yes/yes	
	Deliverable Volume	USP <697> Ph. Eur. <2.9.17> (mL)		5:12:12	0.616-0.63	0.62-0.63	0.62-0.628	Yes/yes/yes	
	Subvisible Particles	Micro Flow Imaging (MFI)	≥ 2 µm		10:6:6	24472 – 35653	751– 12254	7128 – 78252	Yes*/yes/yes
			≥ 5 µm		10:6:6	2618 – 8250	143 – 2643	1139 – 15286	Yes/yes/yes
≥ 10 µm			10:6:6	149 – 1588	16 – 519	97 – 2351	Yes/yes/yes		
≥ 25 µm			10:6:6	2 – 38	0 – 18	0 – 16	Yes/yes/yes		

	pH	Ph. Eur. <2.2.3> and USP <791>	10:3:3	3.7	4.0-4.1	3.8-4.0	Yes/yes/yes
	Osmolality	Ph. Eur. <2.2.35> and USP <785> (mOsmol/kg)	8:5:5	297 – 310	267 – 309	296 – 311	Yes*/yes/yes
	Polysorbate 20	RP-HPLC (% w/v)	10:3:3	0.003	0.004-0.005	0.003-0.004	Yes/yes/yes
	Appearance, Color, and Clarity	Ph. Eur. <2.2.2> and <2.2.1>	10:3:3	Clear, colorless solution	Clear, colorless solution	Clear, colorless solution	Yes/yes/yes
	Visible Particles	USP <790> Ph. Eur. <2.9.20>	10:3:3	Practically free of visible particles	Practically free of visible particles	Practically free of visible particles	Yes/yes/yes

1. Yes = met acceptance criteria, Yes*= differences were noted but do not preclude a determination of highly similar and is further discussed below, No = did not meet acceptance criteria
2. Lower levels of impurities in the proposed biosimilar product do not impact a determination of highly similar when there is no expected impact on product quality, e.g. potency, or clinical performance.

Comparative Analytical Assessment Results

1. Functional Activity

Three functional assays were utilized to assess pegfilgrastim biological activity as part of the comparative analytical assessment, including in vitro cell-based proliferation assay, a competitive receptor binding assay (CRBA), and a Surface Plasmon Resonance (SPR) assay for determination of receptor binding affinity (KD and Relative KD) and the binding rate kinetics (k_{on} and k_{off}).

Assessor's Comment:

The applicant used an appropriate panel of tests for assessing functional activities.

1a. In-Vitro Cell-based Potency Assay

In vitro potency of PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U. was determined using a cell-based assay that measures the induction of receptor-activated proliferation of hematopoietic cells. The comparative analytical assessment included 10 PF-06881894 DP lots, 14 pegfilgrastim-U.S. lots, and 14 pegfilgrastim-E.U. lots. Ages at time of test for PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U. lots ranged from 4-27, 12-29, and 9-33 months, respectively. The in-vitro potency results were provided in Table 3.2.R.5.5-3 of the BLA but are not shown here for brevity. Graphical comparison of in vitro potency is shown below in the applicant's Fig. 3.2.R.5.5-1. A summary of the number of lots, mean, standard deviation and range is provided below in the applicant's Table 3.2.R.5.5-4.

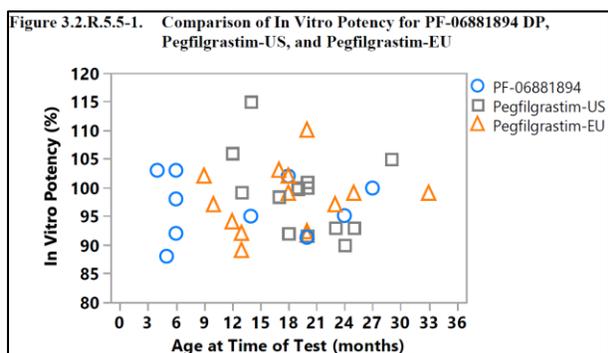


Table 3.2.R.5.5-4. Summary of Descriptive Statistics for In Vitro Potency for PF-06881894 DP, Pegfilgrastim-US, and Pegfilgrastim-EU

Product	Number of Lots Tested	In Vitro Potency (% Relative Potency)		
		Mean	Standard Deviation	Range
PF-06881894	10	97	5.3	88 – 103
Pegfilgrastim-US	14	99	6.8	90 – 115
Pegfilgrastim-EU	14	98	5.6	89 – 110

Pair-wise equivalence testing was performed for the three products. Two statistical methods were used based on how the equivalence margin was defined from the pegfilgrastim-U.S. reference product standard deviation: the conventional two one-sided test (TOST) for a fixed margin approach (TOST-1) and the modified approach for a random margin (TOST-2). For the imbalanced sample sizes (10 PF-06881894 lots vs. 14 pegfilgrastim-U.S. lots, 10 PF-06881894 lots vs. 14 pegfilgrastim-E.U. lots), the formulas for adjusted sample size were used for PF-06881894 as shown in Table 3.2.R.5.4-9 (data not shown). However, 14 pegfilgrastim-U.S. lots

vs. 14 pegfilgrastim-E.U. lots were balanced and no adjustment was required for sample sizes. The 90% confidence interval of the mean difference was used for the equivalence testing.

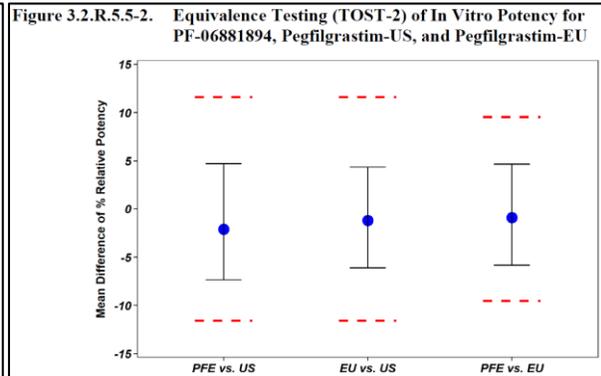
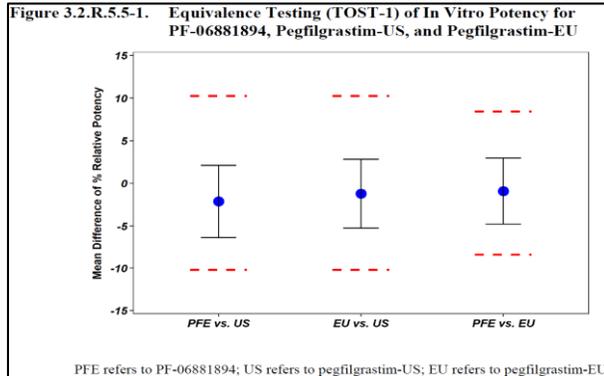


Table 3.2.R.5.5-1. Summary of In Vitro Potency Equivalence Testing (TOST-1) for PF-06881894 , Pegfilgrastim-US, and Pegfilgrastim-EU

Comparison	Number of Lots	Method	Mean Difference	90% Confidence Interval	Equivalence Margin ^a	Equivalent
PF-06881894 vs Pegfilgrastim-US	10, 14	TOST-1	-2.13	(-6.38, 2.12)	(-10.23, 10.23)	Yes
Pegfilgrastim-EU vs Pegfilgrastim-US	14, 14		-1.21	(-5.25, 2.82)	(-10.23, 10.23)	Yes
PF-06881894 vs Pegfilgrastim-EU	10, 14		-0.91	(-4.79, 2.96)	(-8.42, 8.42)	Yes

a. Upper and lower equivalence margins were defined as $\pm 1.5 SD_{ref}$ where SD_{ref} is the standard deviation of the pegfilgrastim-US or pegfilgrastim-EU lot results.

Table 3.2.R.5.5-2. Summary of In Vitro Potency Equivalence Testing (TOST-2) for PF-06881894 , Pegfilgrastim-US, and Pegfilgrastim-EU

Comparison	Number of Lots	Method	Mean Difference	90% Confidence Interval	Equivalence Margin ^a	Equivalent
PF-06881894 vs Pegfilgrastim-US	10, 14	TOST-2	-2.13	(-7.37, 4.72)	(-11.59, 11.59)	Yes
Pegfilgrastim-EU vs Pegfilgrastim-US	14, 14		-1.21	(-6.12, 4.33)	(-11.59, 11.59)	Yes
PF-06881894 vs Pegfilgrastim-EU	10, 14		-0.91	(-5.84, 4.66)	(-9.54, 9.54)	Yes

a. Upper and lower equivalence margins were defined as $\pm 1.7 SD_{ref}$ where SD_{ref} is the standard deviation of the pegfilgrastim-US or pegfilgrastim-EU lot results.

Assessor’s Comment:

The potency results were similar among the three products, independent of product age. The PF-06881894 lots and reference product lots were selected randomly and covered the proposed product shelf-life of 36 months, which is appropriate.

The potency results for 9 out of 10 PF-06881894 lots (88 – 103%) and 13 out of 14 pegfilgrastim-E.U. lots (89 – 110%) are within the range of pegfilgrastim-U.S. lots (90 – 115%). The single lots of PF-06881894 and pegfilgrastim-E.U. outside the range of pegfilgrastim-U.S. are not meaningful because the differences are very small and the results passed equivalence testing. The distribution of the in vitro potency assay data in Figure 3.2.R.5.5-1 above did not show any trends indicating this attribute is stable over time.

The applicant indicated that the conventional TOST-1 method was used as a default method. The alternative TOST-2 method was used to support the analysis because this method mitigates the impact on both the reduction of power and the inflation of the type I error rate due to small number of lots. The multiplier of 1.5 for TOST-1 method was used to achieve the power of at

least of 85% for sample sizes greater than 10 lots for each product as recommended by Tsong (Tsong et al, 2017). In the TOST-2 method, the multiplier was increased from 1.5 to 1.7 to ensure the power of at least 85% for the number of lots different from sample size of 10 as suggested by Dong and Wen (Dong & Bian et al, 2017 and Weng et al 2018). Therefore, the selection of different multipliers for different equivalency tests is reasonable. The 90% confidence intervals of mean difference fall within the corresponding equivalence acceptance limits for both the TOST-1 and TOST-2 methods. CMC stats also confirmed that the potency passed the 3-ways equivalence testing using TOST-1 and TOST-2 methods.

1b. Competitive Receptor Binding Assay

The binding of pegfilgrastim to the G-CSF receptor was measured using the Competitive Receptor Binding Assay (CRBA), which measures the binding of biotin-labeled pegfilgrastim to an immobilized G-CSF receptor. Results are expressed as percent relative binding based on direct comparison of the dose response curves of PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U. to the dose response curve for the PF-06881894 reference standard (lot ASN107). Ages at time of test for PF-06881894, pegfilgrastim-U.S. lots, and pegfilgrastim-E.U. lots were estimated to be 3-30, 12-30, and 9-33 months, respectively. Graphical comparisons of competitive receptor binding results of PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U. lots are shown below in the applicant’s Figure 3.2.R.5.5-9. The upper and lower limits of quality range were defined as Mean ± 3xSD of the pegfilgrastim-U.S. lots. A summary of the number of lots, mean, standard deviation, range, and quality range is provided below in the applicant’s Table 3.2.R.5.5-9.

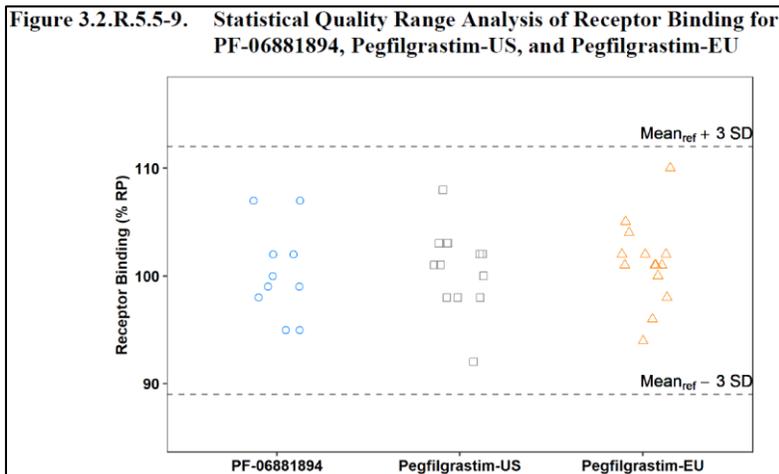


Table 3.2.R.5.5-9. Summary of Statistical Quality Range Analysis for Receptor Binding for PF-06881894, Pegfilgrastim-US, and Pegfilgrastim-EU

Test Articles	Number of Lots Tested	Receptor Binding (% Relative Potency)				Lots within Quality Range (%)
		Mean	Standard Deviation	Range	Quality Range	
PF-06881894	10	100	4.2	95 – 107	NA	100
Pegfilgrastim-EU	14	101	3.8	94 – 110	NA	100
Pegfilgrastim-US	13	101	3.8	92 – 108	89 – 112	NA

NA, not applicable

Assessor’s Comments:

The results of receptor binding assay were similar among the three products, independent of product age. The use of multiplier $k=3$ for quality range analysis translates to acceptance criteria of 89 – 112% relative potency, which is a conservative relative potency range when both

assay and manufacturing variability are considered. No meaningful differences in relative potency ranges were observed between PF-06881894 lots (95-107%), pegfilgrastim-U.S. lots (92-108%), and pegfilgrastim-E.U. lots (94-110%). The quality range analysis showed that 100% of the PF-06881894 lots and the pegfilgrastim-E.U. lots are within the quality range of pegfilgrastim-U.S. lots.

1c. Surface Plasmon Resonance (SPR) Assay

Changes in receptor binding affinity parameters and binding rates are indicative of structural changes that may impact receptor binding. Graphical comparisons of Relative K_D , K_{on} , K_{off} , K_D are provided below in the applicant's Figures 3.2.R.5.5-10, 3.2.R.5.5-3, 3.2.R.5.5-4, and 3.2.R.5.5-5 below, respectively. Ages at time of test for PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U. lots were estimated to be 3-25, 12-29, and 9-25 months, respectively. Summaries of the number of lots, mean, standard deviation, range, or quality range for each parameter or kinetics are provided below in the applicant's Tables 3.2.R.5.5-8, 3.2.R.5.5-9, and 3.2.R.5.5-10. Quality range analysis was only conducted for the Relative K_D .

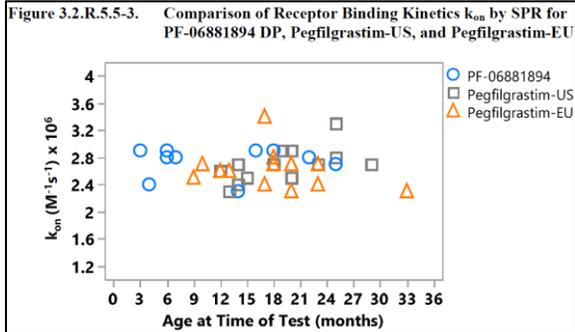
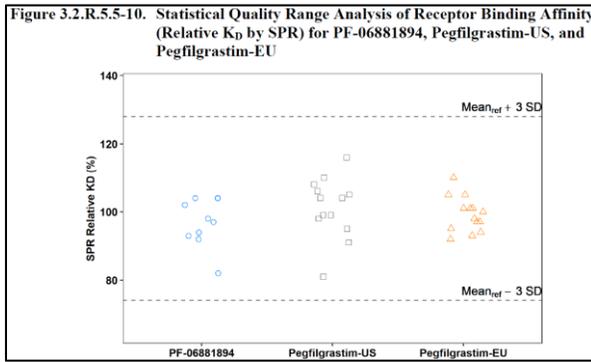


Table 3.2.R.5.5-8. Summary of Descriptive Statistics for Receptor Binding Kinetics k_{on} for PF-06881894 DP, Pegfilgrastim-US and Pegfilgrastim-EU

Product	Number of Lots Tested	$k_{on} \times 10^6 (M^{-1}s^{-1})$		
		Mean	Standard Deviation	Range
PF-06881894	10	2.7	0.22	2.3 - 2.9
Pegfilgrastim-US	13	2.7	0.26	2.3 - 3.3
Pegfilgrastim-EU	14	2.6	0.28	2.3 - 3.4

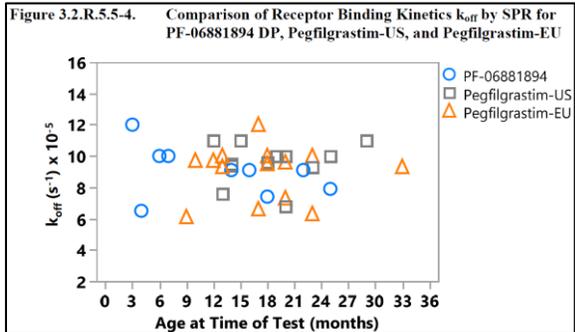


Table 3.2.R.5.5-9. Summary of Descriptive Statistics for Receptor Binding Kinetics k_{off} for PF-06881894 DP, Pegfilgrastim-US and Pegfilgrastim-EU

Product	Number of Lots Tested	$k_{off} \times 10^5 (s^{-1})$		
		Mean	Standard Deviation	Range
PF-06881894	10	9.1	1.56	6.5 - 12
Pegfilgrastim-US	13	9.6	1.24	6.8 - 11
Pegfilgrastim-EU	14	9.0	1.71	6.1 - 12

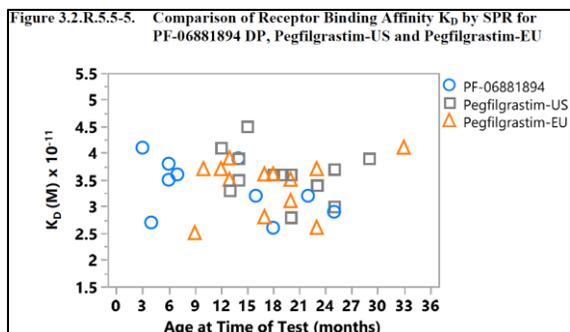


Table 3.2.R.5.5-10. Summary of Descriptive Statistics for Receptor Binding Affinity K_D for PF-06881894 DP, Pegfilgrastim-US and Pegfilgrastim-EU

Product	Number of Lots Tested	$K_D \times 10^{-11}$ (M)		
		Mean	Standard Deviation	Range
PF-06881894	10	3.4	0.51	2.6 – 4.1
Pegfilgrastim-US	13	3.6	0.45	2.8 – 4.5
Pegfilgrastim-EU	14	3.4	0.48	2.5 – 4.1

Assessor’s Comment:

The SPR results were similar among the three products, independent of product age. The relative K_D for PF-06881894 (82 – 104%) and pegfilgrastim-E.U. (92 – 110%) lots fall within the relative K_D range for pegfilgrastim-U.S. lots (81 – 116%). The applicant proposed using a multiplier $k=3$ for quality range analysis of Relative K_D which translates to a range of 74-128%. This range may be slightly broader than preferred; however, the data were all within 81 – 116% so the multiplier isn’t relevant. The dataset of pegfilgrastim-U.S. lots is normally distributed. The ranges of PF-06881894 lots and pegfilgrastim-E.U. lots are within the quality range of pegfilgrastim-U.S. lots. The comparison of G-CSF receptor affinity (relative K_D and K_D), and on and off rates (K_{on} and K_{off}) support a determination that the higher order structure required for binding to the receptor and receptor binding kinetics are similar between PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U. The distribution of on and off rates and K_D showed no trends indicating this attribute is stable over time.

The in-house PF-06881894 reference standard (RS) lot ASN107 was used for the three functional activity assays throughout the comparative analytical assessment. RS lot ASN107 was produced from PF-06881894 DS lot 1805074, which was manufactured using the commercial DS manufacturing process. The potency of RS lot ASN107 was calibrated against the NIBSC 12/188 pegfilgrastim international standard. The qualification data demonstrated that the relative potency of ASN107 was comparable to the NIBSC standard as discussed in Section 3.2.S.5 Reference Standard or Materials.

Functional Assays Summary:

Results from multiple orthogonal analytic studies to assess functional activities support a determination that PF-06881894 is highly similar to pegfilgrastim-U.S. and that the analytical part of the scientific bridge was established.

3.2.R.5.5.1 Structural Analysis

The structural characterization includes the evaluation of primary structure and high order structure, which covers secondary and tertiary structure.

3.2.R.5.5.1.1 Primary Structure:

The analysis of primary structure included assessment of amino acid sequence, pegylation site and linker composition, molecular weight (dispersity), verification of one free cysteine residue (Cys18), and isoelectric point (pI).

Assessor comment:

The applicant used an appropriate panel of tests for assessing primary structure.

3.2.R.5.5.1.1 Amino Acid Sequence by RP-UPLC

The amino acid sequences of PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U. lots were determined using the Glu-C peptide mapping method. Glu-C digested samples were separated by the RP-UPLC and the comparative total ion chromatograms (TIC) results are shown below in the applicant's Figure 3.2.R.5.5-1. A comparison of theoretical monoisotopic mass and measured monotonopic mass for each peptide in the three products are presented below in the applicant's Table 3.2.R.5.5-3.

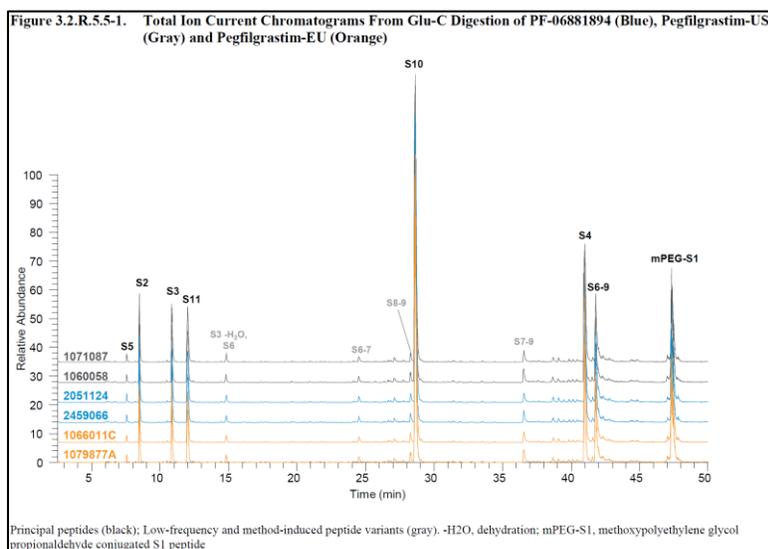


Table 3.2.R.5.5-3. Primary Structure Confirmation by Glu-C Peptide Mapping of PF-06881894, Pegfilgrastim-US, and Pegfilgrastim-EU

Product	Lot	Age at Time of Test (months) ^a	Date of Test	S1	S2	S3	S4	S5	S6-9	S10	S11
Theoretical	NA	NA	NA	2131.106	1511.806	1533.732	4941.629	501.244	2834.368	4025.077	1437.820
PF-06881894	2051124	42	Jun 2018	2131.107	1511.806	1533.730	4941.627	501.244	2834.365	4025.063	1437.821
	2573125	30	Jun 2018	2131.107	1511.806	1533.731	4941.624	501.243	2834.361	4025.059	1437.820
	2459066	24	Jun 2018	2131.109	1511.807	1533.730	4941.632	501.244	2834.364	4025.068	1437.822
	213047	14	Jun 2018	2131.107	1511.807	1533.731	4941.632	501.244	2834.360	4025.067	1437.821
	3058V	6	Nov 2018	2131.106	1511.806	1533.732	4941.632	501.244	2834.369	4025.077	1437.820
	4058V	5	Nov 2018	2131.108	1511.806	1533.733	4941.634	501.244	2834.369	4025.075	1437.821
Pegfilgrastim-US	2068V	5	Nov 2018	2131.106	1511.806	1533.733	4941.631	501.244	2834.372	4025.074	1437.821
	1060058	36	Jun 2018	2131.107	1511.805	1533.727	4941.621	501.243	2834.352	4025.070	1437.819
	1071087	29	Jun 2018	2131.105	1511.806	1533.727	4941.620	501.243	2834.359	4025.064	1437.820
	1072044	28	Nov 2018	2131.107	1511.806	1533.732	4941.633	501.243	2834.367	4025.081	1437.821
	1078875	25	Jun 2018	2131.105	1511.805	1533.729	4941.626	501.243	2834.360	4025.062	1437.819
	1083446	19	Nov 2018	2131.108	1511.806	1533.733	4941.637	501.244	2834.371	4025.074	1437.821
Pegfilgrastim-EU	1094104	17	Nov 2018	2131.107	1511.806	1533.732	4941.634	501.243	2834.368	4025.077	1437.821
	1066011C	33	Jun 2018	2131.106	1511.806	1533.730	4941.622	501.243	2834.358	4025.064	1437.820
	1069490C	30	Jun 2018	2131.107	1511.805	1533.730	4941.627	501.243	2834.356	4025.061	1437.820
	1079877A	20	Jun 2018	2131.108	1511.807	1533.732	4941.635	501.244	2834.366	4025.071	1437.822
	1090139B	12	Nov 2018	2131.108	1511.807	1533.732	4941.630	501.243	2834.370	4025.082	1437.821
	1093686K	11	Nov 2018	2131.108	1511.806	1533.733	4941.635	501.243	2834.370	4025.075	1437.821
1094582	9	Nov 2018	2131.107	1511.806	1533.732	4941.637	501.244	2834.370	4025.081	1437.821	

NA, not applicable

a. Date of manufacture for pegfilgrastim-US and pegfilgrastim-EU is estimated by subtracting 36 months from the date of expiry.

Assessor's Comment:

Amino acid sequence analysis showed that the amino acid sequences are identical between the three products. All the resolved peptides have the same retention time and similar intensity between the three products. The measured monoisotopic mass of all the separated peptides are consistent with the theoretical mass of the expected sequences for all the separated peptides with a mass accuracy of 10 parts per million (ppm) or lower for all the three products. Appropriate lots were used in the studies. The PF-06881894 lots 2051124 and 2459066 used for amino acid sequence analyses were also used in comparative clinical as well as stability studies. The pegfilgrastim-U.S. lot 1071087 used in amino acid sequence analyses was used in the comparative clinical study. Fewer than 10 pegfilgrastim-U.S. and pegfilgrastim-E.U. lots were analyzed. This is acceptable because amino acid sequence is identical for the products and statistical analyses are not needed.

As discussed below, the conjugation site for pegylation is located on the N-terminal S1 peptide for all the products, which was further confirmed by focused peptide mapping of the pegylated S1 peptide.

3.2.R.5.5.1.1.2 Pegylation Site and Linker Composition by Peptide Mapping of Pegylated Peptide

The pegylation site and linker composition were assessed using the Glu-C peptide mapping of pegylated peptide. The mass spectra of the mPEG-S1 peptide peak containing fragment ions corresponding to PEG 350-1200m/z and PEG-S1 (1240-2000m/z) with different number of ethylene oxide (EO) units produced by Source Induced Dissociation (SID) of the intact mPEG-S1 peptide are provided in the applicant's Figures 3.2.R.5.5-4 and 3.2.R.5.5-5 (data not shown). The mass spectrum results showed that the major S1 peptide y-ion fragments at N-terminus of Pro3 (y18 ion) and Pro11 (y10 ion) and additional y-ion fragments containing the S1 peptide sequence (y4-9, y15-16). The y-ion fragment spectra are consistent with pegylation at the N-terminus of the S1 peptide. In the applicant's Figure 3.2.R.5.5-6, the MS results from further fragmentation of PEG-S1 peptide with 20 EO units by Higher-energy Collisional Dissociation (HCD) are consistent with a-ion fragments with corresponding 20 EO units attached to peptide sequence position 1, which indicates that the pegylation site is at the N-terminal amine of Met1. The mass of the PEG-S1 fragments are consistent with the expected mass of the -CH₂CH₂CH₂-linker.

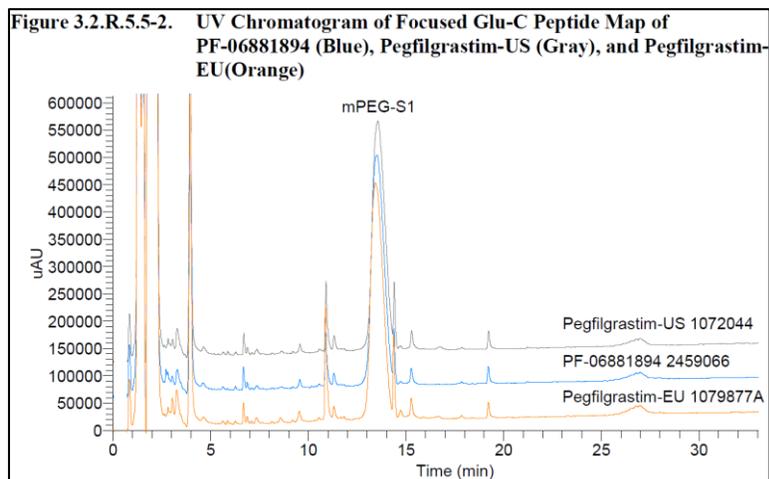
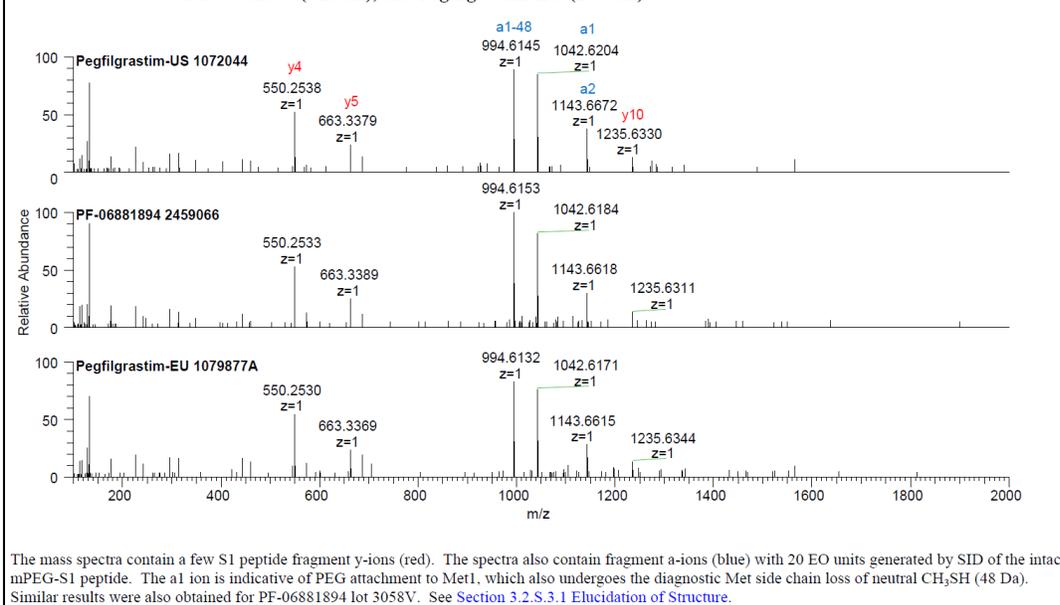


Figure 3.2.R.5.5-6. MS/MS Spectra of PEG-S1 Peptide Fragment Ion with 20 EO Units in Pegfilgrastim-US(Top), PF-06881894 (Middle), and Pegfilgrastim-EU (Bottom)



The mass spectra contain a few S1 peptide fragment y-ions (red). The spectra also contain fragment a-ions (blue) with 20 EO units generated by SID of the intact mPEG-S1 peptide. The a1 ion is indicative of PEG attachment to Met1, which also undergoes the diagnostic Met side chain loss of neutral CH₃SH (48 Da). Similar results were also obtained for PF-06881894 lot 3058V. See Section 3.2.S.3.1 Elucidation of Structure.

Assessor's Comment:

The pegylation site and linker composition were similar among the three products, independent of product age. The MS results of pegylated S1 peptide confirmed that the S1 peptide sequence, pegylation site at the N-terminal amine of Met1, and linker composition of -CH₂CH₂CH₂- are the same for the three products.

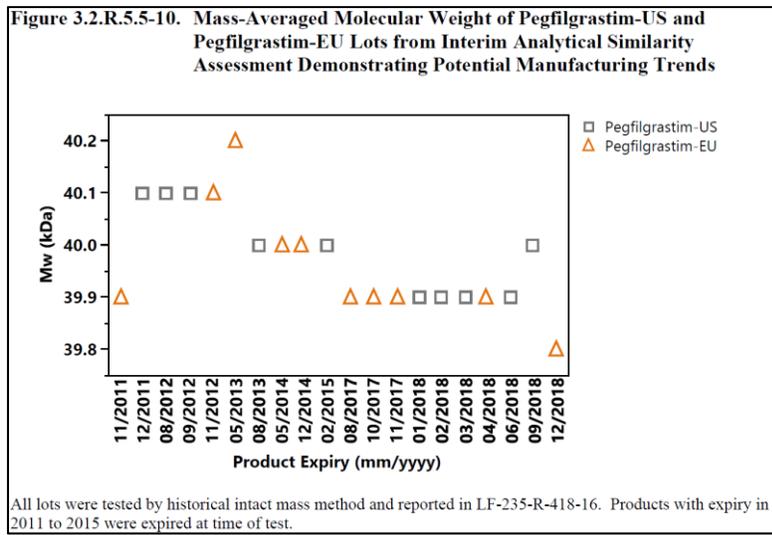
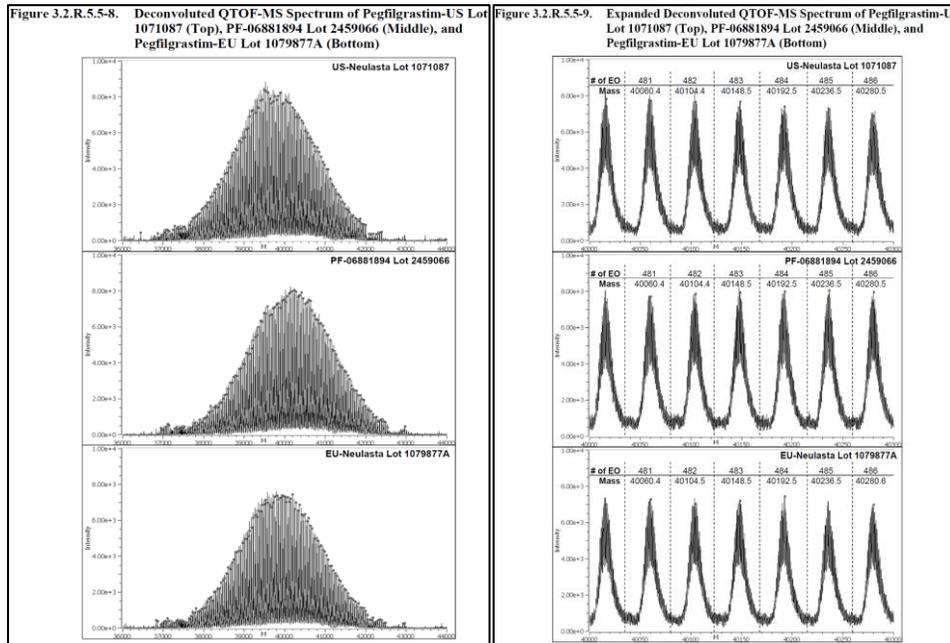
3.2.R.5.5.1.1.3 Molecular Weight by Intact Mass

Intact mass was measured by RP-UPLC-MS for PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U. lots. Representative raw mass spectra were provided in the applicant's Figure 3.2.R.5.5-7 (data not shown). The representative full scale deconvoluted and expanded deconvoluted mass spectra are shown below in the applicant's Figures 3.2.R.5.5-8 and 3.2.R.5.5-9. The deconvoluted MS results show that the mass of pegylated protein range from 37-42.5 kDa with different number of EO units. The expanded mass spectra show that the conjugated mPEG moieties in each pegylated protein contain 480-486 EO units. The observed mass of 40148.4 Da for PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U. with 483 EO units is consistent with the theoretical mass of pegfilgrastim of 401481.1 Da.

The data presented below in the applicant's Figure 3.2.R.5.5-8 shows a slight shift in the molecular weight of PF-06881894, which caused the calculated mass-averaged molecular weight of PF-06881894 lots to be higher by approximately 0.3-0.4 kDa or 8 EO units as compared to those of pegfilgrastim-U.S. lots and pegfilgrastim-E.U. lots. The applicant stated that due to the long shelf-life of mPEG, analysis of pegfilgrastim-U.S. and pegfilgrastim-E.U. lots cover a broad manufacturing history. As indicated in a BPD Type 2 Briefing Document for IND 124793 (SDN 0009), the data showed an apparent shift towards lower molecular weight with lot-to-lot difference of 0.4 kDa for pegfilgrastim-U.S. and pegfilgrastim-E.U. lots manufactured from November 2011 to December 2018 as shown in Figure 3.2.R.5.5-10 below. Therefore, the

observed shift in the mass distribution is due to shift in the number of EO units but not due to product degradation.

The molecular-weight dispersity is used to evaluate the heterogeneity of sizes of molecules and defined as the mass-averaged molecular weight (Mw) vs the number-average molecular weight (Mn). The molecular weight dispersity (Mw/Mn) was 1.001 for all PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U. lots. A comparison of the molecular weights for three products is summarized in Table 3.2.R.5.5-5.



Attribute	Product	Number of Lots	Mean	Standard Deviation	Range
Average Mass (483 EO Units, Da)	PF-06881894	7	40148.4	0.05	40148.4-40148.5
	Pegfilgrastim-US	6	40148.4	0.05	40148.4-40148.5
	Pegfilgrastim-EU	6	40148.4	0.08	40148.3-40148.5
Mass-Averaged MW (kDa)	PF-06881894	7	40.18	0.026	40.15-40.21
	Pegfilgrastim-US	6	39.85	0.066	39.77-39.93
	Pegfilgrastim-EU	6	39.82	0.073	39.77-39.92
Molecular Weight Dispersity	PF-06881894	7	1.001	0.0000	1.001-1.001
	Pegfilgrastim-US	6	1.001	0.0000	1.001-1.001
	Pegfilgrastim-EU	6	1.001	0.0000	1.001-1.001

EO, ethylene oxide; MW, molecular weight

Assessor's Comment:

A minor difference in MW indicating the use of mPEG raw material with slightly lower numbers of EO repeats in the manufacture of PF-06881894 as compared to pegfilgrastim-U.S. and pegfilgrastim-E.U. was observed. However, this difference does not preclude a determination that PF-06881894 and pegfilgrastim-U.S. are highly similar or a determination that the analytical part of the scientific bridge was established. The 20 kDa mPEG contains a mixture of mPEG molecules with variable EO repeat units (44 Da/EO unit). Data indicate that mPEG raw materials with lower numbers of EO units were used to manufacture the pegfilgrastim-U.S. and pegfilgrastim-E.U. lots compared to those used in PF-06881894 lots in the comparative analytical studies. However, the results provided in the applicant's Figure 3.2.R.5.5-10 above indicate that there is variability in average mPEG MW used to manufacture pegfilgrastim-U.S. and pegfilgrastim-E.U. over time, most likely reflecting variability between mPEG lots. mPEG impacts pharmacokinetics and potency of filgrastim. The use of mPEG with different EO units had no impact on potency of the products as demonstrated in the comparative analytical assessment. In addition, the clinical pharmacology reviewer confirmed that the comparative clinical study demonstrated the equivalence of PD and PK between PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U. The molecular weight of the product with 483 EO units and molecular weight dispersity are nearly identical. The molecular weight of the intact molecule is similar with minor difference due to the use of mPEG raw material with the different EO units between PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U.

3.2.R.5.5.1.1.4 Free Thiol by Ellman's Assay

The number of free thiol in PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U. lots were determined by Ellman's Assay. The results were provided in the applicant's Table 3.2.R.5.5-6 (data not shown). A comparison of the mean, standard deviation, and range of free thiol results for the three products are summarized below in the applicant's Table 3.2.R.5.5-7.

Product	Number of Lots	Free Thiol (mol Thiol/mol Pegfilgrastim)		
		Mean	Standard Deviation	Range
PF-06881894	10	1.09	0.061	1.01-1.17
Pegfilgrastim-US	9	1.07	0.054	1.00-1.15
Pegfilgrastim-EU	9	1.08	0.052	1.01-1.15

Assessor's Comment:

Free-thiol content was similar among the three products, independent of product age.

3.2.R.5.5.1.1.5 Isoelectric Point by Capillary Isoelectric Focusing

The isoelectric point (pI) for PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U. lots was measured by the capillary isoelectric focusing (cIEF) method. Representative electropherograms are shown below in the applicant's Figure 3.2.R.5.5-11. A comparison of the number of lots, mean, standard deviation, and range of pI results for three products are summarized below in the applicant's Table 3.2.R.5.5-9.

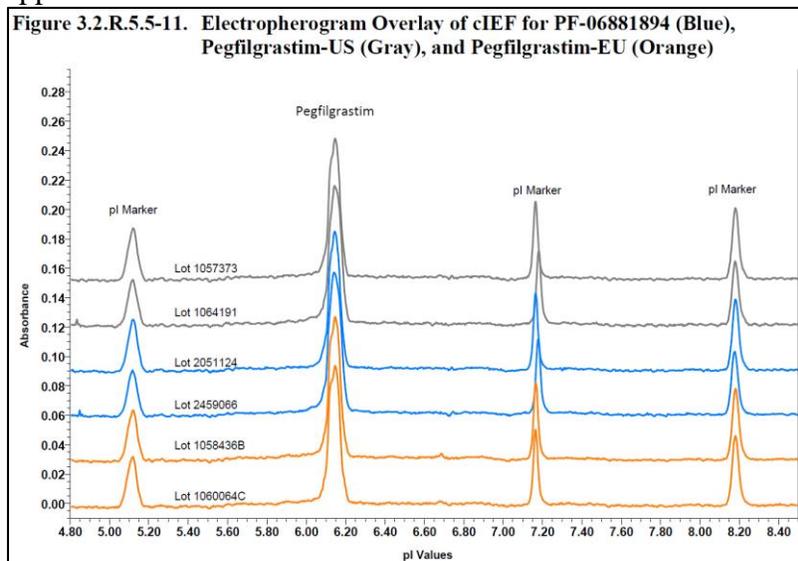


Table 3.2.R.5.5-9. Comparison of Isoelectric Point for PF-06881894, Pegfilgrastim-US, and Pegfilgrastim-EU

Product	Number of Lots	pI		
		Mean	Standard Deviation	Range
PF-06881894	10	6.17	0.030	6.14 – 6.22
Pegfilgrastim-US	6	6.17	0.031	6.14 – 6.22
Pegfilgrastim-EU	6	6.17	0.031	6.14 – 6.22

Assessor's Comment:

The pI was similar among the three products, independent of product age. Appropriate lots were used in the studies. Pegfilgrastim-U.S. lot 1057373 was used in the comparative clinical and nonclinical studies, and pegfilgrastim-E.U. lot 1061466C and PF-05881894 lots 2051124 and 2459066 were used in the comparative clinical and stability studies.

Primary Structure Summary

Results from multiple orthogonal analytical studies to assess primary structure support a determination that the PF-06881894 is highly similar to pegfilgrastim-U.S., and a determination that the analytical part of the scientific bridge was established.

3.2.R.5.5.1.2 Higher Order Structure

The higher order structure comparison of PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U. lots was assessed using multiple orthogonal LC-MS and biophysical methods. The secondary structure was determined by Far-UV circular dichroism (CD) and the tertiary structure was evaluated by peptide mapping, hydrogen-deuterium exchange (HDX), analytical ultracentrifugation for sedimentation velocity (SV-AUC), differential scanning calorimetry (DSC), for melting temperature (T_m), and nuclear magnetic resonance spectroscopy (NMR).

Assessor comment: The applicant used an appropriate panel of tests for assessing HOS.

3.2.R.5.5.1.2.1 Secondary Structure by Far-UV CD

Secondary structure elements, α -helix, β -sheet, β -turn, and random coil were measured by Far-UV CD. The representative spectra for PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U. lots are shown below in the applicant's Figure 3.2.R.5.5-12. The percent of α -helix, β -sheet, β -turn, and random coil for each lot of the three products are provided below in the applicant's Table 3.2.R.5.5-12. A comparison of the mean, standard deviation, and range of each secondary structure element for the three products are summarized below in the applicant's Table 3.2.R.5.5-13.

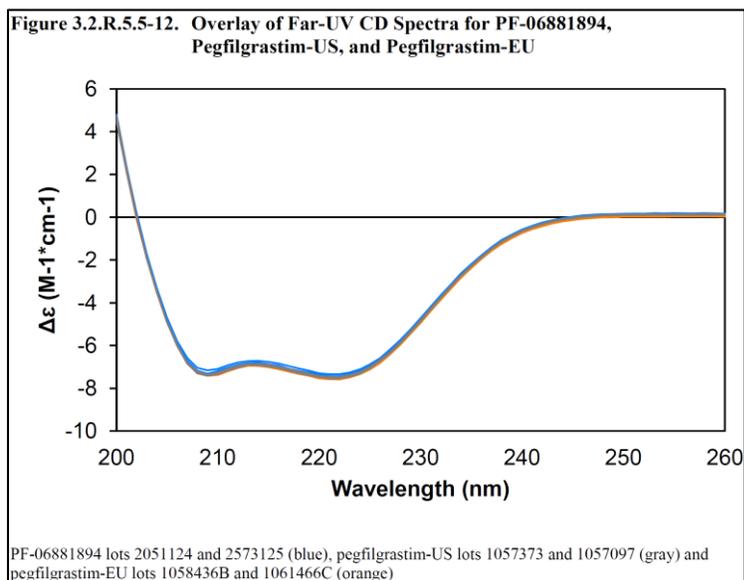


Table 3.2.R.5.5-13. Comparison of Percent Secondary Structure Content Determined by Far-UV CD

Attribute	Product	Mean (%)	Standard Deviation (%)	Range (%)
α -helix	PF-06881894	73	0.8	72-74
	Pegfilgrastim-US	74	0.5	73-74
	Pegfilgrastim-EU	74	1.0	72-75
β -structure	PF-06881894	16	0.5	15-16
	Pegfilgrastim-US	15	0.5	15-16
	Pegfilgrastim-EU	15	0.5	15-16
Random Coil	PF-06881894	14	0.5	13-14
	Pegfilgrastim-US	13	0.5	13-14
	Pegfilgrastim-EU	14	0.5	13-14

Assessor's Comment:

The ranges for β -structure and random coil are essentially the same between the three products. The percent α -helix, β -pleated sheet, and random coil was consistent in products of different ages.

Appropriate lots were used for these studies. PF-05881894 lots 2051124 and 2573125 were used in the comparative clinical and/or stability studies. Pegfilgrastim-U.S. lot 1057373 was used in the comparative clinical and nonclinical studies. Pegfilgrastim-E.U. lot 1058436B was used in

the comparative non-clinical and stability studies, and pegfilgrastim-E.U. lot 1061466C was used in the comparative clinical and stability studies.

3.2.R.5.5.1.2.2 Disulfide Linkages by Disulfide Mapping

The disulfide linkages in PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U. were determined using a non-reduced peptide mapping method. The samples were digested by pepsin and separated by RP-UPLC. A comparison of representative total ion chromatograms (TIC) is provided below in the applicant's Figure 3.2.R.5.5-13. A comparison of the theoretical monoisotopic mass and the measured monoisotopic mass of the non-reduced peptides for each lot is provided below in the applicant's Table 3.2.R.5.5-14.

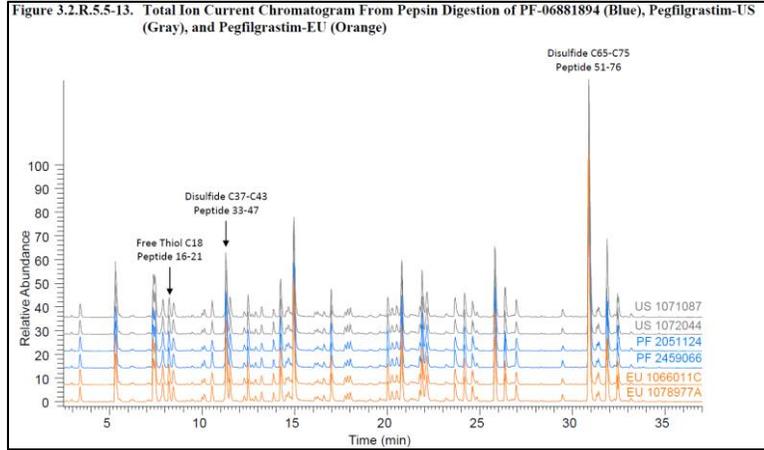


Table 3.2.R.5.5-14. Monoisotopic Mass of Cysteine Containing Pegfilgrastim Non-Reduced Peptic Peptides^a

Product	Lot Number	Age at Time of Test ^b (months)	Date of Test	Free Thiol Cys18 (Peptide 16-21)	Disulfide Cys37-Cys43 (Peptide 33-47)	Disulfide Cys65-Cys75 (Peptide 51-76)
Theoretical	NA	NA	NA	732.384	1788.817	2616.319
PF-06881894	2051124	45	Sep 2018	732.385	1788.820	2616.323
	2459066	27	Sep 2018	732.384	1788.819	2616.323
	3058V	4	Sep 2018	732.384	1788.818	2616.319
Pegfilgrastim-US	1071087	32	Sep 2018	732.383	1788.816	2616.318
	1072044	26	Sep 2018	732.385	1788.820	2616.321
	1083446	17	Sep 2018	732.385	1788.823	2616.326
Pegfilgrastim-EU	1066011C	36	Sep 2018	732.386	1788.821	2616.327
	1069490C	33	Sep 2018	732.385	1788.820	2616.325
	1079877A	23	Sep 2018	732.383	1788.817	2616.320

NA, not applicable

a. Pepsin digestion results in a mixture of peptides containing the same cysteine residues for each disulfide linkage. The most abundant peptic peptide containing cysteine residues were chosen for comparison.

b. Date of manufacture for pegfilgrastim-US and pegfilgrastim-EU is estimated by subtracting 36 months from the date of expiry.

Assessor's Comment:

The peptide mapping results show that the same disulfide bonds are present in the three products. The TIC chromatograms show the same retention time and similar intensity between the three products. In addition, the measured masses of non-reduced cysteine containing peptides are consistent with the theoretical masses of the sequences. Monoisotopic mass shows the products have the same non-oxidized free thiol at Cys18 and the same disulfide linkages—four oxidized cysteines at Cys37-Cys43 and Cys65 and Cys75, which form 2 disulfide bonds. The applicant indicated that no peptides related to mismatched disulfide bonds were observed based on peptide mapping. Disulfide bonding was similar among the three products, independent of

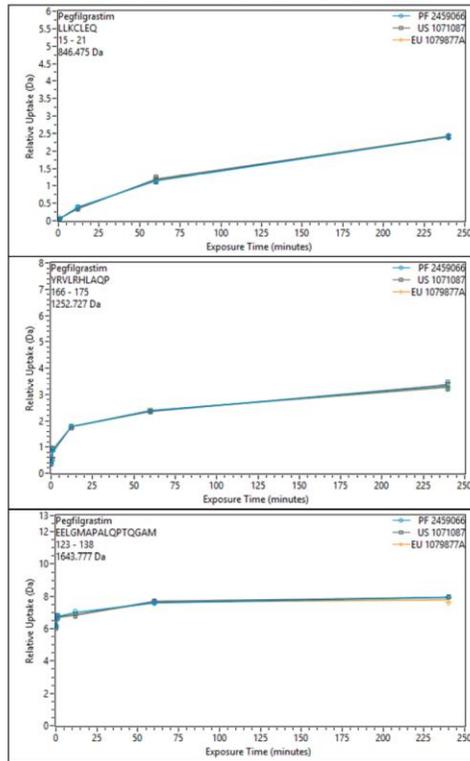
product age. Pegfilgrastim-U.S. lots 1071087 and 1072044, and PF-06881894 lots 2051124 and 2459066 were used in the comparative clinical study.

3.2.R.5.5.1.2.3 Structure Dynamics by Hydrogen-Deuterium Exchange

Structure dynamics of pegfilgrastim based on the overall deuterium content of molecules that have undergone hydrogen-deuterium exchange (HDX) was determined by HDX-mass spectrometry (MS). The level of deuterium incorporated for each region of the pegfilgrastim sequence is calculated using the average mass difference between the deuterated peptide at a given time and T0 time point. The number of exchanged protons measured at the different time point for every digested peptide was plotted as a deuterium uptake curve for the three products. The plots for three different digested peptides with slow, medium, and fast rates of exchange are shown below in the applicant's Figure 3.2.R.5.5-14.

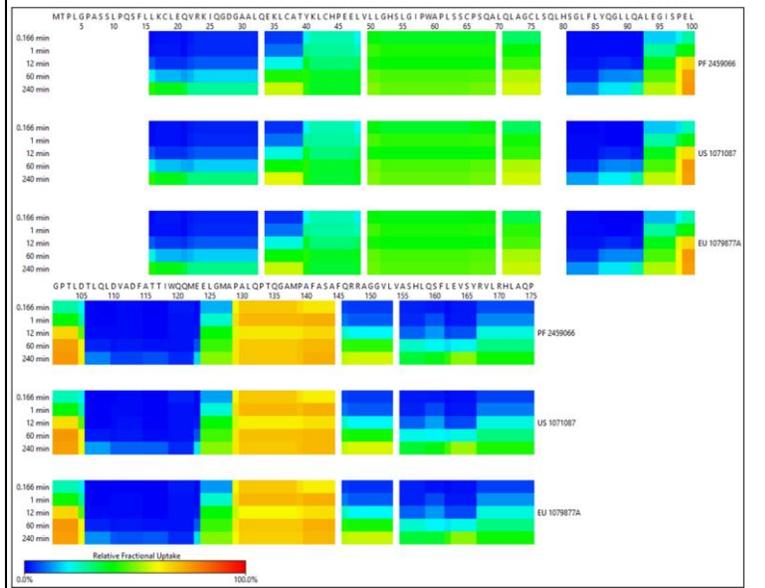
Deuterium uptake curves were used by the applicant to produce a heat map diagrams, which show the percent deuterium uptake relative to the T0 as a function of time. The extent of deuteration for each peptide is presented by a color scale in the heat map. An example of heat maps for the peptides from residues 15-100 and residues 101-175 is shown below in the applicant's Figure 3.2.R.5.5-15. The applicant stated that 90.3% of the pegfilgrastim protein sequence is covered except for the N-terminal 14 amino acids due to the pegylation and residues 77-79 due to poor retention of the small peptide on the C18 RP-HPLC column, and each N-terminal amino acid of the peptides with rapid back exchange after backbone amide cleavage. The region lacking peptide coverage and N-terminal amino acids are shown in white in the heat map.

Figure 3.2.R.5.5-14. Deuterium Uptake Curves of Peptides with Slow (Top), Medium (Middle) and Fast (Bottom) Exchange for PF-06881894 (Blue), Pegfilgrastim-US (Gray) and Pegfilgrastim-EU (Orange)



Top, peptide 15-21 corresponding to slow exchange; middle, peptide 167-175, corresponding to medium exchange; bottom, peptide 123-138 corresponding to fast exchange. The deuterium uptake curves of pegfilgrastim-US lot 1071087 (gray) and pegfilgrastim-EU lot 1079877A are superimposed with those of PF-06881894 DP lot 2459066 (blue) and therefore not readily visible.

Figure 3.2.R.5.5-15. HDX-MS Heat Maps for PF-06881894, Pegfilgrastim-US and Pegfilgrastim-EU



Assessor's Comment:

The deuterium uptake curve and heat map results indicate consistent higher order structure for PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U. lots. The deuterium uptake curves are superimposable at slow, medium, and fast rates of exchange for PF-06881894, pegfilgrastim-U.S. and pegfilgrastim-E.U. lots. The blue regions for the slow rate of exchange area in pegfilgrastim represent the α -helices secondary structure in pegfilgrastim, which are consistent with the X-ray crystallography structure reported in literature. The applicant also provided differential heat map results to show the differences in the rates of deuterium uptake at all the time points between the product samples are less than 5%. HDX results were similar among the three products, independent of product age.

3.2.R.5.5.1.2.4 Sedimentation Coefficient by Sedimentation Velocity Analytical Ultracentrifugation

The sedimentation coefficient (S) of pegfilgrastim was measured by the sedimentation velocity analytical ultracentrifugation (SV-AUC) using the C (s) method developed by Peter Schuck at the NIH. The SV-AUC results for PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U. lots at full and expanded scales are shown below in the applicant's Figures 3.2.5.5-25 and 3.2.R.5.5-26, respectively. The results for sedimentation coefficient of monomer, and the percent of monomer and HMWS greater than dimer are provided in the applicant's Table 3.2.R.5.5-17 but are not shown here for brevity. The applicant stated that the dimer can not be resolved by SV-AUC method at a low level. A comparison of the means, standard deviations, and ranges of the sedimentation coefficient for PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U. lots is summarized in Table 3.2.R.5.5-18.

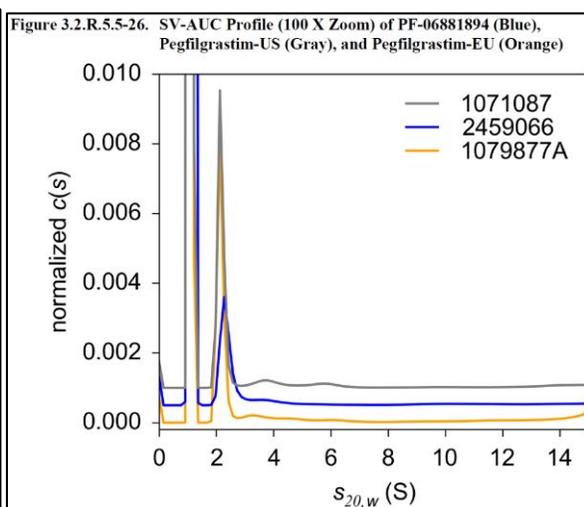
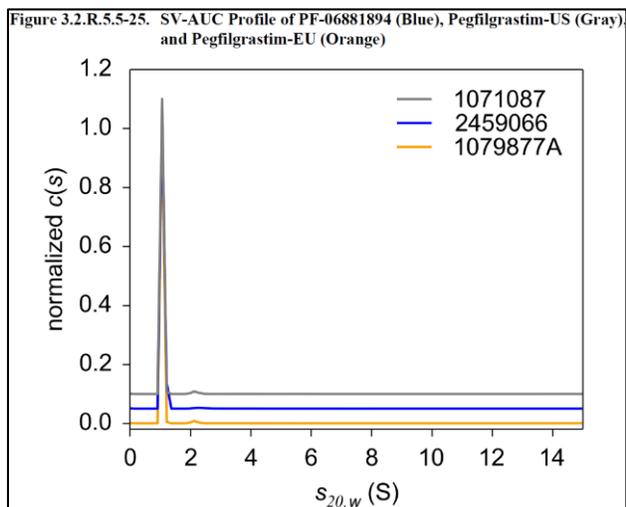


Table 3.2.R.5.5-18. Comparison of Sedimentation Coefficient, Monomer, and HMWS For PF-06881894, Pegfilgrastim-US, and Pegfilgrastim-EU

Attribute	Product	Number of Lots	Mean	Standard Deviation	Range
Sedimentation Coefficient (S)	PF-06881894	7	1.1	0.04	1.0-1.1
	Pegfilgrastim-US	6	1.1	0.05	1.0-1.1
	Pegfilgrastim-EU	6	1.1	0.05	1.0-1.1
Monomer (%)	PF-06881894	7	99.7	0.31	99.1-100.0
	Pegfilgrastim-US	6	99.5	0.69	98.3-100.0
	Pegfilgrastim-EU	6	99.5	0.66	98.3-100.0
HMWS (%)	PF-06881894	7	0.3	0.31	0.0-0.9
	Pegfilgrastim-US	6	0.5	0.69	0.0-1.7
	Pegfilgrastim-EU	6	0.5	0.66	0.0-1.7

HMWS, high molecular weight species

Assessor’s Comment:

The SV-AUC results indicate the size and shape and therefore, tertiary structure, of PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U. are consistent with each other. The mean and range of sedimentation coefficients for PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U. lots were identical. Mean %monomer species was greater than 99% for all three products and the range of PF—06881894 was within the ranges of pegfilgrastim-U.S. and pegfilgrastim EU. With the exceptions of pegfilgrastim-U.S. lot 1071087 (HMW content 1.7%) and pegfilgrastim-E.U. lot 1079877A (HMW content 1.7%), HMW content was less than 1% for all three products. Percent monomer and HMW species was similar among the three products, independent of product age.

3.2.R.5.5.1.2.5 Melting Temperature by Differential Scanning Calorimetry (DSC)

Melting temperature (T_m) was determined by differential scanning calorimetry (DSC). T_m for PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U. lots were compared using the quality range, mean ± 3 x SD, of pegfilgrastim-U.S. lots. The quality range analysis is provided below in the applicant’s Figure 3.2.R.5.5-3 and results are summarized below in the applicant’s Table 3.2.R.5.5-3. An overlay of DSC thermograms of PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U. was provided in the applicant’s Figure 3.2.R.5.5-27 (data not shown). A summary of the number of lots, mean, standard deviation, range, and quality range is provided below in the applicant’s Table 3.2.R.5.5-3.

Figure 3.2.R.5.5-3. Statistical Quality Range Analysis of T_m by DSC for PF-06881894, Pegfilgrastim-US, and Pegfilgrastim-EU

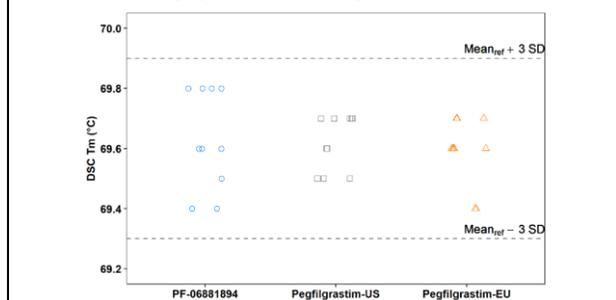


Table 3.2.R.5.5-3. Summary of Statistical Quality Range Analysis for T_m by DSC for PF-06881894, Pegfilgrastim-US, and Pegfilgrastim-EU

Product	Number of Lots Tested	Melting Temperature (T _m)			Quality Range	Lots within Quality Range (%)
		Mean	Standard Deviation	Range		
PF-06881894	10	69.6	0.16	69.4 – 69.8	NA	100
Pegfilgrastim-EU	10	69.6	0.11	69.4 – 69.7	NA	100
Pegfilgrastim-US	10	69.6	0.09	69.5 – 69.7	69.3 – 69.9	NA

NA, Not Applicable

Assessor’s Comment:

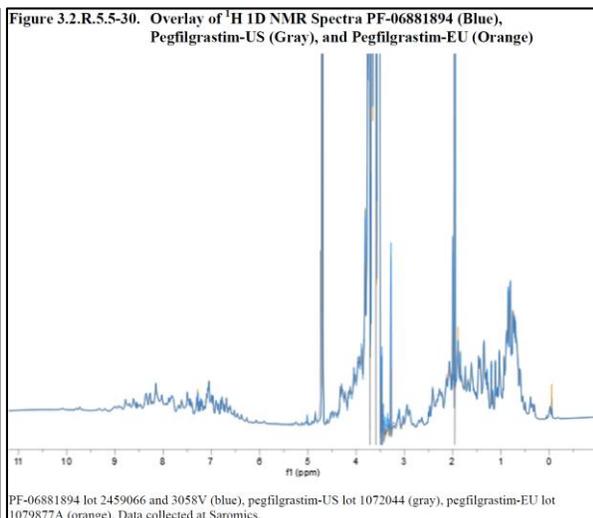
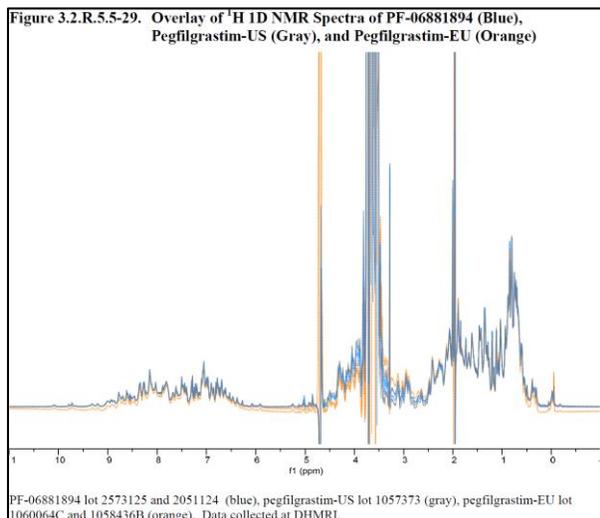
The T_m results indicate that thermal stability and therefore, higher order structure is consistent between PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U. The mean T_m is the same for PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U. lots. In addition, the overlay of the thermograms from the three products are superimposable. The T_m range of PF-06881894 is

within the *Quality Range* limits, which were determined using a multiplier $k=3$. This multiplier is reasonable because the range is less than 1°C , which indicates that there are not meaningful structural differences between the products.

3.2.R.5.5.1.2.6 Protein Structure by Nuclear Magnetic Resonance Spectroscopy

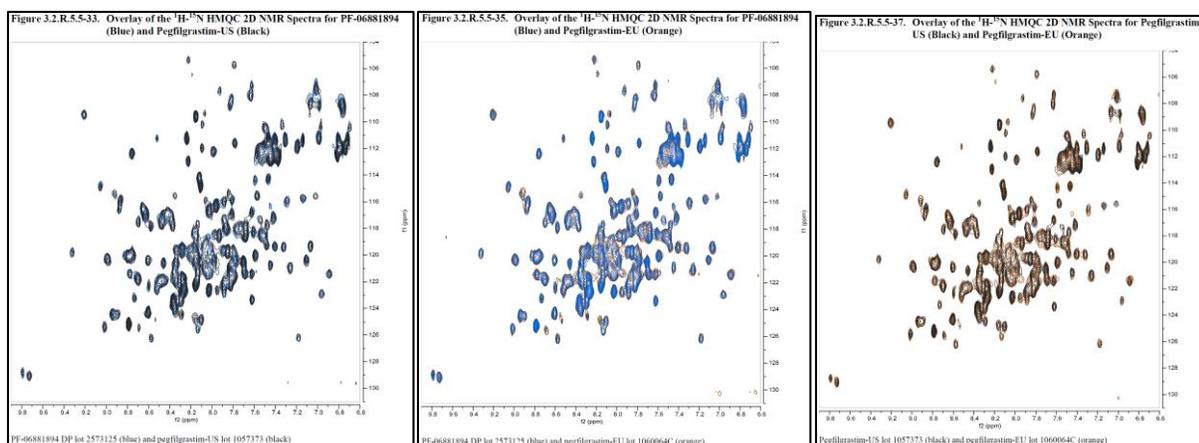
One and two-dimensional Nuclear Magnetic Resonance Spectroscopy (NMR) was used to compare the tertiary structure of the proteins at atomic resolution. One dimensional (1D) NMR spectra provide the chemical shifts and intensity of peaks for different types of protons in a molecule but peaks are not easily assignable to the specific amino acid residues. The two-dimensional (2D) NMR can determine the specific sequence using isotope labeled proteins.

Overlays of ^1H 1D NMR spectra of PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U. lots are provided by two testing facilities: DHMRI and Saromics, as shown below in the applicant's Figures 3.2.R.5.5-29 and 3.2.R.5.5-30, respectively. The horizontal axis of the NMR spectrum is called chemical shift measured in parts per million (ppm). The NMR spectra include two main regions: aromatic/backbone amide region (~ 10.5 to 6 ppm) and the aliphatic region (~ 5.0 to 0.0 ppm).



The peaks at ~ 4.8 ppm, ~ 4.0 - 3.5 ppm, and ~ 2.0 ppm is residual water remaining after water suppression, sorbitol, and acetate, respectively. The sorbitol and acetate are the components of formulation buffer. The peaks from 1 ppm or less are the pegfilgrastim protein. These peaks correspond to methyl groups in the interior of the protein.

The ^1H - ^{15}N HMQC NMR spectra by DHMRI and Saromics were provided (data not shown). Some difference in NMR spectra were observed at the two testing facilities due to folding of lysine side chains caused by use of different sweep width and processing parameters. The lysine sidechain fold leads to a negative peak that impact the signal for the Val49 cross peak. The pegfilgrastim-U.S. lot 1072044 showed a lower signal intensity compared to other pegfilgrastim-E.U. lots and PF-06881894 lots at the same facility. Therefore, only pegfilgrastim-U.S. lot 1057373 was used for the graphical comparison. The overlays of ^1H - ^{15}N NMR spectra for pairwise comparison of PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U. lots are provided by DHMRI in Figures 3.2.R.5.5-33, 3.2.R.5.5-35 and Figure 3.2.R.5.5-37, respectively.



Assessor's Comment:

The 1D and 2D NMR spectrum results demonstrated that tertiary structure is consistent between PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U. Although some differences were observed in the 2D NMR spectra at the two testing sites due to the use of different processing parameters, all the main cross-peaks showed consistent patterns between the two sites. The low signal intensity of the pegfilgrastim-U.S. lot 1072044 also exhibited the similar cross peak profile as those of the pegfilgrastim-E.U. lots and PF-06881894 lots. The ^1H ^1D NMR spectra exhibited a complete overlay of PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U. lots. the ^1H - ^{15}N 2D NMR spectra showed that all the main cross peaks are consistent and there were no chemical shift differences observed.

Higher Order Structure Summary:

Results from multiple orthogonal analytic studies to assess primary structure support a determination that PF-06881894 is highly similar to pegfilgrastim-U.S., and a determination that the analytical part of the scientific bridge was established.

3.2.R.5.5.2 Product-Related Substances and Impurities

Pegfilgrastim is susceptible to different types of degradation, including oxidation of methionine and tryptophan, deamidation of glutamine residues, and des-pegylation. Product-related impurities can be generated during fermentation, pegylation of filgrastim intermediate, and storage of DS and DP. The comparative studies included the evaluation of total related proteins, total charge variants, size variants (HMWS, residual PEG, oxidation, deamidation, reduced species, des-pegylated species, N-terminal des-pegylated species), and other pegfilgrastim-related species. The PF-06881894 DP lots used for the comparative analytical assessment included the development, clinical, and process validation lots. The total related proteins, total charge variants and oxidation are discussed in Quality Range Attributes Section above.

Assessor comment: The panel of assays used is appropriate.

3.2.R.5.5.2.1 Total Related Proteins

Total Related Proteins include product related impurities, including various degradation products, e.g. oxidized and deamidated species, product variants introduced during fermentation, e.g., amino acid misincorporation, and pegylation variants, e.g., des-pegylated species and pegylation site variants, dimers, and high molecular weight species (HMWS). Comparison of

Total Related Proteins by RP-HPLC for PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U. are shown below in the applicant’s Figure 3.2.R.5.5-4. A summary of the number of lots, mean, standard deviation, range, and quality range are provided below in the applicant’s Table 3.2.R.5.5-4.

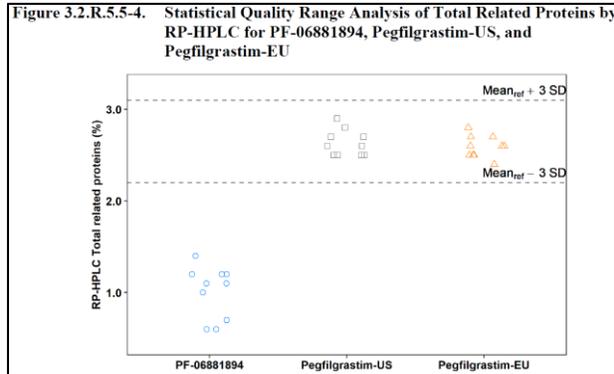


Table 3.2.R.5.5-4. Summary of Statistical Quality Range Analysis of Total Related Proteins by RP-HPLC for PF-06881894, Pegfilgrastim-US, and Pegfilgrastim-EU

Test Articles	Number of Lots Tested	Total Related Proteins (%)			Lots within Quality Range (%)
		Mean	Standard Deviation	Range	
PF-06881894	10	1.0	0.28	0.6 – 1.4	NA
Pegfilgrastim-EU	10	2.6	0.12	2.4 – 2.8	100
Pegfilgrastim-US	10	2.6	0.14	2.5 – 2.9	2.2 – 3.1

NA, Not Applicable

Assessor’s Comment:

All the results of Total Related Protein in PF-06881894 lots tested are below the lower limit (2.2%) of the quality range, which was defined as Mean ± 3xSD of pegfilgrastim-U.S. lots. This is acceptable because the increased purity of PF-06881894 is not expected to have clinical consequences. The use of multiplier k=3 for quality range analysis is reasonable because the RP-HPLC assay is fairly precise, the range for pegfilgrastim-U.S. is narrow (2.5 – 2.9), and studies showed that Total Related Protein by RP-HPLC increased up to 10% with no significant change to potency under stressed temperature of 40°C (see Section 3.2.P.8.3 Stability Data.). The comparative analytical assessment for each individual product related impurity is discussed in Section 3.2.R.5.5.2 Product-Related Substances and Impurities below.

3.2.R.5.5.2.2 Total Charge Variants

Graphical comparison of Total Charge Variants in PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U. lots are shown below in the applicant’s Figure 3.2.R.5.5-5. A summary of the number of lots, and mean, standard deviation, range, and quality range is provided below in the applicant’s Table 3.2.R.5.5-5 below. The quality range was defined as Mean ± 3x SD of Total Charge Variant results of the pegfilgrastim-U.S. lots.

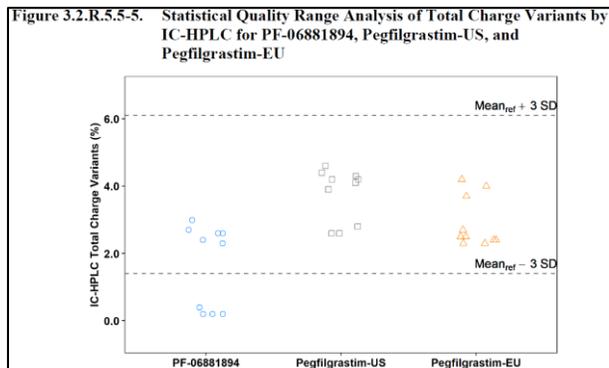


Table 3.2.R.5.5-5. Summary of Statistical Quality Range Analysis of Total Charge Variants for PF-06881894, Pegfilgrastim-US, and Pegfilgrastim-EU

Test Articles	Number of Lots Tested	Total Charge Variants (%)			Lots within Quality Range (%)
		Mean	Standard Deviation	Range	
PF-06881894	10	1.7	1.23	0.2 – 3.0	60
Pegfilgrastim-EU	10	2.9	0.75	2.3 – 4.2	100
Pegfilgrastim-US	10	3.8	0.78	2.6 – 4.6	1.4 – 6.1

NA, Not Applicable

Assessor's Comment:

As shown in Section 3.2.S.3 Characterization, the majority of charge variants are acidic variants. The Total Charge Variants in PF-06881894 lots were consistently lower than those in pegfilgrastim-U.S. lots and pegfilgrastim-E.U. lots, with only 60% of PF-06881894 lots falling within the quality range. The PF-06881894 results outside of the quality range are either below or at the lower range limit of the pegfilgrastim-U.S. lots. This is acceptable because the increased purity of PF-06881894 is not expected to have clinical consequences.

The use of multiplier $k=3$ for quality range analysis is reasonable because the Total Charge Variant range of pegfilgrastim-U.S. lots (2.6-4.6%) is similar to the range of pegfilgrastim-E.U. lots (2.3-4.2%). In addition, the Total Charge Variants by IC-HPLC increased about 8% but with no significant change to potency under stressed temperature of 40°C as shown in Section 3.2.P.8.3 Stability Data; therefore, any change within the quality range of Total Related Proteins based on $3xSD$ would not have impact on the potency.

3.2.R.5.5.2.3 Size Variants

3.2.R.5.5.2.3.1 Size Variants by SEC-HPLC

The SEC-HPLC method is used to detect HMWS larger than dimer in the range of RRT 0.73 to 0.84, dimer in the range of 0.85 to 0.88, N-terminal des-pegylated species and des-Met1 species (Des-PEGMet1) at RRT 1.5. The results of dimer, other HMWS, and Des-pegylated species for PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U. lots are found in the applicant's Table 3.2.R.5.5-7, which is not shown here for brevity. The graphical comparison of total size variants and representative SEC chromatograms are provided below in the applicant's Figures 3.2.R.5.5-5 and 3.2.R.5.5-6, respectively. A summary of mean, standard deviation, and range is provided below in the applicant's Table 3.2.R.5.5-8.

Figure 3.2.R.5.5-5. Comparison of Total Size Variants for PF-06881894, Pegfilgrastim-US, and Pegfilgrastim-EU

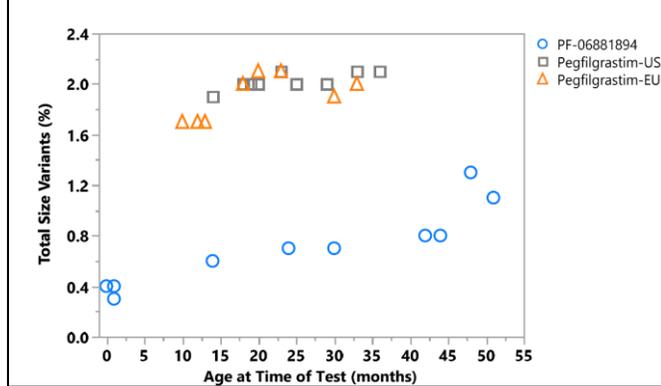


Figure 3.2.R.5.5-6. Example SEC Chromatograms for PF-06881894, Pegfilgrastim-US, and Pegfilgrastim-EU

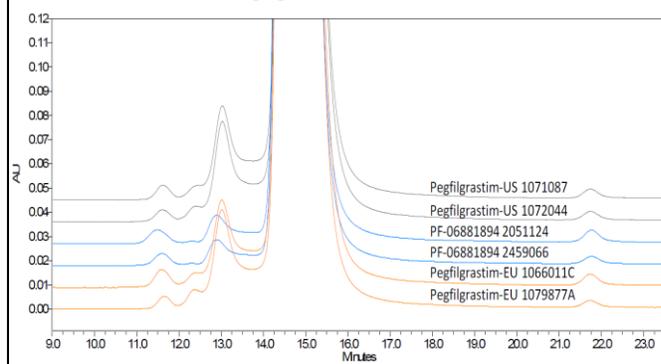


Table 3.2.R.5.5-8. Summary of Descriptive Statistics for Total Size Variants by SEC for PF-06881894, Pegfilgrastim-US and Pegfilgrastim-EU

Product	Number of Lots Tested	Total Size Variants (%)		
		Mean	Standard Deviation	Range
PF-06881894	10	0.7	0.31	0.3 – 1.3
Pegfilgrastim-US	10	2.0	0.06	1.9 – 2.1
Pegfilgrastim-EU	10	1.9	0.17	1.7 – 2.1

Assessor’s Comment:

All three products showed increase in total size variants over time. However, the PF-06881894 lots showed significantly lower levels of total size variants than those of pegfilgrastim-U.S. lots and pegfilgrastim-E.U. lots. The ages of PF-06881894 lots at the time of testing ranged from 0 – 51 months, which provides confidence that size variants content will remain acceptable throughout the dating period. The SEC chromatogram results of PF-06881894 lots did not show additional peaks compared to the pegfilgrastim-U.S. and pegfilgrastim-E.U. lots. The applicant did not conduct the quality range analysis for each individual size variant in pegfilgrastim, such as dimer, other HMWS (not the dimer), and des-pegylated species. This is acceptable because the levels of dimer and other HMWS in PF-06881894 lots are much lower than those in pegfilgrastim-U.S. and pegfilgrastim-E.U. lots. The levels of des-pegylated species in three products are either equal to method LOQ of 0.1% or <LOQ. An orthogonal SV-AUC method was used to further confirm the low levels of HMWS in PF-06881894 and showed low levels of HMWS.

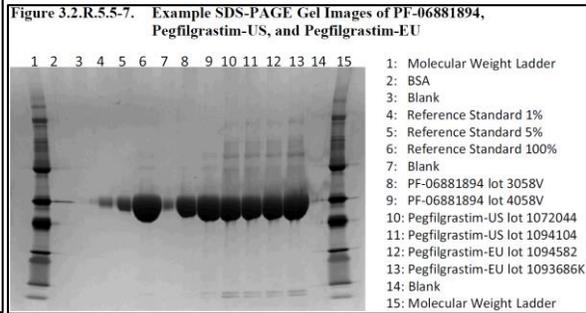
3.2.R.5.5.2.3.2 Size Variants by Non-reducing SDS-PAGE

The size variants were further evaluated by non-reducing SDS-PAGE method. A comparison of the SDS-PAGE samples for PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U. is provided below in the applicant's Figure 3.2.R.5.5-7.

Table 3.2.R.5.5-9. Size Variants as Determined by SDS-PAGE in PF-06881894, Pegfilgrastim-US, and Pegfilgrastim-EU

Product	Lot Number	Age at Time of Test ^a (Months)	Date of Test	Result ^b
PF-06881894	2078064	48	Jun 2018	< 1%
	2082094	44	Jun 2018	< 1%
	2051124	42	Jun 2018	< 1%
	2573125	30	Jun 2018	< 1%
	2459066	24	Jun 2018	< 1%
	213047	14	Jun 2018	< 1%
	3058V	6	Nov 2018	< 1%
Pegfilgrastim-US	4058V	5	Nov 2018	< 1%
	2068V	5	Nov 2018	< 1%
	1060058	36	Jun 2018	< 1%
	1071087	29	Jun 2018	< 1%
	1072044	28	Nov 2018	< 1%
	1078875	25	Jun 2018	< 1%
	1083446	19	Nov 2018	< 1%
Pegfilgrastim-EU	1094104	17	Nov 2018	< 1%
	1066011C	33	Jun 2018	< 1%
	1069490C	30	Jun 2018	< 1%
	1079877A	20	Jun 2018	< 1%
	1090139B	12	Nov 2018	< 1%
	1093686K	11	Nov 2018	< 1%
	1094582	9	Nov 2018	< 1%

a. Date of manufacture for pegfilgrastim-US and pegfilgrastim-EU is estimated by subtracting 36 months from the date of expiry.
b. The result is reported as complies if no impurity band is more intense than the principal band of the 1% standard solution.



Assessor's Comment:

The silver stained SDS-PAGE gel results showed that all products met the applicant's electropherogram criteria of no impurity band more intense than the principal band of the 1% standard solution, as shown in Table 3.2.R.5.5-9. The PF-06881894 lots exhibited similar band patterns compared to the pegfilgrastim-U.S. lots and pegfilgrastim-E.U. lots. The lane 7 with blank sample showed a small band is most likely due to the overloaded sample in lane 6. The intensity of non-specific bands in PF-06881894 lots are lower than those of pegfilgrastim-U.S. lots and pegfilgrastim-E.U. lots, which is consistent with the SEC-HPLC results.

3.2.R.5.5.2.4 Residual PEG

The levels of residual PEG in PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U. lots were determined by RP-HPLC-ELSD method. The residual PEG results are presented in the applicant's Table 3.2.R.5.5-10, which is not shown for brevity. The comparison of the levels of residual PEG over the shelf life for three products are provided below in the applicant's Figure 3.2.R.5.5-8 and the comparison results are summarized in the applicant's Table 3.2.R.5.5-11.

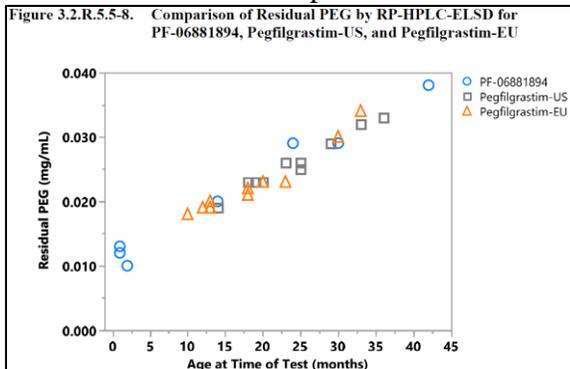


Table 3.2.R.5.5-11. Summary of Descriptive Statistics for Residual PEG by RP-HPLC-ELSD for PF-06881894, Pegfilgrastim-US and Pegfilgrastim-EU

Product	Number of Lots Tested	Residual PEG (mg/mL)		
		Mean	Standard Deviation	Range
PF-06881894	7	0.022	0.0107	0.010 – 0.038
Pegfilgrastim-US	10	0.026	0.0044	0.019 – 0.033
Pegfilgrastim-EU	10	0.023	0.0052	0.018 – 0.034

Assessor's Comment:

The residual PEG was similar among the three products, independent of product age. All the three products had low levels of free PEG that increased over time under long-term storage condition. The applicant did not conduct a quality range analysis for the residual PEG. This is acceptable because the levels of residual PEG in PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U. lots are all low and not expected to impact product safety. The mean of the residual PEG level in PF-06881894 lots is lower than those in the pegfilgrastim-U.S. and pegfilgrastim-E.U. lots. The upper range limit (0.0038) of PF-06881894 lots is slightly higher than the upper range limit (0.033) of pegfilgrastim-U.S. lots, however this is likely due to the age of that lot at the time of testing, ~42 months.

3.2.R.5.5.2.5 Oxidation

Oxidized pegfilgrastim species were measured by RP-HPLC and Glu-C Peptide Mapping methods.

3.2.R.5.5.2.5.1 Oxidation by RP-HPLC

The results of Met127 oxidation in PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U. lots are shown in the applicant's Figure 3.2.R.5.5-6 below, and the number of lots, mean, standard deviation, range, and quality range are summarized in the applicant's Table 3.2.R.5.5-6 below. The quality range was defined as Mean \pm 3x SD of Met127 oxidation results of the pegfilgrastim-U.S. lots.

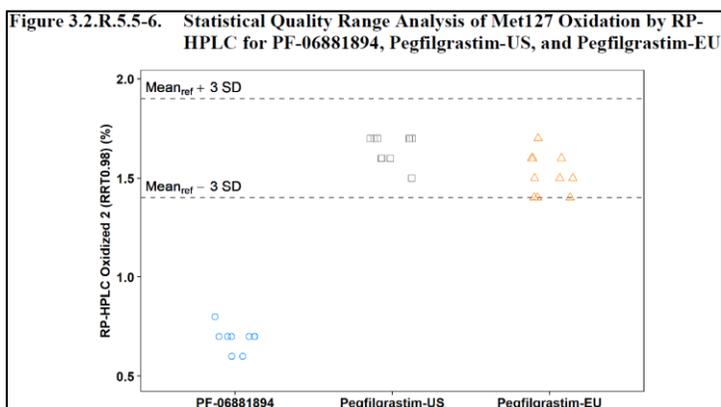


Table 3.2.R.5.5-6. Summary of Statistical Quality Range Analysis of Met127 Oxidation by RP-HPLC for PF-06881894, Pegfilgrastim-US, and Pegfilgrastim-EU

Test Articles	Number of Lots Tested	Met127 Oxidation (%)				Lots within Quality Range (%)
		Mean	Standard Deviation	Range	Quality Range	
PF-06881894	10	0.7	0.06	0.6 – 0.8	NA	0
Pegfilgrastim-EU	10	1.5	0.10	1.4 – 1.7	NA	100
Pegfilgrastim-US	10	1.6	0.07	1.5 – 1.7	1.4 – 1.9	NA

NA, Not Applicable

Assessor's Comment:

Oxidized species with a relative retention time (RRT) between 0.81-0.85 were all below the LOQ (0.3%) of the RP-HPLC method for all PF-06881894, pegfilgrastim-U.S., and pegfilgrastim -E.U. lots tested. Therefore, it is acceptable that the quality range analysis was only conducted for Met127 oxidation (RRT 0.96-0.98). The levels of oxidized Met127 in PF-06881894 lots are consistently lower than those in pegfilgrastim-U.S. lots and pegfilgrastim-E.U. lots, independent of the age of the products. Therefore, it is acceptable that PF-06881894 Met127 oxidation levels are outside the quality range of pegfilgrastim-U.S. and pegfilgrastim E.U.

The use of multiplier $k=3$ for quality range analysis is reasonable because the oxidized Met127 ranges of pegfilgrastim-U.S. and pegfilgrastim-E.U. lots 1.5-1.7% and 1.4 – 1.7 are small and the method has good accuracy and precision. In addition, as shown in Section 3.2.S.3.2 Impurities, peroxide treatment showed that stressed PF-06881894 samples with approximately 8% oxidized Met127 did not have significant change to potency; therefore, any change within the quality range (1.4-1.9%) of Met127 oxidation would not impact potency. The results demonstrate that the Met127 oxidation (RRT 0.96-0.98) is similar between PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U.

3.2.R.5.5.2.5.2 Oxidation by Glu-C Peptide Mapping

Site specific oxidation in pegfilgrastim was measured by the Glu-C peptide mapping method. The oxidation results on four potential residues in PF-06881894, pegfilgrastim-U.S., pegfilgrastim-E.U. are provided below in the applicant’s Table 3.2.R.5.5-14. Descriptive statistical analysis of Trp59 oxidation results are shown below in the applicant’s Table 3.2.R.5.5-15. Graphical comparison of Trp59 oxidation over time is provided in the applicant’s Figure 3.2.R.5.5-10.

Table 3.2.R.5.5-14. Oxidized Species for PF-06881894, Pegfilgrastim-US, and Pegfilgrastim-EU Determined by Glu-C Peptide Mapping

Product	Lot Number	Age at Time of Test ^a (months)	Date of Test	Oxidation ^b (%)			
				M122	M127	M138	Trp59
PF-06881894	2051124	42	Jun 2018	<0.5	<0.5	0.5	1.0
	2573125	30	Jun 2018	<0.5	<0.5	<0.5	0.8
	2459066	24	Jun 2018	<0.5	<0.5	0.5	0.8
	213047	14	Jun 2018	<0.5	<0.5	<0.5	0.7
	3058V	6	Nov 2018	<0.5	<0.5	<0.5	<0.5
	4068V	5	Nov 2018	<0.5	<0.5	<0.5	<0.5
	2068V	5	Nov 2018	<0.5	<0.5	<0.5	<0.5
Pegfilgrastim-US	1060058	36	Jun 2018	<0.5	<0.5	0.5	0.9
	1071087	29	Jun 2018	<0.5	<0.5	<0.5	0.8
	1072044	28	Nov 2018	<0.5	<0.5	<0.5	<0.5
	1078875	25	Jun 2018	<0.5	<0.5	<0.5	0.7
	1083446	19	Nov 2018	<0.5	<0.5	0.5	0.5
	1094104	17	Nov 2018	<0.5	<0.5	<0.5	0.5
Pegfilgrastim-EU	1066011C	33	Jun 2018	<0.5	<0.5	0.5	0.8
	1069490C	30	Jun 2018	<0.5	<0.5	<0.5	0.7
	1079877A	20	Jun 2018	<0.5	<0.5	0.5	0.9
	1090139B	12	Nov 2018	<0.5	<0.5	<0.5	<0.5
	1093686K	11	Nov 2018	<0.5	<0.5	<0.5	0.5
	1094582	9	Nov 2018	<0.5	<0.5	<0.5	<0.5

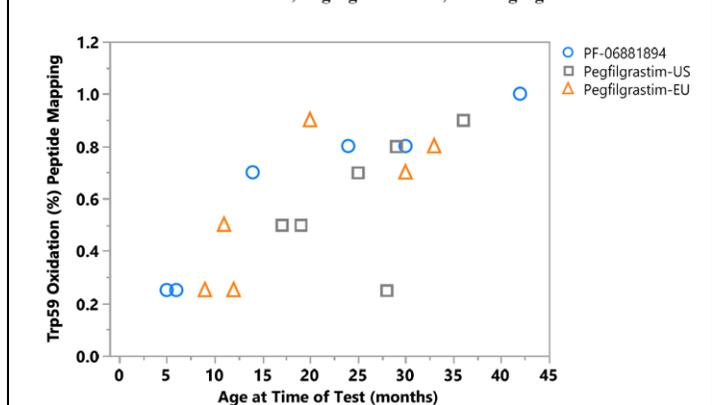
a. Date of manufacture for pegfilgrastim-US and pegfilgrastim-EU is estimated by subtracting 36 months from the date of expiry
b. Method reporting limit is 0.5%.

Table 3.2.R.5.5-15. Summary of Descriptive Statistics for Trp59 Oxidation by Glu-C Peptide Mapping for PF-06881894, Pegfilgrastim-US, and Pegfilgrastim-EU

Product	Number of Lots Tested	Trp Oxidation (%)		
		Mean ^a	Standard Deviation ^a	Range
PF-06881894	7	0.8	0.13	<0.5 – 1.0
Pegfilgrastim-US	6	0.7	0.18	<0.5 – 0.9
Pegfilgrastim-EU	6	0.7	0.17	<0.5 – 0.9

<0.5, Less than the reporting limit of 0.5%
a. Calculations did not include values below the reporting limit (0.5%)

Figure 3.2.R.5.5-10. Comparison of Trp59 Oxidation by Glu-C Peptide Mapping for PF-06881894, Pegfilgrastim-US, and Pegfilgrastim-EU



Assessor’s Comment:

The oxidized species by peptide mapping were similar among the three products, independent of product age. Glu-C peptide mapping results indicated that Trp59 was the major site of oxidation. However, Reidhaar-Olson et al (Reidhaar-Olson JF, De Souza-Hart JA, Selick HE. Identification of residues critical to the activity of human granulocyte colony-stimulating factor. *Biochemistry* 1996; 35 (28): 9034-41) report that Trp59 is not critical to the binding and biological activity of G-CSF. All the three products exhibited age dependent increases in Trp59 oxidation over time under long-term storage condition. The levels of oxidation at Met122, Met127, and Met138 are all below the LOQ of 0.5% for three products. These results indicate oxidation is not a significant degradation pathway under long-term storage condition.

3.2.R.5.5.2.6 Deamidation

Deamidation at Glutamine (Gln) and Asparagine residues commonly occurs in human therapeutic proteins. Pegfilgrastim has 17 glutamine residues and no asparagine. The deamidation of pegfilgrastim is measured by RP-HPLC, IC-HPLC, and Glu-C peptide mapping.

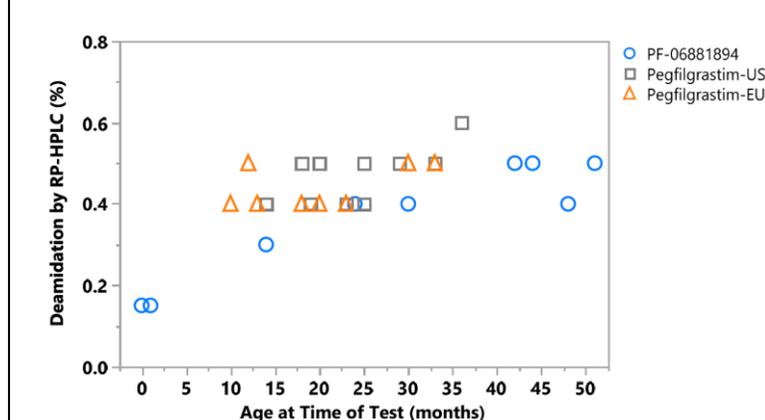
3.2.R.5.5.2.6.1 Deamidation by RP-HPLC

The results for Gln108 deamidation in PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U. lots as determined by RP-HPLC are provided in the applicant’s Table 3.2.R.5.5-16 but are not shown for brevity. A comparison of mean, standard deviation, and range of the three products is summarized below in the applicant’s Table 3.2.R.5.5-17. Graphical comparison of Gln108 deamidation over time is provided below in the applicant’s Figure 3.2.R.5.5-11.

Product	Number of Lots Tested	Gln108 Deamidation (%)		
		Mean	Standard Deviation	Range
PF-06881894	10	0.4 ^a	0.08 ^a	<LOQ – 0.5
Pegfilgrastim-US	10	0.5	0.07	0.4 – 0.6
Pegfilgrastim-EU	10	0.4	0.05	0.4 – 0.5

a. Results below the limit of quantitation (LOQ) were not included in the calculation.

Figure 3.2.R.5.5-11. Comparison of Deamidation by RP-HPLC for PF-06881894, Pegfilgrastim-US, and Pegfilgrastim-EU



Assessor’s Comment:

One peak corresponding to deamidation of Gln108 at RRT 1.07 was observed by RP-HPLC analysis, as discussed in Section 3.2.S.3.2 Impurities. A trend of increased deamidation was observed for PF-06881894 but not pegfilgrastim-U.S. or pegfilgrastim-E.U. lots. The applicant attributes this PF-06881894 lots having a broader age range, 0-51 months, than pegfilgrastim-U.S. lots, 14-36 months, and pegfilgrastim-E.U. lots, 10-33 months. This explanation is supported by the data provided (see above). The levels of Gln108 deamidation of PF-06881894 lots are either <LOQ or within the range of pegfilgrastim-U.S. lots. The RP-HPLC method LOQ is 0.3% and a value of 0.15% was used for the results <LOQ in the graphical comparison. In addition, the age ranges of tested are narrower than that of PF-06881894 Therefore, the results demonstrate that the deamination at Gln108 by RP-HPLC is similar between PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U.

3.2.R.5.5.2.6.2 Deamidation by IC-HPLC

An orthogonal method IC-HPLC was used to measure the Gln deamidation. The levels of deamidated species in PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U. lots are provided in the applicant’s Table 3.2.R.5.5-18 but are not shown for brevity. A comparison of mean, standard deviation, and range of the three products is summarized below in the applicant’s Table 3.2.R.5.5-19. Graphical comparison of deamidated species at RRT 0.85 and RRT 0.89-0.9 over time are provided below in the applicant’s Figures 3.2.R.5.5-12 and 3.2.R.5.5-13, respectively. The LOQ is 0.4% for the IC-HPLC method and a value of 0.2% was used for the results <LOQ in the graphical comparison.

Table 3.2.R.5.5-19. Summary of Descriptive Statistics for Deamidation by IC-HPLC for PF-06881894, Pegfilgrastim-US, and Pegfilgrastim-EU

Deamidation	Product	Number of Lots Tested	Peak Area (%)		
			Mean ^a	Standard Deviation ^a	Range
RRT 0.85	PF-06881894	10	<LOQ	NA	<LOQ
	Pegfilgrastim-US	10	0.9	0.11	0.6 – 1.0
	Pegfilgrastim-EU	10	0.8	0.07	0.7 – 0.9
RRT 0.89-0.90	PF-06881894	10	0.5	0.04	<LOQ – 0.5
	Pegfilgrastim-US	10	0.8	0.12	0.7 – 1.0
	Pegfilgrastim-EU	10	0.7	0.12	0.6 – 1.0

LOQ, limit of quantitation; NA, not applicable; RRT, relative retention time
a. Results below LOQ were not included in the calculation

Figure 3.2.R.5.5-12. Comparison of Deamidated Species RRT 0.85 by IC-HPLC for PF-06881894, Pegfilgrastim-US, and Pegfilgrastim-EU

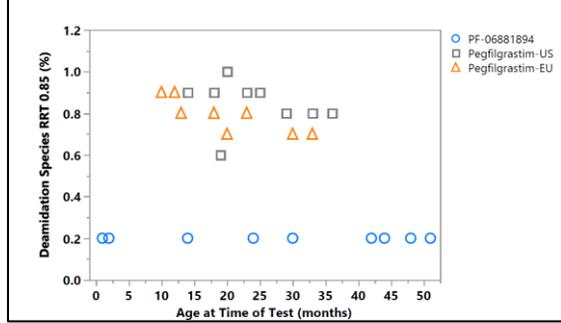
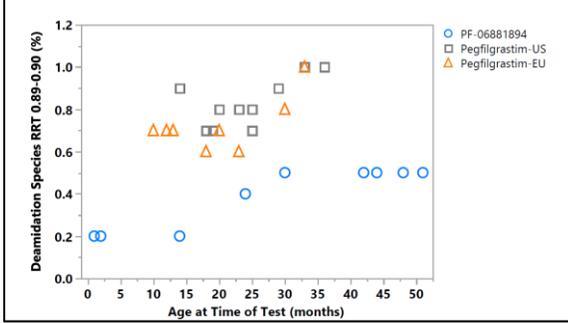


Figure 3.2.R.5.5-13. Comparison of Deamidated Species RRT 0.89-0.90 by IC-HPLC for PF-06881894, Pegfilgrastim-US, and Pegfilgrastim-EU



Assessor’s Comment:

The results of deamidation by IC-HPLC were similar among the three products, independent of product age. Two peaks with RRT of 0.85 and 0.89-0.90 were observed by IC-HPLC as discussed in Section 3.2.S.3.2 Impurities. The levels of deamidated species at RRT 0.85 and RRT 0.89-0.90 in pegfilgrastim-U.S. lots are comparable to those in pegfilgrastim-E.U. lots. However, the levels of deamidated species at RRT 0.85 and RRT 0.89-0.90 in PF-06881894 lots are significantly lower than those in pegfilgrastim-U.S. lots and pegfilgrastim-E.U. lots, which is acceptable, as it results in a product with lower levels of this impurity.

3.2.R.5.5.2.6.3 Deamidation by Glu-C Peptide Mapping

Site specific deamidation in pegfilgrastim was measured using an additional orthogonal method, Glu-C peptide mapping. The deamidation results for PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U. lots are provided in the applicant’s Table 3.2.R.5.5-20 but are not shown here for brevity. A comparison of mean, standard deviation, and range of the three products for deamidation sites at Gln21 and Gln91 is summarized below in the applicant’s Table 3.2.R.5.5-21. Graphical comparisons of deamidated species at Gln21 and Gln91 over time are provided below in the applicant’s Figures 3.2.R.5.5-14 and 3.2.R.5.5-15, respectively.

Deamidation Site	Product	Number of Lots Tested	Peak Area (%)		
			Mean ^a	Standard Deviation ^a	Range
Gln21	PF-06881894	7	NA	NA	<0.5 – 0.7
	Pegfilgrastim-US	6	0.7	0.11	0.6 – 0.9
	Pegfilgrastim-EU	6	0.6	0.15	0.5 – 0.8
Gln91	PF-06881894	7	NA	NA	<0.5 – 1.0
	Pegfilgrastim-US	6	1.0	0.26	0.8 – 1.5
	Pegfilgrastim-EU	6	0.9	0.21	0.7 – 1.2

<0.5, Less than the reporting limit of 0.5%; NA, not applicable
a. Calculations did not include values below the reporting limit (0.5%)

Figure 3.2.R.5.5-14. Comparison of Deamidation at Gln21 by Glu-C Peptide Mapping for PF 06881894, Pegfilgrastim-US, and Pegfilgrastim-EU

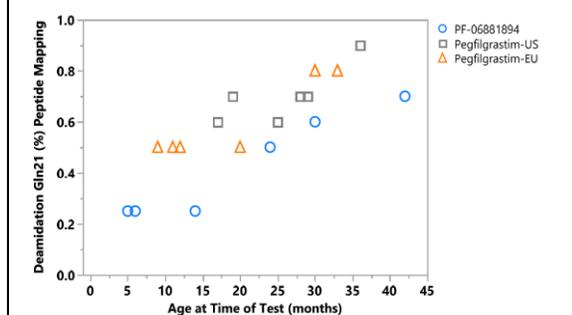
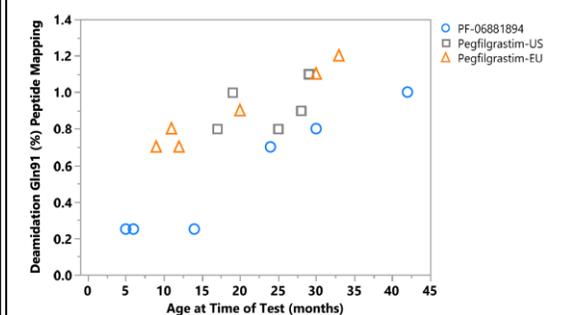


Figure 3.2.R.5.5-15. Comparison of Deamidation at Gln91 by Glu-C Peptide Mapping for PF 06881894, Pegfilgrastim-US, and Pegfilgrastim-EU



Assessor's Comment:

Of the 17 Gln residues present in pegfilgrastim, Gln21 and Gln91 were identified as the primary deamidated species with low levels of deamidation also observed at Gln120 and Gln135. The levels of other deamidated Gln sites are all below LOQ of 0.5% for the three products. A value of 0.2% was used for the results <LOQ in the graphical comparison.

Deamidated species detected by RP-HPLC, IC-HPLC and Glu-C peptide mapping showed similar levels and sites of deamidation for PF-06881894, pegfilgrastim-U.S. and pegfilgrastim-E.U. and demonstrate that the deamidation is not a major degradation pathway under recommended long-term storage conditions.

3.2.R.5.5.2.7 Reduced Species

Assessor's Comment:

Pegfilgrastim contains 5 cysteines and four of which oxidize to form two intrachain disulfide bonds: Cys37-Cys43 and Cys65-Cys75. Reduced pegfilgrastim is a product related impurity that can be detected at RRT1.05 by RP-HPLC. The percent reduced species in PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U. lots are at or below the LOQ of 0.3% as shown in the applicant's Table 3.2.R.5.5-22 but are not shown here for brevity. The results demonstrate that the levels of reduced pegfilgrastim as determined by RP-HPLC are similar between PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U.

3.2.R.5.5.2.8 Des-Pegylated Species

Des-pegylated pegfilgrastim is generated due to incomplete conjugation between filgrastim and PEG. The levels of des-pegylated species determined by the RP-HPLC are summarized in Table below summarized by assessor. The des-pegylated species determined by SEC-HPLC is discussed in Size Variants by SEC Section above. The levels of the des-pegylated species determined by RP-HPLC are higher than that by SEC-HPLC.

Product	Number of lots Tested	Des-Pegylated Species		
		Mean	Standard Deviation	Range (%)
PF-06881894	10	N/A	N/A	<LOQ
Pegfilgrastim-U.S.	10	0.53	0.17	0.4-0.8
Pegfilgrastim-E.U.	10	0.63	0.16	0.4-0.8

Assessor's Comment:

Des-pegylated species detected by RP-HPLC and SEC-HPLC are primarily N-terminal des-pegylated and des-PEGMet1 species as discussed in Characterization Section 3.2.S.3.2 Impurities. The applicant states that in the RP-HPLC chromatogram, the des-pegylated species at RRT 1.04 were not well resolved from the main peak, which led to over-estimation of the des-pegylated species. The applicant did not conduct the quality range analysis for the des-pegylated species at Met1 (Des-PEGMet1) in pegfilgrastim. This is acceptable because the levels of Des-PEGMet1 in PF-06881894 lots are all below the method LOQ (0.3%) and are lower than those in pegfilgrastim-U.S. and pegfilgrastim-E.U. lots. The range of des-pegylated species in pegfilgrastim-U.S. lots is identical to the range of pegfilgrastim-E.U. lots.

Since di-pegylated species cannot be detected in PF-06881894 DP as demonstrated in Section 3.2.S.3.2 Impurities, the analysis of di-pegylated species is not included in the comparative analytical assessment.

3.2.R.5.5.2.9 N-terminal Des-Pegylated Species by Glu-C Peptide Mapping

The results of the pegylated Lys35 by Glu-C peptide mapping for three products are shown in the applicant's Table 3.2.R.5.5-24 but not provided here for brevity.

Assessor's Comment:

As discussed in Section 3.2.S.3.2 Impurities, the results from RP-HPLC fractions by Glu-C peptide mapping showed that the Des-PEG S1 peptide is most likely Lys35 pegylated pegfilgrastim. The percent N-terminal des-pegylated species in PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U. lots are at or below the LOQ of 0.5%. The applicant stated that although free filgrastim would also generate the des-PEG S1 peptide with Glu-C digestion, the des-pegylated species separated by RP-HPLC and SEC-HPLC and identified by Glu-C peptide mapping is primarily Des-PEGMet1 species lacking the N-terminal Met1 residue and not the free filgrastim. The applicant did not conduct a quality range analysis for the N-terminal des-pegylated species at Lys35 (des-PEGLys35) in pegfilgrastim. This is acceptable because the levels of des-PEGLys35 in PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U. lots are all at or below the method LOQ (0.5%).

3.2.R.5.5.2.10 Other Low Abundant Pegfilgrastim Related Species

Assessor's Comment:

Quantitative comparisons of other product related species reported in the literature, including Asp isomerization, amino acid mis-incorporations (e.g., Asp to Glu) and atypical amino acids (e.g., norvaline) and N-terminal modifications (e.g., methyl, propyl), were not performed. This is acceptable because levels were all below the method LOQ of 05%.

Product-Related Substances and Impurities Summary

Results from multiple orthogonal analytical studies to assess product-related substances and impurities support a determination that PF-06881894 is Highly similar to pegfilgrastim-U.S., and a determination that the analytical part of the scientific bridge was established.

3.2.R.5.5.3 Drug Product Attributes

DP attribute testing was conducted as part of the comparative analytical assessment of PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U. The results of the first 5 PF-06881894 DP lots manufactured during the early development program were slightly higher in Protein Concentration and lower in Deliverable Volume compared to that from pegfilgrastim-U.S. and pegfilgrastim-E.U. lots. Therefore, target protein concentration and fill weights of PF-06881894 lots were slightly adjusted to better match those of pegfilgrastim-U.S. and pegfilgrastim-E.U. The revised attributes were implemented for the 6th PF-06881894 DP lot (lot 2459066) and all subsequent PF-06881894 DP lots. Quality range analysis of these attributes only include the 5 PF-06881894 DP lots with revised manufacturing targets. For osmolality, release results were used for the comparative analytical assessment. The osmolality results from the first two DP lots were not included in the comparative analytical assessment because osmolality was not part of the release specifications at that time.

Assessor Comments:

It is acceptable to include only post-manufacturing change lots in similarity assessment because they reflect the attributes of the to-be-marketed product.

3.2.R.5.5.3.1 Protein Concentration

Protein concentrations of PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U. lots were determined by UV-Vis Spectrometry. The quality range was defined as Mean \pm 3x SD of Protein Concentration results of the pegfilgrastim-U.S. lots. Graphical Protein Concentration comparisons of PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U. lots are provided below in the applicant’s Figure 3.2.R.5.5-7 and the number of lots, mean, standard deviation, and quality range are summarized below in the applicant’s Table 3.2.R.5.5-7.

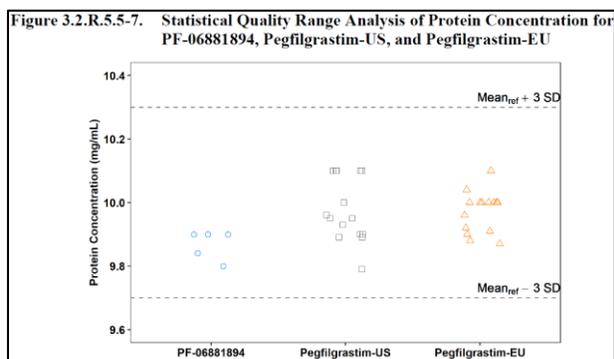


Table 3.2.R.5.5-7. Summary of Statistical Quality Range Analysis of Protein Concentration for PF-06881894, Pegfilgrastim-US, and Pegfilgrastim-EU

Product	Number of Lots Tested	Protein Concentration (mg/mL)			Quality Range	Lots within Quality Range (%)
		Mean	Standard Deviation	Range		
PF-06881894	5	9.9	0.05	9.8 – 9.9	NA	100
Pegfilgrastim-US	15	10.0	0.06	9.87 – 10.1	NA	100
Pegfilgrastim-EU	15	10.0	0.10	9.79 – 10.1	9.7 – 10.3	NA

NA: not applicable

Assessor’s Comment:

The means and ranges of the PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U. lots are similar. The quality range analysis showed that all the protein concentration results from the PF-06881894 and pegfilgrastim-E.U. lots are within the quality range of pegfilgrastim-U.S. lots. The use of multiplier k=3 for quality range analysis is reasonable because the method has excellent precision and results show low variability. The extinction coefficient was determined both experimentally and theoretically, with little difference between the results. Therefore, the method is acceptable.

3.2.R.5.5.3.2 Deliverable Volume

The deliverable volume of each prefilled syringe was calculated by dividing the weight of the discharged content by the density of PF-06881894 DP lots (1.020 g/mL). Graphical comparison results of the deliverable volume for PF-06881894 DP lots with the revised fill weight target and reference products are provided below in the applicant’s Figure 3.2.R.5.5-2. A summary of the number of lots, mean, standard deviation, and range are provided below in the applicant’s Table 3.2.R.5.5-6.

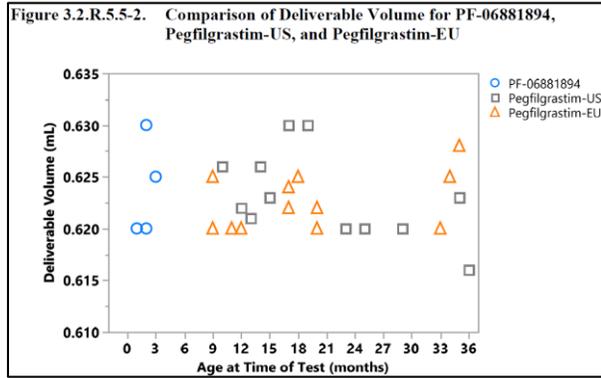


Table 3.2.R.5.5-6. Summary of Descriptive Statistics for Deliverable Volume for PF-06881894, Pegfilgrastim-US and Pegfilgrastim-EU

Product	Number of Lots	Deliverable Volume (mL)		
		Mean	Standard Deviation	Range
PF-06881894	5	0.62 ^a	0.004 ^a	0.62 – 0.63 ^a
Pegfilgrastim-US	12	0.62	0.004	0.616 – 0.63
Pegfilgrastim-EU	12	0.62	0.003	0.62 – 0.628

a. Lots manufactured prior to the revised target fill weight are not included in calculations.

Assessor’s Comment:

There is no trend in deliverable volume for PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U. lots of different ages. The means and ranges of deliverable volume for three products are nearly identical.

3.2.R.5.5.3.3 Deliverable Content

The deliverable content was determined by multiplying the protein concentration by the deliverable volume. Graphical comparisons of the deliverable content results for PF-06881894 DP lots with revised protein concentration and fill weight targets, pegfilgrastim-U.S. lots, and pegfilgrastim-E.U. lots are provided below in the applicant’s Figure 3.2.R.5.5-8 and the data are summarized below in the applicant’s Table 3.2.R.5.5-8.

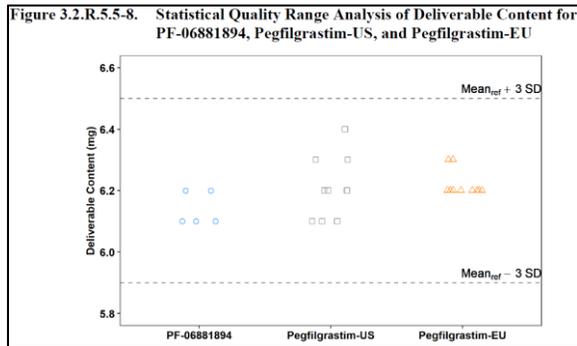


Table 3.2.R.5.5-8. Summary of Statistical Quality Range Analysis of Deliverable Content for PF-06881894, Pegfilgrastim-US, and Pegfilgrastim-EU

Product	Number of Lots Tested	Deliverable Content (mg)				Lots within Quality Range (%)
		Mean	Standard Deviation	Range	Quality Range	
PF-06881894	5	6.1	0.05	6.1 – 6.2	NA	100
Pegfilgrastim-EU	10	6.2	0.04	6.2 – 6.3	NA	100
Pegfilgrastim-US	10	6.2	0.10	6.1 – 6.4	5.9 – 6.5	NA

NA, not applicable

Assessor’s Comment:

The means and ranges of the PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U. lots are similar. The quality range analysis showed that all the deliverable content results from the PF-06881894 lots and the pegfilgrastim-E.U. lots are within the quality range of pegfilgrastim-U.S.

lots. The use of multiplier $k=3$ for quality range analysis is reasonable because the assays have good precision and the results have low variability.

3.2.R.5.5.3.4 Subvisible Particles

Subvisible particles were measured by Microflow Imaging (MFI). The numbers of particle per milliliter for sizes $\geq 2 \mu\text{m}$, $\geq 5 \mu\text{m}$, $\geq 10 \mu\text{m}$, and $\geq 25 \mu\text{m}$ were evaluated at different product ages at the time of testing as shown below in the applicant's Figure 3.2.R.5.5-4 and results for PF-06881894 DP, pegfilgrastim-U.S., and pegfilgrastim-E.U. are summarized below in the applicant's Table 3.2.R.5.5-10.

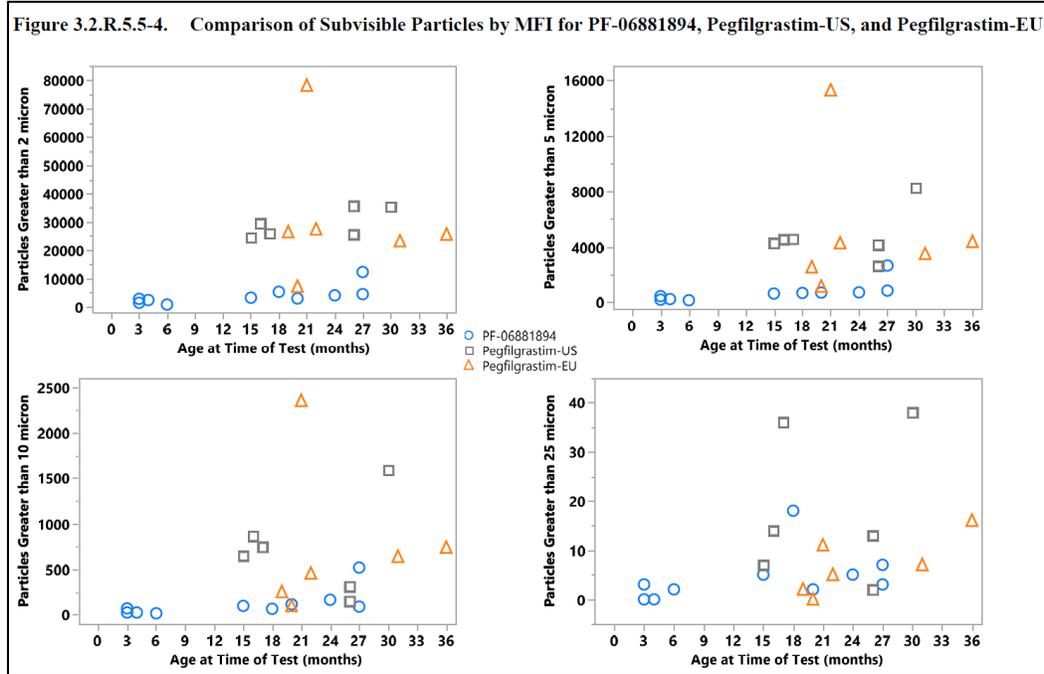


Table 3.2.R.5.5-10. Summary of Descriptive Statistics for Subvisible Particles by MFI for PF-06881894, Pegfilgrastim-US, and Pegfilgrastim-EU

Particle Size	Product	Number of Lots Tested	Particles per mL		
			Mean	Standard Deviation	Range
$\geq 2 \mu\text{m}$	PF-06881894	10	3913	3225.6	751 – 12254
	Pegfilgrastim-US	6	29406	4999.5	24472 – 35653
	Pegfilgrastim-EU	6	31316	24195.3	7128 – 78252
$\geq 5 \mu\text{m}$	PF-06881894	10	716	720.1	143 – 2643
	Pegfilgrastim-US	6	4730	1869.3	2618 – 8250
	Pegfilgrastim-EU	6	5176	5096.6	1139 – 15286
$\geq 10 \mu\text{m}$	PF-06881894	10	119	148.0	16 – 519
	Pegfilgrastim-US	6	718	504.8	149 – 1588
	Pegfilgrastim-EU	6	755	817.3	97 – 2351
$\geq 25 \mu\text{m}$	PF-06881894	10	5	5.23	0 – 18
	Pegfilgrastim-US	6	18	15.1	2 – 38
	Pegfilgrastim-EU	6	7	5.9	0 – 16

Assessor's Comment:

The number of subvisible particles for PF-06881894 DP lots are significantly lower than those for pegfilgrastim-U.S. lots and pegfilgrastim-E.U. lots for all the different size groups. There is

no correlation between the subvisible partible numbers and the product ages. Lower levels of sub-visible particles are acceptable for this product. It should be noted that although the applicant conducted the subvisible particle study, this study is not required for comparative analytical assessment.

3.2.R.5.5.3.5 pH

The pH of PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U. lots were measured per USP <791> and Ph. Eur. <2.2.3> as shown in Table 3.2.R.5.5-11.

Table 3.2.R.5.5-11. pH Results for PF-06881894 DP, Pegfilgrastim-US, and Pegfilgrastim-EU

Product	Lot Number	Age at Time of Test* (Months)	Date of Test	pH
PF-06881894	2056034	1	Apr 2014	4.0
	2078064	4	Oct 2014	4.1
	2082094	0	Oct 2014	4.0
	2051124	1	Jan 2015	4.0
	2573125	1	Jan 2016	4.0
	2459066	0	Jun 2016	4.0
	213047	1	May 2017	4.0
	3058V	1	Jun 2018	4.1
	4058V	0	Jun 2018	4.1
	2068V	1	Jul 2018	4.0
Pegfilgrastim-US	1064191	33	Jun 2018	3.7
	1072044	23	Jun 2018	3.7
	1078875	25	Jun 2018	3.7
Pegfilgrastim-EU	1066011C	33	Jun 2018	3.8
	1090139B	13	Dec 2018	3.8
	1094582	10	Dec 2018	4.0

a. Date of manufacture for pegfilgrastim-US and pegfilgrastim-EU is estimated by subtracting 36 months from the date of expiry.

Assessor's Comment:

Results show that the pH of four out of five pegfilgrastim-U.S. and pegfilgrastim-E.U. lots are between 3.7 and 3.8 whereas the pH of PF-06881894 was 4.0 – 4.1. Neulasta prescribing information states the product is pH = 4. The applicant indicated that a range of ± 0.3 pH is in alignment with or more conservative than the typical commercial pH specification for a biological product. Note that the formulation differences are allowed for biosimilars. The comparative analytical studies demonstrated that other product quality attributes were not impacted by this difference in pH.

3.2.R.5.5.3.6 Osmolality

The osmolality of PF-06881894 DP, pegfilgrastim-U.S., and pegfilgrastim-E.U. lots are determined per USP <785> and Ph. Eur. <2.2.35>. Results for the three products are provided below in the applicant's Tables 3.2.R.5.5-12 and Table 3.2.R.5.5-13.

Table 3.2.R.5.5-12. Osmolality Results for PF-06881894 DP, Pegfilgrastim-US, and Pegfilgrastim-EU

Product	Lot Number	Age at Time of Test ^a (Months)	Date of Test	Osmolality (mOsmol/kg)
PF-06881894	2082094	5	Mar 2015	309
	2051124	0	Dec 2014	289
	2573125	2	Feb 2016	298
	2459066	0	Jun 2016	267
	213047	2	Jun 2017	295
	3058V	2	Jul 2018	289
	4058V	0	Jun 2018	297
Pegfilgrastim-US	2068V	1	Jul 2018	302
	1035686	36	Sep 2015	307
	1036285	35	Sep 2015	310
	1064191	33	Jun 2018	301
	1072044	23	Jun 2018	305
Pegfilgrastim-EU	1078875	25	Jun 2018	297
	1039830D	35	Sep 2015	305
	1041021D	34	Sep 2015	311
	1066011C	3	Jun 2018	304
	1090139B	13	Jun 2018	296
	1094582	10	Jun 2018	298

a. Date of manufacture for pegfilgrastim-US and pegfilgrastim-EU is estimated by subtracting 36 months from the date of expiry

Table 3.2.R.5.5-13. Summary of Descriptive Statistics for Osmolality for PF-06881894, Pegfilgrastim-US and Pegfilgrastim-EU

Product	Number of Lots	Osmolality (mOsmol/kg)		
		Mean	Standard Deviation	Range
PF-06881894	8	293	12.5	267 – 309
Pegfilgrastim-US	5	304	5.1	297 – 310
Pegfilgrastim-EU	5	303	6.0	296 – 311

Assessor’s Comment:

The osmolality ranges of pegfilgrastim-U.S. and pegfilgrastim-E.U. lots are nearly the same. Only 8 PF-06881894 lots were used in the comparative analytical assessment because osmolality testing was not implemented as a release test for PF-06881894 lots 2056034 and 2078064. One PF-06881894 lot has slightly lower osmolality 267 mOsmol/kg, which is not clinically meaningful, and the other 7 lots range from 289-309 mOsmol/kg.

3.2.R.5.5.3.7 Polysorbate 20

Polysorbate 20 levels in PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U. lots were determined by RP-HPLC with evaporative light scattering detection (ELSD). The polysorbate 20 results are provided below in the applicant’s Table 3.2.R.5.5-14.

Table 3.2.R.5.5-14. Polysorbate 20 Concentration Results for PF-06881894 DP, Pegfilgrastim-US, and Pegfilgrastim-EU

Product	Lot Number	Age at Time of Test ^a (Months)	Date of Test	Polysorbate 20 (% w/v)
PF-06881894	2056034	9	Dec 2014	0.004
	2078064	3	Sep 2014	0.005
	2082094	2	Dec 2014	0.004
	2051124	1	Jan 2015	0.005
	2573125	5	May 2016	0.004
	2459066	2	Aug 2016	0.005
	213047	2	Jun 2017	0.004
	3058V	2	Jul 2018	0.004
	4058V	1	Jul 2018	0.004
	2068V	1	Jul 2018	0.004
Pegfilgrastim-US	1064191	33	Jun 2018	0.003
	1072044	23	Jun 2018	0.003
	1078875	25	Jun 2018	0.003
Pegfilgrastim-EU	1066011C	33	Jun 2018	0.003
	1090139B	13	Dec 2018	0.004
	1094582	10	Dec 2018	0.004

a. Date of manufacture for pegfilgrastim-US and pegfilgrastim-EU is estimated by subtracting 36 months from the date of expiry

Assessor's Comment:

The ranges of the polysorbate 20 content are 0.004-0.005%, 0.003%, and 0.003-0.004% for PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U. lots, respectively.

The target polysorbate 20 concentration in PF-06881894 DP is 0.004% (w/v). The pegfilgrastim-U.S. labeling indicates that the polysorbate 20 concentration is 0.02 mg/0.6mL, which is equal to 0.0033% (w/v). The formulation DOE studies demonstrated that stability profile of PF-06881894 using polysorbate 20 at concentrations of 0.002 and 0.006% are comparable to that at the target polysorbate 20 concentration of 0.004%. Therefore, there is no meaningful difference on polysorbate content.

3.2.R.5.5.3.8 Appearance, Color, and Clarity

The appearance of the PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U. is determined by visual inspection. Color and Clarity are determined per Ph.Eur.<2.2.2> and Ph.Eur.<2.2.1>, respectively. Results of Appearance, Color, and Clarity are provided in Table 3.2.R.5.5-15 but not shown for brevity.

Assessor's Comment:

All the lots of three product are identified as "clear, colorless solution". The methods used for color and clarity testing are compendial.

3.2.R.5.5.3.9 Visible Particles

Visible Particles of approximately $\geq 125\mu\text{m}$ in PF-06881894 DP, pegfilgrastim-U.S., and pegfilgrastim-E.U. lots were measured per USP <790> and Ph. Eur. <2.9.20>.

Assessor's Comment:

The results from the PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U. lots are "Practically free of visible particles". The methods used for visible particles testing are compendial.

Drug Product Attributes Summary

Results from multiple orthogonal analytical studies to assess drug product attributes support a determination that the PF-06881894 is highly similar to pegfilgrastim-U.S., and a determination that the analytical part of the scientific bridge was established.

Comparative Forced Degradation Studies

A comparative forced degradation study was conducted by testing two lots each of PF-06881894 DP, pegfilgrastim-U.S., and pegfilgrastim-E.U. The stress conditions include peroxide, heat, light, and high pH. A summary of the lots used in the comparative forced degradation studies is listed below in the applicant's Table 3.2.R.5.5-1. The forced degradation conditions and tested time points are described below in the applicant's Table 3.2.R.5.5-2. The analytical methods selected to assess the potential changes in product related proteins, size variants, charge variants, and chemical modifications of the stressed samples are shown in Table 3.2.R.5.5-3. A summary of comparative forced degradation results is provided in Appendix 2.

Table 3.2.R.5.5-1. PF-06881894 DP, Pegfilgrastim-US and Pegfilgrastim-EU lots Used in the Comparative Forced Degradation Studies

Product	Lot Number	Expiry	Age (Months) at Time of Stress ^a
PF-06881894	2459066	NA	29
	3058V	NA	6
Pegfilgrastim-EU	1094582	Feb 2021	9
	1090139B	Nov 2020	12
Pegfilgrastim-US	1071087	Jan 2019	34
	1078875	May 2019	30

NA, Not applicable
a. Age of pegfilgrastim-US and pegfilgrastim-EU lots are estimated based on the date of expiry

Table 3.2.R.5.5-2. Forced Degradation Conditions and Time Points

Type of Stress	Stress Condition	Temperature	Stress Intervals
Peroxide	0.015% H ₂ O ₂ (150 ppm)	RT	0, 1, 2, 3, 4 hours
Heat	Elevated temperature	50°C	0, 4, 8, 24 hours
Light	ICH Q1B Option 2	25°C	0X, 0.2X, 0.4X, and 0.6X ^a
High pH	pH 10.0	RT	0, 2, 5, 7 days

ICH, International Conference on Harmonisation; ppm, parts per million; RT, room temperature
a. X represents 1X ICH Q1B light exposure level, ie, 1.2 million lux hours of white light and 200 watt hours per square meter of UV energy.

Table 3.2.R.5.5-3. Analytical Testing Plan

Attributes	Analytical Methods	Peroxide	Heat	Light	High pH
Pegfilgrastim Related Proteins	RP-HPLC (LAB-31364)	X	X	X	X
Size Variants	SEC (LAB-31156)	--	X	X	X
Charge Variants	IC-HPLC (LAB-31157)	--	X	--	X
Chemical Degradation	Glu-C Peptide Mapping (RP-UPLC-MS)	X	X	X	X

X, All time points for RP-HPLC, SEC, and IC-HPLC and selected time points for Glu-C Peptide Mapping (RP-UPLC-MS)
--, Not analyzed
RP, reversed-phase; HPLC, high performance liquid chromatography; SEC, size exclusion chromatography; IC, ion chromatography; UPLC, ultra performance liquid chromatography; MS, mass spectrometry

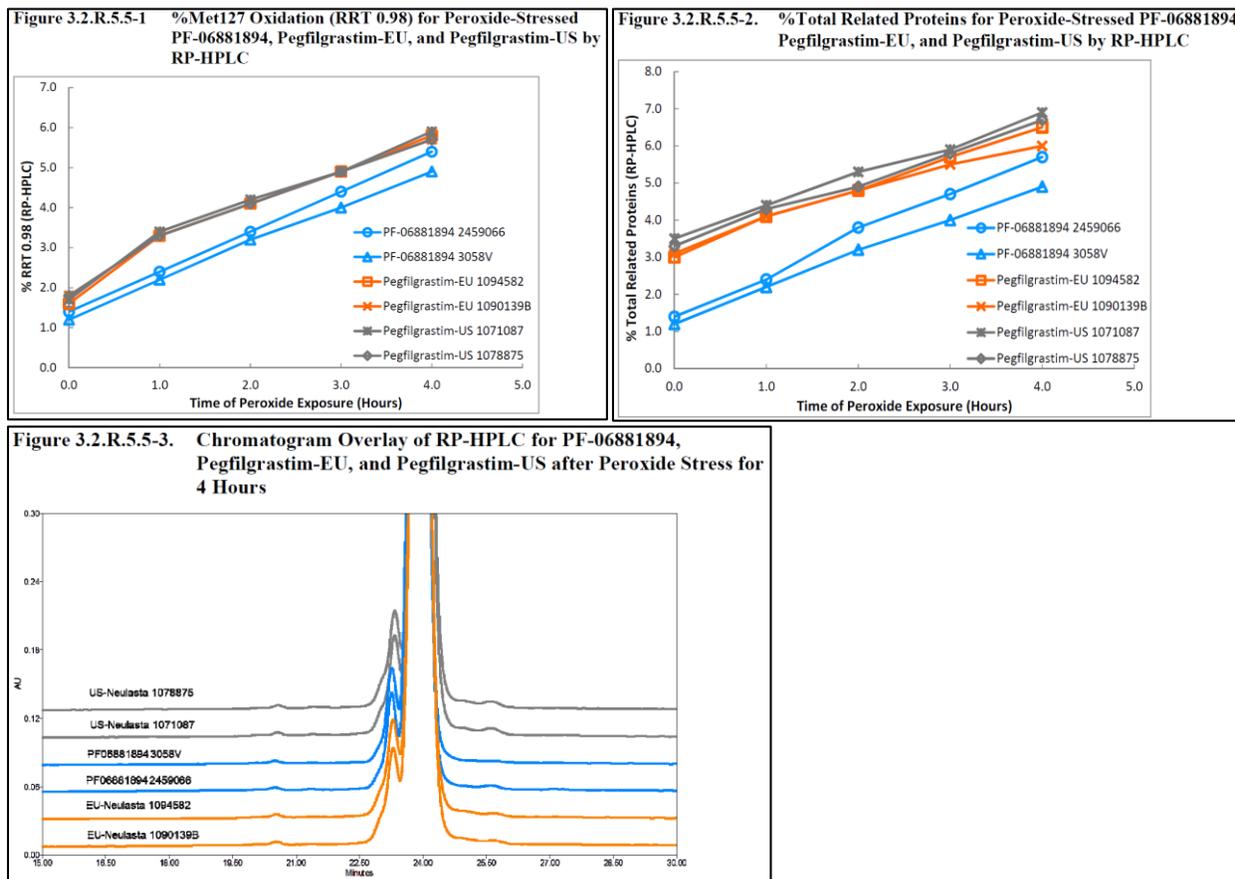
Assessor's comment:

The selection of lots in the comparative forced degradation study is appropriate because the PF-06881894 lot 2459066 and pegfilgrastim-U.S. lot 1071087 were used in the comparative clinical study and PF-06881894 lot 3058V was the process validation lot. The selected stressed conditions are typical forced degradation study conditions with the exception that a low pH condition is missing. An IR was sent to the applicant to justify the missing low pH condition. In response to the IR, the applicant provided results showing that at pH 2.0, no changes to product quality of PF-06881894 DP were observed by SEC-HPLC, CEX-HPLC, and RP-HPLC methods and the low pH condition was not included in comparative forced degradation studies. Therefore, the selected stressed conditions are adequate to cover the potential protein degradation conditions and the test methods are suitable for detecting protein degradation. The analytical methods chosen in the comparative forced degradation study were capable of detecting the degradants of pegfilgrastim protein under the stress conditions as demonstrated in section 3.2.S.3 Characterization and method validation reports.

3.2.R.5.5.3.1. Peroxide Stress

Samples of PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U. were incubated with 0.015% H₂O₂ at room temperature and samples were collected at 0, 1, 2, 3, and 4 hours. Under oxidation stress, the oxidized variants and Total Related Protein were separated by RP-HPLC

and levels of oxidized methionine at different relative retention time (RRT) were determined by LC-MS peptide mapping. The results are provided in Tables 3.2.R.5.5-4 and 3.2.R.5.5-5 but not shown here for brevity. The levels of Met127 at RRT 0.98 and Total Relative Proteins at different exposure time are shown below in the applicant's Figures 3.2.R.5.5-1 and 3.2. R.5.5-2. The chromatogram overlay of RP-HPLC results for PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U. lots is shown below in the applicant's Figure 3.2.R.5.5-3.

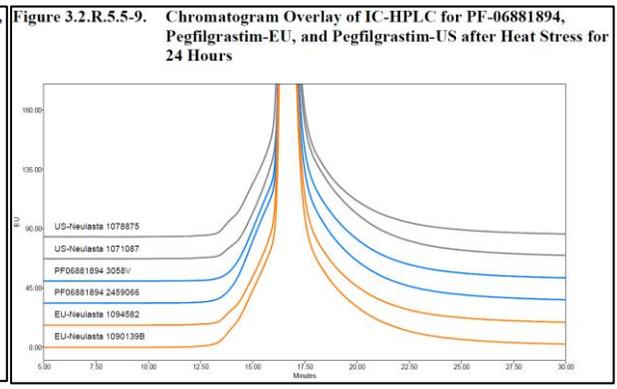
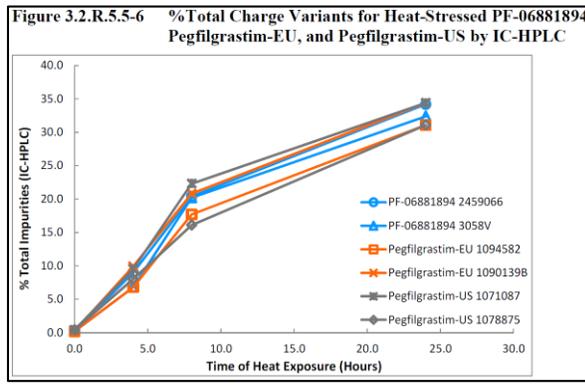
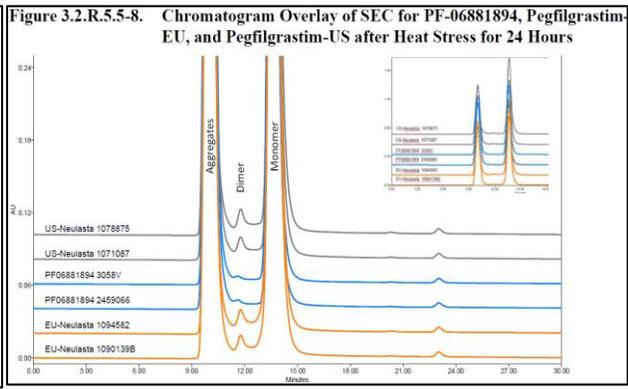
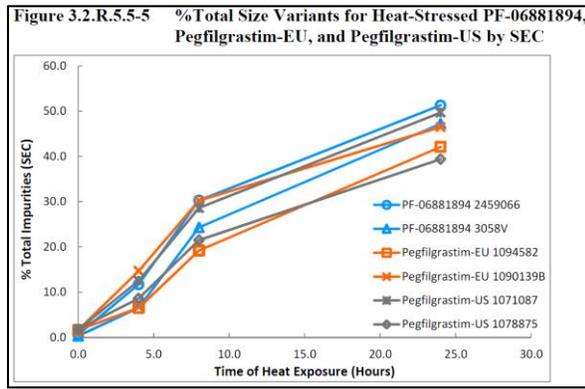
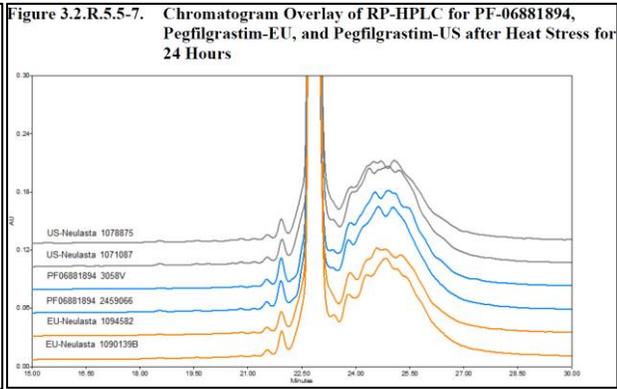
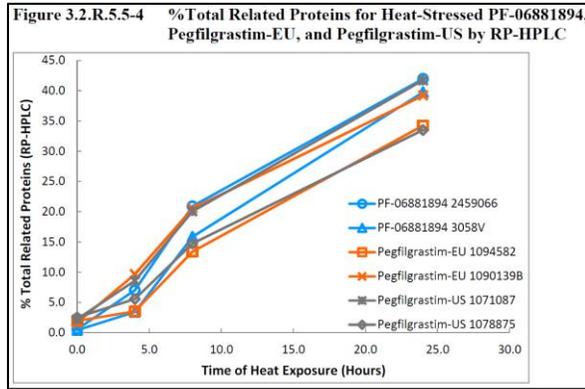


Assessor's Comment:

Oxidized Met127, Met138 (in table 3.2.R.5.5-5), and Total Related Proteins increased over the 4 hours of exposure to peroxide for all six tested lots. However, the oxidized methionine species in PF-06881894 lots at each tested time point are consistently lower than those in pegfilgrastim-U.S. lots and pegfilgrastim-E.U. lots. The degradation profile of pegfilgrastim-U.S. lots is comparable to that of pegfilgrastim-E.U. lots. The levels of other variants as results of oxidation, deamidation, and isomerization in three products were either under LOD/LOQ or no change compared to untreated samples. Overlay of RP-HPLC chromatograms are superimposable at 4-hour post peroxide treatment for PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U. lots. Therefore, the PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U. lots exhibit a similar trend of degradation for product related impurities in response to the oxidative stress.

3.2.R.5.5.3.2. Heat Stress

The samples from PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U. lots were heated at 50°C for 0, 4, 8, and 24 hours to compare the degradation profiles of the three products. The purity of the three products were measured for the product related proteins by RP-HPLC, size variants by SEC-HPLC and charge variants by IC-HPLC and graphical results are shown below in the applicant's Figures 3.2.R.5.5-4, 3.2.R.5.5-5, and 3.2.R.5.5-6, respectively. The results are provided in Table 3.2.R.5.5-6 but are not shown here for brevity. The chromatogram overlays of Total Related Protein, Total Size Variants, and Total Charge Variants at 50°C for 24 hours for the three products are shown below in the applicant's Figures 3.2.R.5.5-7, 3.2.R.5.5-8, and 3.2.R.5.5-9, respectively.



Assessor's Comment:

The heat stress condition of 50°C was selected because it was possible to observe gradual changes in product-related impurities, charge variants and potency of pegfilgrastim under that condition. The RP-HPLC results in Table 3.2.R.5.5-6 showed that intensity of impurity peaks at RRT 1.05, RRT 1.07, and RRT 1.13 were significantly increased and the peak at RRT 1.13 was the major impurity peak in the three products. We noted that for RP-HPLC results at RRT 0.98 for untreated samples (0 hour), the two PF-06881894 lots in peroxide stress study showed lower abundance of impurity peaks at 0.4% and 0.6% compared to the results at 1.4% and 1.2% in heat stress study. This is mostly like due to the method variability. The SEC results showed that aggregation is the major heat induced degradation pathway. There is no significant change in dimer and des-pegylated species. The IC-HPLC results indicated that the levels of acidic and basic variants were significantly increased under the heat stress. There was a slight increase in aspartate isomerization determined by peptide mapping and the three products exhibited similar trend of increased isomerization. Overlay of chromatograms for RP-HPLC, SEC-HPLC, and IC-HPLC show the results are superimposable at 24-hour heat exposure for three products. Therefore, PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U. showed similar degradation rates and pathways in response to heat stress.

3.2.R.5.5.3.3. Light Stress

PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U. lots were exposed to different intensities of combined white and UV light based on ICH Q1B option 2 as shown in the applicant's Table 3.2.R.5.5-2 above. Graphical results for Total Related Protein by RP-HPLC and size variants by SEC-HPLC are shown below in the applicant's Figures 3.2.R.5.5-10 and 3.2.R.5.5-11, respectively. The light stress results are provided in the applicant's Tables 3.2.R.5.5-7 but are not shown here for brevity. The chromatogram overlays of Total Related Protein and Total Size Variants for the three products under light stress of 0.6x are shown below in the applicant's Figures 3.2.R.5.5-12 and 3.2.R.5.5-13, respectively.

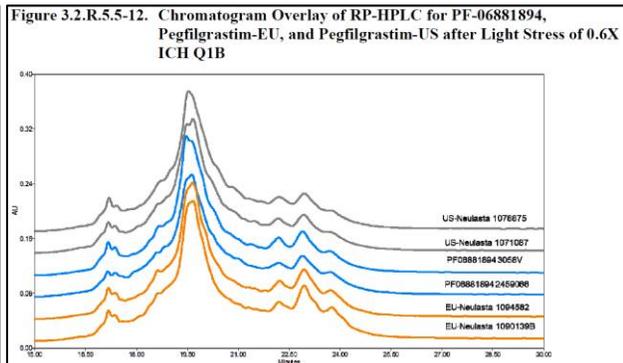
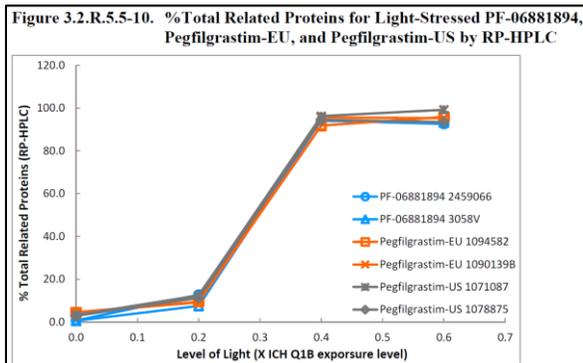


Figure 3.2.R.5.5-11. % Total Size Variants for Light-Stressed PF-06881894, Pegfilgrastim-EU, and Pegfilgrastim-US by SEC

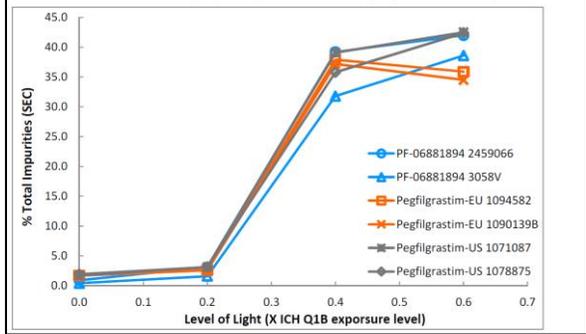
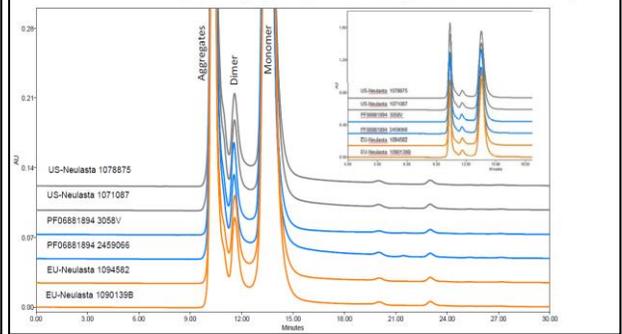


Figure 3.2.R.5.5-13. Chromatogram Overlay of SEC for PF-06881894, Pegfilgrastim-EU, and Pegfilgrastim-US after Light Stress of 0.6X ICH Q1B



Assessor's Comment:

The RP-HPLC results showed significant increase in Total Related Protein under the light stress. The SEC results indicated that the major degradation pathway in response to light stress is aggregation. Significantly increased oxidation at Met122, Met127 and Met138, W59 and W119 in response to light stress were identified by LC-MS peptide mapping. There was a slight increase in deamidation observed at Q108. The three products exhibited similar increased rates of aggregation, oxidation and product related impurities. Overlay of chromatograms from RP-HPLC and SEC-HPLC show the results are superimposable after exposure to 0.6x ICH1B option 2 light stress conditions for PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U. lots. Therefore, the three products showed similar degradation rates and pathways in response to the light stress.

3.2.R.5.5.3.4. High pH Stress

PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U. were incubated at pH 10 solution at room temperature and collected for testing at 0, 2, 5, and 7 days. High pH induced degradation was evaluated for Total Related Protein, Total Size Variants, and Total Charge Variants, and graphical results are shown below in the applicant's Figures 3.2.R.5.5-14, 3.2.R.5.5-15, and 3.2.R.5.5-16 below, respectively. The results for each product are provided in the applicant's Table 3.2.R.5.5-8 but not shown for brevity. The chromatogram overlays of Total Related Protein, Total Size Variants, and Total Charge Variants are shown below in the applicant's Figures 3.2.R.5.5-17, 3.2.R.5.5-18 and 3.2.R.5.5-19, respectively.

Figure 3.2.R.5.5-14. % Total Related Proteins for High pH-Stressed PF-06881894, Pegfilgrastim-EU, and Pegfilgrastim-US by RP-HPLC

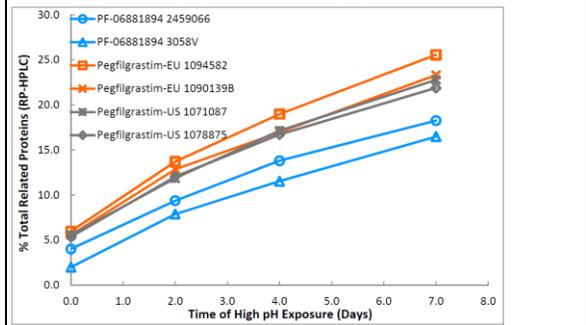


Figure 3.2.R.5.5-17. Chromatogram Overlay of RP-HPLC for PF-06881894, Pegfilgrastim-EU, and Pegfilgrastim-US after High pH Stress for 7 Days

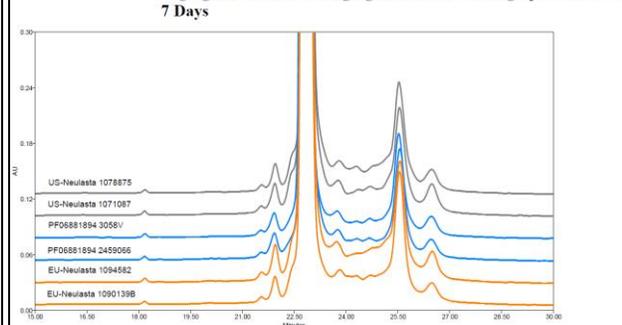


Figure 3.2.R.5.5-15. %Total Size Variants for High pH-Stressed PF-06881894, Pegfilgrastim-EU, and Pegfilgrastim-US by SEC

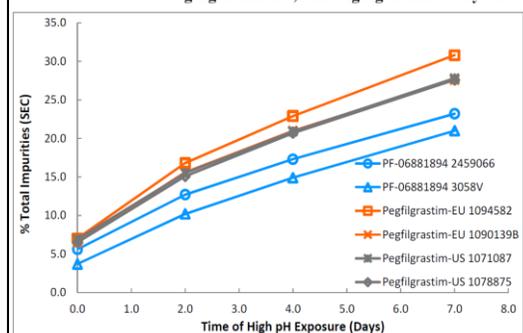


Figure 3.2.R.5.5-18. Chromatogram Overlay of SEC for PF-06881894, Pegfilgrastim-EU, and Pegfilgrastim-US after High pH Stress for 7 Days

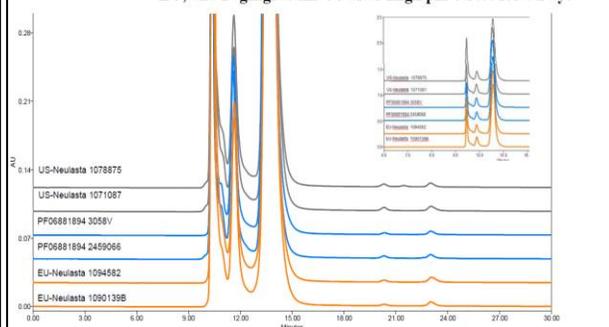


Figure 3.2.R.5.5-16. %Total Charge Variants for High pH-Stressed PF-06881894, Pegfilgrastim-EU, and Pegfilgrastim-US by IC-HPLC

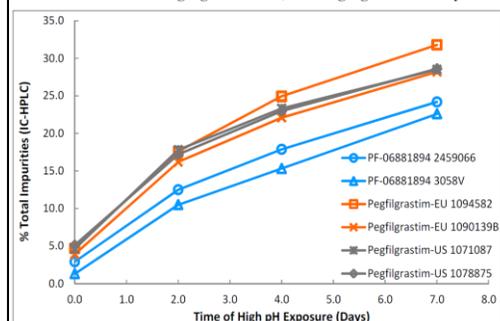
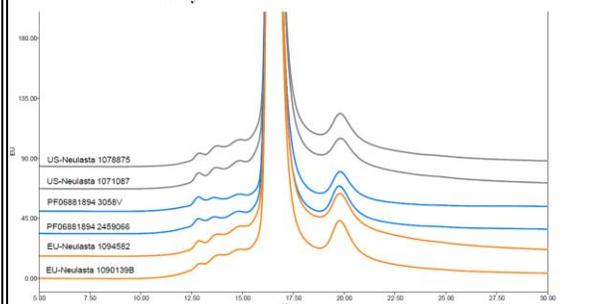


Figure 3.2.R.5.5-19. Chromatogram Overlay of IC-HPLC for PF-06881894, Pegfilgrastim-EU, and Pegfilgrastim-US after High pH Stress for 7 Days



Assessor's comment:

Under the high pH condition, the RP-HPLC results show significant increase in Total Related Protein at RRTs 1.05, 1.07 and 1.13. The SEC-HPLC results show significant increase in aggregates (the first peak from left) and dimers (the second peak from left). Significant increase in acidic and basic charge variants were observed by IC-HPLC. There was no significant change for oxidation, deamidation, and isomerization as determined by LC-MS peptide mapping. Overlay of chromatograms from RP-HPLC, SEC-HPLC, and IC-HPLC are superimposable after 7-days exposure to high pH stress for PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U. lots. All three products showed similar degradation rate and pathways in response to the high pH condition.

Comparative Forced Degradation Summary

Results from multiple orthogonal analytical studies to assess product quality under forced degradation conditions support a determination that PF-06881894 is highly similar to pegfilgrastim-U.S. are, and a determination that the analytical part of the scientific bridge was established.

Comparative Stability Study

Stability studies were conducted to compare the stability profiles of PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U. Six lots of PF-06881894 DP, 2 lots of pegfilgrastim-U.S., and 2 lots of pegfilgrastim-E.U. were tested in the comparative stability study. Three storage conditions were evaluated: 1) the long-term storage condition of 2-8°C (5°C) for up to 36 months; 2) accelerated storage conditions of 25 ± 2°C/60 ± 5% RH for up to 6 months; and 3) stress conditions of 40 ± 2°C/ 75 ± 5% RH for up to 3 months for PF-06881894 DP and up to 6 weeks for reference products. The tested attributes include protein concentration by UV-Vis method,

potency by in vitro cell-based assay, size variants by SEC-HPLC, and product-related proteins by RP-HPLC. The stability results for PF-06881894 DP lots are provided in Section 3.2.P.8.3 Stability Data. The comparative stability results for two reference products are provided below. Summaries of the stability study design are provided below for PF-06881894 in the applicant's Table 3.2.R.5.5-1, and for pegfilgrastim-U.S. and pegfilgrastim-E.U. in the applicant's Table 3.2.R.5.5-2.

Product	Lot Number	DP Manufacturing Date	Stability Initiation Date	Stability Conditions and Time Points	
				Conditions	Time Points
PF-06881894	2056034	Mar 2014	Apr 2014	5°C 25°C/60% RH 40°C/75% RH	0, 3, 6, 9, 12, 18, 24, 36 months 0, 1, 2, 3, 6 months 0, 1, 2, 3 months
	2078064	Jun 2014	Oct 2014	5°C 25°C/60% RH 40°C/75% RH	0, 3, 6, 9, 12, 18, 24, 36 months 0, 1, 2, 3, 6 months 0, 1, 2, 3 months
	2082094	Oct 2014	Oct 2014	5°C 25°C/60% RH 40°C/75% RH	0, 2, 3, 6, 9, 12, 18, 24, 36 months 0, 1, 2, 3, 6 months 0, 1, 2, 3 months
	2051124	Dec 2014	Feb 2015	5°C 25°C/60% RH 40°C/75% RH	0, 2, 3, 6, 9, 12, 18, 24, 36 months 0, 1, 2, 3, 6 months 0, 1, 2, 3 months
	2573125	Dec 2015	Feb 2016	5°C 25°C/60% RH 40°C/75% RH	0, 2, 3, 6, 9, 12, 18, 24 months 0, 1, 2, 3, 6 months 0, 2, 4, 6 weeks
	2459066	Jun 2016	Jun 2016	5°C	0, 9, 12, 18, 24 months

Product	Lot Number	Expiry Date	Estimated Date of Manufacture	Stability Conditions and Time Points	
				Conditions	Time Points
Pegfilgrastim-US	1057096	Nov 2017	Nov 2014	5°C 25°C/60% RH 40°C/75% RH	10, 14, 18, 24, 36 months 0, 1, 2, 3, 6 months 0, 2, 4, 6 weeks
	1057133	Oct 2017	Oct 2014	5°C 25°C/60% RH 40°C/75% RH	11, 14, 18, 24, 36 months 0, 1, 2, 3, 6 months 0, 2, 4, 6 weeks
Pegfilgrastim-EU	1058436B	Aug 2017	Aug 2014	25°C/60% RH 40°C/75% RH	0, 1, 2, 3, 6 months 0, 2, 4, 6 weeks
	1060064C	Oct 2017	Oct 2014	5°C 25°C/60% RH 40°C/75% RH	13, 18, 24, 30, 36 months 0, 1, 2, 3, 6 months 0, 2, 4, 6 weeks
	1061466C	Oct 2017	Oct 2014	5°C	13, 18, 24, 30, 36 months

RH=relative humidity

The comparative stability assessment was conducted based on trend analysis of stability-indicating attributes followed by comparison of the average slopes from the linear regression analysis. The significance of the average slope for each product is determined if a p-value is less than 0.05. However, the applicant stated that, due to the limited data for pegfilgrastim-U.S., and pegfilgrastim-E.U. lots, the slope significance is presented for information only.

Assessor's Comment:

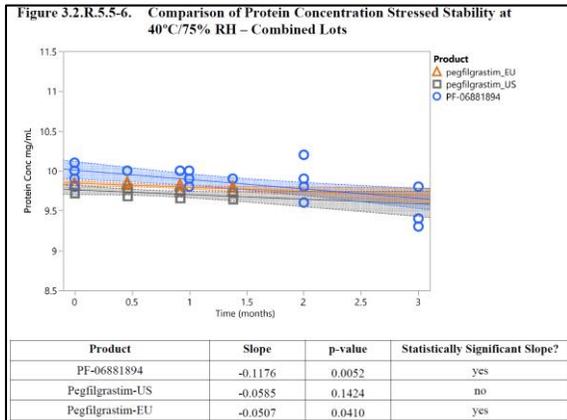
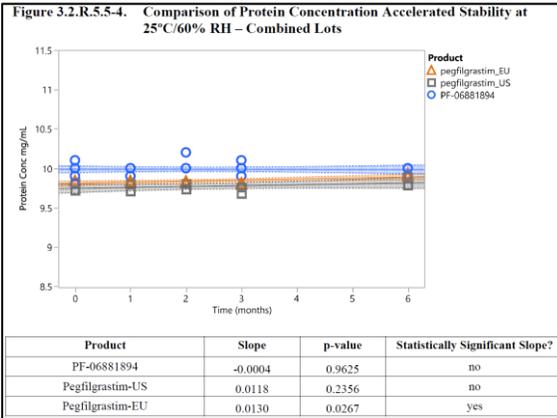
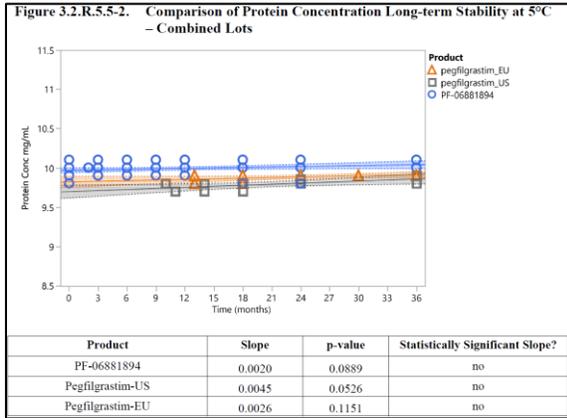
The applicant indicated that historical validated versions of the SEC and RP-HPLC methods were used in comparative stability studies, whereas revised SEC and RP-HPLC methods were used for the forced degradation studies. This does not impact the acceptability of the results from the comparative stability assessment as all the methods were shown to be suitable for their intended purposes and the bridging data provided by the applicant demonstrated the comparability of the methods. All the stability-indicating attributes of PF-06881894 DP, except for charge variants, were tested in the comparative stability study. However, charge variants were tested as part of the comparative forced degradation study and the charge variants stability

profile of PF-06881894 DP was evaluated under long-term, accelerated, and stressed storage conditions. Therefore, the proposed stability attributes, conditions, and time points in the comparative stability assessment are appropriate.

The PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U. lots used in the comparative stability study were properly selected. For example, PF-06881894 lots 2051124 and 2459066, pegfilgrastim-U.S. lot 1057133, and pegfilgrastim-E.U. lots 1060064C and 1061466C used in the comparative stability study were also used in the comparative clinical study; pegfilgrastim-E.U. lot 1058436B was used in the comparative non-clinical study. The applicant estimated that the product ages at the initiation time of long-term stability studies were approximately 10 - 13 months for pegfilgrastim-U.S. and pegfilgrastim-E.U. lots based on the products' expiration dates. The difference in ages of the lots at the time 0 comparisons is considered when interpreting the stability results.

3.2.R.5.5.4.1 Protein Concentration (UV-Vis)

The combined linear regression plots, slopes, p-values, and statistical significance in slopes of the protein concentrations for three products under long-term (5°C), accelerated (25°C), and stress (40°C) conditions are shown below in the applicant's Figure 3.2.R.5.5-2, Figure 3.2.R.5.5-4, and Figure 3.2.R.5.5-6, respectively. The shaded areas are the two-sided 95% confidence regions of the average slopes for each product. This statistical approach was also used for other tested attributes in the comparative stability study.

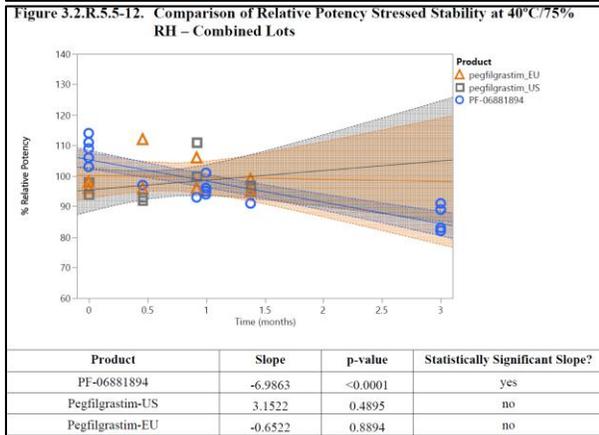
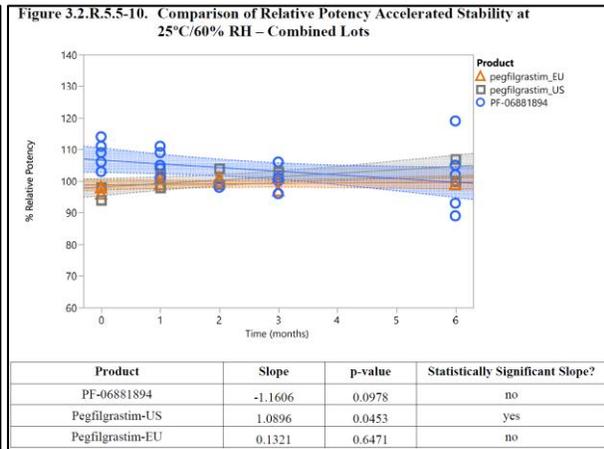
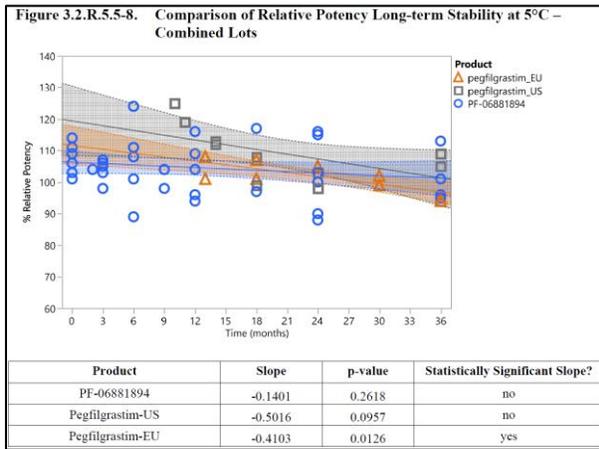


Assessor's Comment:

At the recommended long-term storage condition, the three products showed similar stability trends and the changes of average slope for three products were not statistically significant. At the accelerated storage condition there were no practical changes to the average slopes for PF-06881894 lots, pegfilgrastim-U.S. lots, and pegfilgrastim-E.U. At the stress condition, there is a slight decrease in protein content for PF-06881894 and pegfilgrastim-E.U. and no change to the average slope of pegfilgrastim-U.S. lots. However, due to the limited numbers of pegfilgrastim-U.S. and pegfilgrastim-E.U. lots, and 6-week of stress stability data, the statistical comparison of average slopes is of limited value. Therefore, there is no practical difference on the stability trend of protein concentration for the three products under the long-term, accelerated, and stress conditions. Although the protein concentrations (10mg/mL) of PF-06881894 are consistently higher than those of pegfilgrastim-U.S. and pegfilgrastim-E.U. lots, the values are aligned with labeling of protein concentration (10mg/mL) for pegfilgrastim-U.S. and pegfilgrastim-E.U. products. Therefore, the results form combined lot linear regression plots demonstrate that stability profile on protein concentration is similar between PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U. lots.

3.2.R.5.5.4.2. Potency (In Vitro Cell-Based Bioassay)

The overlay of the combined linear regression plots, slopes, p-values, and difference significance of potency for three products under long-term, accelerated, and stress conditions are shown in Figure 3.2.R.5.5-8, Figure 3.2.R.5.5-10, and Figure 3.2.R.5.5-12, respectively.



Assessor's Comment:

There was no significant difference in the stability trend for potency under recommended and accelerated storage conditions for the three products. A decrease in potency was observed for PF-06881894 lots under stress condition. However, the potency results of PF-06881894 lots were within the projected potency data for pegfilgrastim-U.S. and pegfilgrastim-E.U. lots at the 3-month time point. Therefore, the potency stability trends under the long-term, accelerated, and stress conditions are similar between PF-06881894, pegfilgrastim-U.S. and pegfilgrastim-E.U. lots,

3.2.R.5.5.4.3. Size Variants (SEC-HPLC)

An overlay of SEC chromatograms of the three products under stress condition at the 6-week time point is provided below in the applicant's Figure 3.2.R.5.5-13. The levels of Total Size Variants are compared between the three products under long-term, accelerated, and stress conditions as shown in Figure 3.2.R.5.5-15, Figure 3.2.R.5.5-17 and Figure 3.2.R.5.5-19, respectively. The dimer and other HMWS between the three products were compared under the accelerated condition as shown in Figure 3.2.R.5.5-21 and Figure 3.2.R.5.5-23, respectively.

Figure 3.2.R.5.5-13. SEC Chromatograms for PF-06881894 DP, Pegfilgrastim-US and Pegfilgrastim-EU Stressed Stability at 40°C/75% RH for 6 Weeks

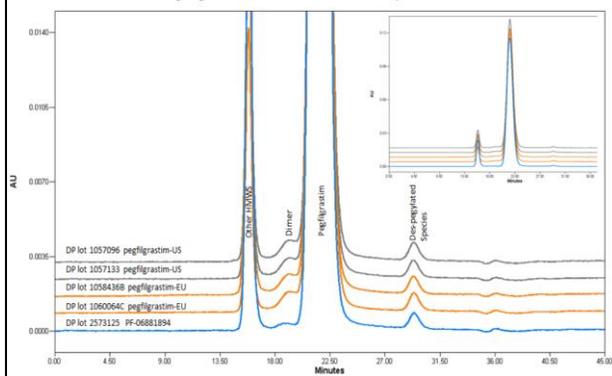


Figure 3.2.R.5.5-15. Comparison of Total Size Variants Long-term Stability at 5°C – Combined Lots

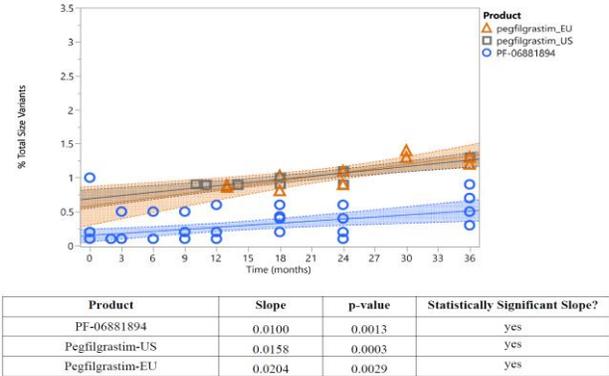


Figure 3.2.R.5.5-17. Comparison of Total Size Variants Accelerated Stability at 25°C/60% RH – Combined Lots

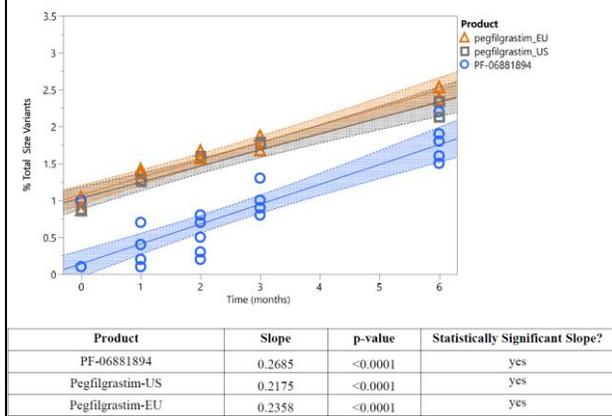
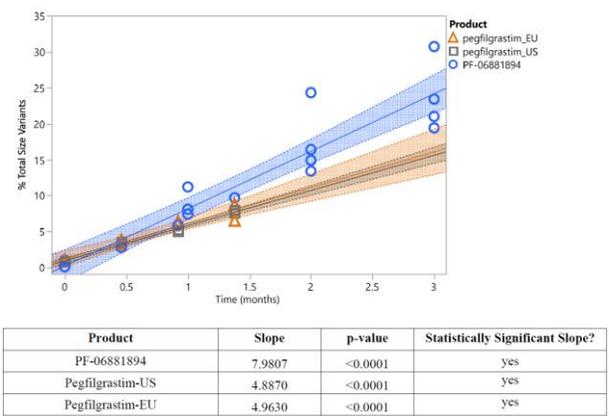
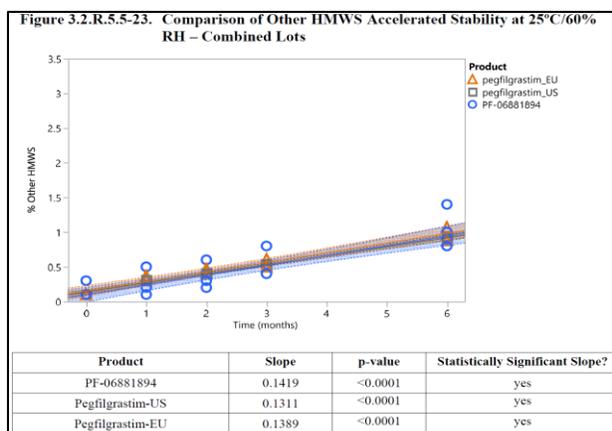
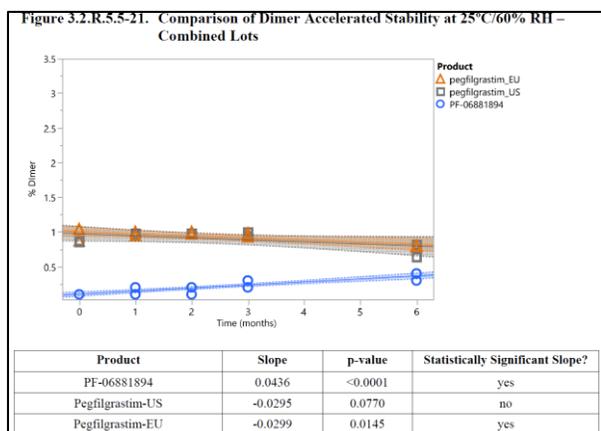


Figure 3.2.R.5.5-19. Comparison of Total Size Variants Stressed Stability at 40°C/75% RH – Combined Lots





Assessor' Comment:

The applicant determined that most size variants in PF-06881894 are dimer and other HMWS larger than dimer. Product related impurities other than dimer and other HMWS are present in low levels under stress stability condition. The PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U. lots showed increased Total Size Variants under accelerated and stressed temperature conditions. Under the stressed stability condition, it appears that Total Size variants increase more rapidly for PF-06881894 than for pegfilgrastim-U.S. or pegfilgrastim-E.U., however, given the totality of the evidence this is most likely an artifact of the small data set. Under the stress condition, the comparison of SEC chromatograms at 6 weeks showed similar levels of dimer, HMWS, and des-pegylated pegfilgrastim and the overlay of SEC chromatograms are superimposable for the three products.

There is no significant change in the dimer levels in the three products under the stressed condition. The levels of other HMWS in PF-06881894 lots over time are similar to that in pegfilgrastim-U.S. lots and pegfilgrastim-E.U. lots.

The size variant stability profiles under long-term, accelerated, and stress storage conditions are similar between PF-06881894, pegfilgrastim-U.S. and pegfilgrastim-E.U. lots,

3.2.R.5.5.4.4. Pegfilgrastim Related Proteins (RP-HPLC)

An overlay of RP-HPLC chromatograms of three products at the 40°C for 6 weeks is shown below in the applicant's Figure 3.2.R.5.5-24. The combined data using linear regression analysis are provided under long-term, accelerated, and stress storage conditions in Figures 3.2.R.5.5-26, 3.2.R.5.5-28, and 3.2.R.5.5-30, respectively.

Figure 3.2.R.5.5-24. RP-HPLC Chromatograms for PF-06881894 DP, Pegfilgrastim-US and Pegfilgrastim-EU Stressed Stability at 40°C/75% RH for 6 Weeks

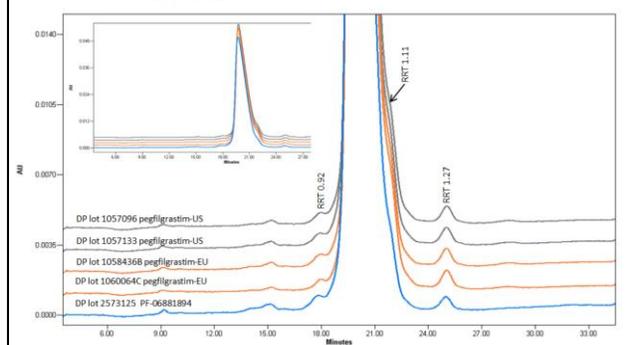


Figure 3.2.R.5.5-26. Comparison of Total Related Proteins Long-Term Stability at 5°C – Combined Lots

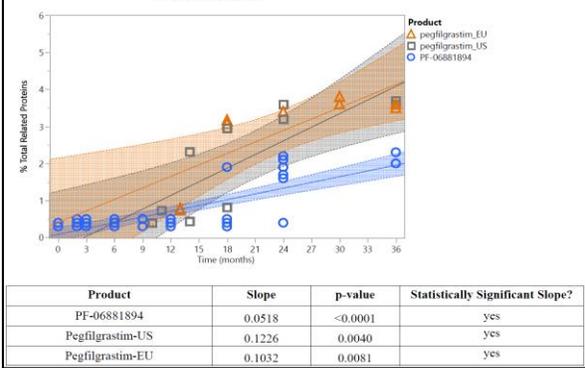


Figure 3.2.R.5.5-28. Comparison of Total Related Proteins Accelerated Stability at 25°C/60% RH – Combined Lots

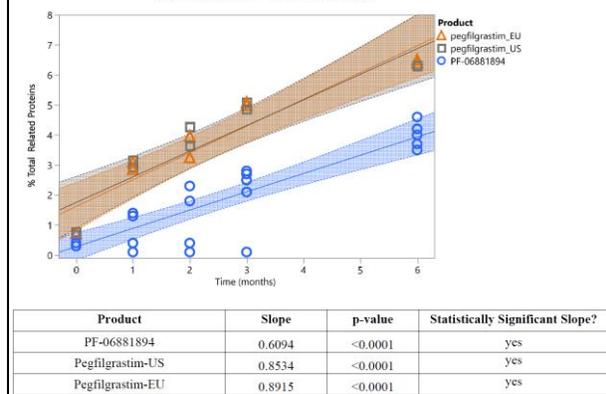
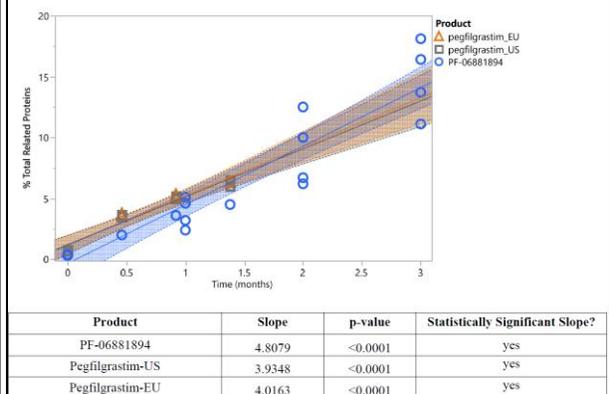


Figure 3.2.R.5.5-30. Comparison of Total Related Proteins Stressed Stability at 40°C/75% RH – Combined Lots



Assessor’s Comment:

The applicant determined that the RP-HPLC pre-main peaks were primarily oxidized species and post-main peaks were primarily reduced and deamidated species. The relative percentage of a post-main peak species at approximately 1.10-1.12 was overestimated due to the partial resolution from the main peak, which contributed to the increase of Total Related Proteins over time. The slopes of pegfilgrastim-U.S. and pegfilgrastim-E.U. lots exhibited a slightly slower rate of increase in the Total Related Proteins than PF-06881894 lots under the long-term condition. Under the stressed condition (40°C), all the three products exhibited similar trend of increase in Total Related Proteins. The two-sided 95% confidence regions of the average slopes are nearly superimposable for all the three products. Therefore, the RP-HPLC results from three products showed similar rates of increase in Total Related Protein under the long-term, accelerated, and stressed stability conditions.

Comparative Stability Summary

Results from multiple orthogonal analytical studies to assess product stability under recommended, stressed, and accelerated storage conditions support a determination that PF-06881894 is highly similar to pegfilgrastim-U.S., and a determination that the analytical part of the scientific bridge was established.

Comparative analytical assessment conclusion:

In summary, the pair-wise analytical comparisons of PF-06881894, pegfilgrastim-U.S., and pegfilgrastim-E.U. support a determination that PF-06881894 is highly similar to pegfilgrastim-U.S. and that the analytical component of the scientific bridge was established.

Appendix 1

Table 3.2.R.5.4-6. Summary of Comparative Primary Structure Characterization Studies and Test Samples

Method	Attribute	Test Article	Number of Lots	Range of Age at Time of Test* (Months)
Intact Mass	Molecular Weight (including Dispersity)	PF-06881894	7	5 – 42
		Pegfilgrastim-US	6	17 – 36
		Pegfilgrastim-EU	6	9 – 33
Glu-C Peptide Mapping	Amino Acid Sequence	PF-06881894	7	5 – 42
		Pegfilgrastim-US	6	17 – 36
		Pegfilgrastim-EU	6	9 – 33
Glu-C Peptide Mapping of Pegylated Peptide	Pegylation Site and Linker Composition	PF-06881894	2	5 – 28
		Pegfilgrastim-US	1	27
		Pegfilgrastim-EU	1	24
Ellman's Assay	Free Thiol	PF-06881894	10	5 – 26
		Pegfilgrastim-US	9	14 – 36
		Pegfilgrastim-EU	9	9 – 33
cIEF	pI	PF-06881894	10	3 – 30
		Pegfilgrastim-US	6	12 – 29
		Pegfilgrastim-EU	6	9 – 36

cIEF, capillary isoelectric focusing; Glu-C, Endoproteinase Glu-C; pI, isoelectric point
a. Age at time of test is estimated for pegfilgrastim-US and pegfilgrastim-EU.

Table 3.2.R.5.4-7. Summary of Comparative Higher Order Structure Characterization Studies and Test Samples

Method	Attribute	Test Article	Number of Lots	Range of Age at Time of Test* (Months)
Far UV-CD	Secondary Structure	PF-06881894	9	4 – 24
		Pegfilgrastim-US	6	14 – 36
		Pegfilgrastim-EU	6	9 – 33
Disulfide Mapping	Disulfide Linkage	PF-06881894	3	4 – 45
		Pegfilgrastim-US	3	17 – 32
		Pegfilgrastim-EU	3	23 – 36
H/D Exchange	Tertiary Structure (Structure Dynamics)	PF-06881894	3	5 – 46
		Pegfilgrastim-US	2	27 – 33
		Pegfilgrastim-EU	2	24 – 34
SV-AUC	Tertiary Structure (Sedimentation Coefficient)	PF-06881894	7	5 – 24
		Pegfilgrastim-US	6	13 – 36
		Pegfilgrastim-EU	6	9 – 33
DSC	Tertiary Structure (Melting Temperature)	PF-06881894	10	3 – 30
		Pegfilgrastim-US	10	12 – 29
		Pegfilgrastim-EU	10	9 – 33
NMR	Tertiary Structure (Protein Structure)	PF-06881894	4	5 – 28
		Pegfilgrastim-US	2	19 – 27
		Pegfilgrastim-EU	3	22 – 33

DSC, differential scanning calorimetry; H/D, hydrogen deuterium; NMR, nuclear magnetic resonance; SV-AUC, sedimentation velocity analytical ultracentrifugation; UV-CD, ultraviolet circular dichroism
a. Age at time of test is estimated for pegfilgrastim-US and pegfilgrastim-EU.

Table 3.2.R.5.4-9. Summary of Comparative Drug Product Attribute Characterization Studies and Test Samples

Method	Attribute	Test Article	Number of Lots	Range of Age at Time of Test* (months)
UV-Vis	Protein Concentration	PF-06881894	5	0 – 3
		Pegfilgrastim-US	15	9 – 29
		Pegfilgrastim-EU	15	9 – 33
Protein Concentration x Deliverable Volume	Deliverable Content	PF-06881894	5	0 – 3
		Pegfilgrastim-US	10	9 – 29
		Pegfilgrastim-EU	10	9 – 33
USP <697> Ph. Eur. <2.9.17>	Deliverable Volume	PF-06881894	5	1 – 3
		Pegfilgrastim-US	12	10 – 36
		Pegfilgrastim-EU	12	9 – 35
MFI	Subvisible Particles	PF-06881894	10	3 – 27
		Pegfilgrastim-US	6	15 – 30
		Pegfilgrastim-EU	6	19 – 36
USP <791> Ph. Eur. <2.2.3>	pH	PF-06881894	10	0 – 4
		Pegfilgrastim-US	3	23 – 33
		Pegfilgrastim-EU	3	10 – 33
USP <785> Ph. Eur. <2.2.35>	Osmolality	PF-06881894	8	0 – 5
		Pegfilgrastim-US	5	23 – 36
		Pegfilgrastim-EU	5	10 – 35
RP-HPLC-ELSD	Polysorbate 20	PF-06881894	10	1 – 9
		Pegfilgrastim-US	3	23 – 33
		Pegfilgrastim-EU	3	10 – 33
Ph. Eur. <2.2.2> and <2.2.1>	Appearance, Color, Clarity	PF-06881894	10	0 – 4
		Pegfilgrastim-US	3	23 – 36
		Pegfilgrastim-EU	3	10 – 30
USP <790> Ph. Eur. <2.9.20>	Visible Particles	PF-06881894	10	0 – 4
		Pegfilgrastim-US	3	23 – 36
		Pegfilgrastim-EU	3	10 – 30

MFI, microflow imaging; Ph. Eur., European Pharmacopoeia; RP-HPLC-ELSD, reversed-phase high performance liquid chromatography evaporative light scattering detector; USP, United States Pharmacopoeia; UV-Vis, ultraviolet-visible
a. Age at time of test is estimated for pegfilgrastim-US and pegfilgrastim-EU.

Table 3.2.R.5.4-8. Summary of Comparative Product-Related Substances and Impurities Characterization Studies and Test Samples

Method	Attribute	Test Article	Number of Lots	Range of Age at Time of Test* (Months)	
RP-HPLC	Total Related Proteins	PF-06881894	10	0 – 51	
	Oxidation	Pegfilgrastim-US	10	14 – 36	
	Deamidation	Pegfilgrastim-EU	10	10 – 33	
	Reduced Species Des-pegylated Species				
IC-HPLC	Total Charge Variants	PF-06881894	10	1 – 51	
	Deamidation	Pegfilgrastim-US	10	14 – 36	
		Pegfilgrastim-EU	10	10 – 33	
SEC	Total Size Variants	PF-06881894	10	0 – 51	
	HMWS	Pegfilgrastim-US	10	14 – 36	
	Des-pegylated Species	Pegfilgrastim-EU	10	10 – 33	
SV-AUC	Size Variants	PF-06881894	7	5 – 24	
		Pegfilgrastim-US	6	13 – 36	
		Pegfilgrastim-EU	6	9 – 33	
SDS-PAGE	Size Variants	PF-06881894	9	5 – 48	
		Pegfilgrastim-US	6	17 – 36	
		Pegfilgrastim-EU	6	9 – 33	
RP-HPLC-ELSD	Residual PEG	PF-06881894	7	1 – 42	
		Pegfilgrastim-US	10	14 – 36	
		Pegfilgrastim-EU	10	10 – 33	
Glu-C Peptide Mapping	Oxidation (Met122, Met127, Met138, Trp59)	PF-06881894	7	5 – 42	
		Pegfilgrastim-US	6	17 – 36	
		Pegfilgrastim-EU	6	9 – 33	
	Deamidation (Gln21, Gln91, Gln120 and Gln135)				
N-terminal Des-pegylated Species Other Low Abundant Pegfilgrastim Related Species					

ELSD, evaporative light scattering detector; Glu-C, Endoproteinase Glu-C; HMWS, High Molecular Weight Species; IC, ion chromatography; PEG, polyethylene glycol; RP-HPLC, reversed-phase high performance liquid chromatography; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; SEC, size exclusion chromatography; SV-AUC, sedimentation velocity analytical ultracentrifugation;
a. Age at time of test is estimated for pegfilgrastim-US and pegfilgrastim-EU.

Appendix 2

A summary of the comparative forced degradation results prepared by the reviewer is provided in the following table.

Stress Conditions	Test Method	Quality Attributes	Duration	U.S.-licensed Neulasta Range	PF Min-Max Range	E.U.-approved Neulasta Range	PF vs. U.S.-licensed Neulasta/ PF vs. E.U.-approved Neulasta/ U.S.-licensed Neulasta vs E.U.-approved Neulasta
Peroxide	RP-HPLC (LOQ= 0.3%)	% (RRT 0.87)	0	<LOQ	<LOQ	<LOQ	Yes/yes/yes
			1 hour	<LOQ	<LOQ	<LOQ	Yes/yes/yes
			2 hours	<LOQ	<LOQ	<LOQ	Yes/yes/yes
			3 hours	<LOQ	<LOQ	<LOQ	Yes/yes/yes
			4 hours	<LOQ	<LOQ	<LOQ	Yes/yes/yes
		% (RRT 0.98)	0	1.7-1.8	1.2-1.4	1.6-1.8	Yes/yes/yes (Note: The oxidized Met127 at RRT 0.98 is a product related impurity and the reduced level of oxidation at Met127 in PF-06881894 is acceptable)
			1 hour	3.3-3.4	2.2-2.4	3.3	Yes/yes/yes (See note above)
			2 hours	4.1-4.2	3.2-3.4	4.1	Yes/yes/yes (See note above)
			3 hours	4.9	4.0-4.4	4.9	Yes/yes/yes (See note above)
			4 hours	5.7-5.9	4.9-5.4	5.7-5.8	Yes/yes/yes (See note above)
		% (RRT 1.05)	0	0.4-0.5	<LOD	0.5	Yes/yes/yes (Note: the oxidized species are product related impurities and the reduced level of oxidized species is acceptable)
			1 hour	0.4-0.5	<LOD	0.5	Yes/yes/yes (See note above)
			2 hours	0.4-0.5	<LOD	0.4-0.5	Yes/yes/yes (See note above)
			3 hours	0.4-0.5	<LOD	0.4-0.5	Yes/yes/yes (See note above)
			4 hours	0.4-0.5	<LOD	<LOD -0.4	Yes/yes/yes (See note above)
		% (RRT 1.07)	0	0.5-0.6	<LOQ	0.4	Yes/yes/yes

						(See note above)	
		1 hour	0.6	<LOD - <LOQ	0.3-0.4	Yes/yes/yes (See note above)	
		2 hours	0.4-0.6	<LOQ- 0.4	0.3	Yes/yes/yes (See note above)	
		3 hours	0.5	<LOQ	0.3	Yes/yes/yes (See note above)	
		4 hours	0.5-0.6	<LOD-0.4	0.3	Yes/yes/yes (See note above)	
	% (RRT 1.13)	0	<LOD	<LOD	<LOD	Yes/yes/yes	
		1 hour	<LOD	<LOD	<LOD	Yes/yes/yes	
		2 hours	<LOD	<LOD	<LOD	Yes/yes/yes	
		3 hours	<LOD	<LOD	<LOD	Yes/yes/yes	
		4 hours	<LOD	<LOD	<LOD	Yes/yes/yes	
	% Total Related Proteins	0	3.3-3.5	1.2-1.4	3.0-3.1	Yes/yes/yes (Note: The Total Related Protein are product related impurities and the reduced level of Total Related Proteins in PF-06881894 is acceptable)	
		1 hour	4.3-4.4	2.2-2.4	4.1	Yes/yes/yes (See note above)	
		2 hours	4.9-5.3	3.2-3.8	4.8	Yes/yes/yes (See note above)	
		3 hours	5.8-5.9	4.0-4.7	5.5-5.7	Yes/yes/yes (See note above)	
		4 hours	6.7-6.9	4.9-5.7	6.0-6.5	Yes/yes/yes (See note above)	
	Peptide Mapping (LOQ=0.5%)	% Q21 Deamidated	0 hour	0.7-0.8	<0.5-0.6	0.5	Yes/yes/yes (Note: The deamidated pegfilgrastim is a product related impurity and the reduced level of deamidated pegfilgrastim in PF-06881894 is acceptable)
		% Q21 Deamidated	4 hours	0.8	<0.5-0.6	0.5	Yes/yes/yes (See note above)
		% D28 Isomerization	0 hour	<0.5	<0.5	<0.5	Yes/yes/yes
		% D28 Isomerization	4 hours	<0.5	<0.5	<0.5	Yes/yes/yes
		% W59	0 hour	0.7	<0.5	0.5-0.7	Yes/yes/yes

		Oxidation					(Note: The oxidized W59 is a product related impurity and the reduced level of oxidation at W59 in PF-06881894 is acceptable)
		% W59 Oxidation	4 hours	0.7-0.8	<0.5	0.6	Yes/yes/yes (See note above)
		% W59 Dioxidation	0 hour	<0.5	<0.5	<0.5	Yes/yes/yes
		% W59 Dioxidation	4 hours	<0.5	<0.5	<0.5	Yes/yes/yes
		% Q91 Deamidated	0 hour	<0.5	<0.5	<0.5	Yes/yes/yes
		% Q91 Deamidated	4 hours	<0.5	<0.5	<0.5	Yes/yes/yes
		% Q108 Deamidated	0 hour	<0.5	<0.5	<0.5	Yes/yes/yes
		% Q108 Deamidated	4 hours	<0.5	<0.5	<0.5	Yes/yes/yes
		% W119 Oxidation	0 hour	<0.5	<0.5	<0.5	Yes/yes/yes
		% W119 Oxidation	4 hours	<0.5	<0.5	<0.5	Yes/yes/yes
		% M122 Oxidation	0 hour	<0.5	<0.5	<0.5	Yes/yes/yes
		% M122 Oxidation	4 hours	<0.5	<0.5	<0.5	Yes/yes/yes
		% M127 Oxidation	0 hour	1.3-1.4	1.3-1.6	1.4	Yes/yes/yes
		% M127 Oxidation	4 hours	5.1-5.4	5.4-5.5	5.0-5.5	Yes/yes/yes
		% M138 Oxidation	0 hour	2.9-3.2	3.0-3.5	3.0-3.2	Yes/yes/yes
		% M138 Oxidation	4 hours	11.9-12.8	12.4-13.5	11.8-12.2	Yes/yes/yes
Heat Stress	RP-HPLC	% (RRT 0.87)	0 hour	<LOQ	<LOQ	<LOQ	Yes/yes/yes
			4 hours	<LOQ	<LOQ	<LOD-<LOQ	Yes/yes/yes
			8 hours	<LOD-<LOQ	<LOD-<LOQ	<LOD-<LOQ	Yes/yes/yes
			24 hours	<LOD-<LOQ	<LOD-<LOQ	<LOD	Yes/yes/yes
		% (RRT 0.98)	0 hour	1.3-1.6	0.4-0.6	1.2-1.4	Yes/yes/yes

						(Note: The oxidized species is a product related impurity and the reduced level of oxidation in PF-06881894 is acceptable)	
		4 hours	1.4	0.3-0.5	1.3	Yes/yes/yes (See note above)	
		8 hours	1.1-1.3	<LOQ-0.4	1.0-1.2	Yes/yes/yes (See note above)	
		24 hours	1.5-2.0	1.3-1.5	1.1-1.5	Yes/yes/yes (See note above)	
	% (RRT 1.05)	0 hour	0.6	<LOD-<LOQ	0.5-0.6	Yes/yes/yes (See note above)	
		4 hours	0.9-1.1	0.6-1.2	0.9-1.8	Yes/yes/yes (See note above)	
		8 hours	2.0	1.7-1.9	1.3-2.1	Yes/yes/yes (See note above)	
		24 hours	3.0-3.7	3.8-4.3	4.0	Yes/yes/yes	
	% (RRT 1.07)	0 hour	0.4	<LOQ	<LOQ	Yes/yes/yes (See note above)	
		4 hours	1.4-2.7	0.6-2.1	1.0-2.6	Yes/yes/yes (See note above)	
		8 hours	3.3-4.4	1.8-3.5	2.8-3.3	Yes/yes/yes (See note above)	
		24 hours	7.1-7.7	4.7-10.1	4.1	Yes/yes/yes	
	% (RRT 1.13)	0 hour	<LOQ	<LOD	<LOD	Yes/yes/yes	
		4 hours	<LOQ-0.6	0.4-0.9	0.3-1.3	Yes/yes/yes	
		8 hours	1.9-4.7	3.2-4.1	2.1-2.5	Yes/yes/yes	
		24 hours	9.4-15.3	12.5-19.9	9.6-15.9	Yes/yes/yes	
	% Total Related Protein	0 hour	2.2-2.5	0.4-0.6	1.7-1.9	Yes/yes/yes	
		4 hours	5.6-8.6	3.4-7.0	3.5-9.7	Yes/yes/yes (See note above)	
		8 hours	14.7-20.1	15.9-20.9	13.4-20.5	Yes/yes/yes	
		24 hours	33.5-41.7	39.8-42.0	34.3-39.2	Yes/yes/yes	
	SEC-HPLC (LOQ=0.1%)	% Aggregates	0 hour	0.1-0.2	<LOQ-0.1	0.1	Yes/yes/yes
			4 hours	6.9-11.1	6.1-11.2	1.9-13.5	Yes/yes/yes
			8 hours	20.1-27.4	23.9-29.9	18.0-29.0	Yes/yes/yes
			24 hours	38.2-48.6	46.8-50.8	41.0-45.5	Yes/yes/yes
		% HMWS A	0 hour	0.1	<LOQ	0.1	Yes/yes/yes
			4 hours	<LOD-0.2	<LOD	<LOD-0.2	Yes/yes/yes

		8 hours	<LOD	<LOD	<LOD	Yes/yes/yes
		24 hours	<LOD	<LOD	<LOD	Yes/yes/yes
	% HMWS B	0 hour	1.3-1.4	0.3-0.4	1.3	Yes/yes/yes (Note: The HMWS is a product related impurity and the reduced level of HMWS is acceptable)
		4 hours	1.2-1.4	0.4-0.5	1.2-1.3	Yes/yes/yes (See note above)
		8 hours	1.2-1.3	0.4-0.5	1.1-1.2	Yes/yes/yes (See note above)
		24 hours	1.1-1.2	0.4-0.5	1.0-1.1	Yes/yes/yes (See note above)
	% Des-Pegylated species	0 hour	0.1	<LOQ-0.1	0.1	Yes/yes/yes
		4 hours	0.1	<LOQ-0.1	<LOQ-0.1	Yes/yes/yes
		8 hours	<LOQ-0.1	<LOQ	<LOQ	Yes/yes/yes
		24 hours	<LOQ	<LOQ	<LOQ	Yes/yes/yes
	% Total Size Variants	0 hour	1.7-1.8	0.3-0.6	1.6	Yes/yes/yes (Note: The Total Size Variants are product related impurities and the reduced level of Total Size Variants is acceptable)
		4 hours	8.6-12.5	6.5-11.7	6.5-14.7	Yes/yes/yes
		8 hours	21.5-28.6	24.3-30.3	19.2-30.2	Yes/yes/yes
		24 hours	39.4-49.7	47.2-51.3	42.1-46.5	Yes/yes/yes
IC-HPLC (LOQ=0.4%)	% Acidic Variants	0 hour	0.4	<LOQ	<LOQ	Yes/yes/yes
		4 hours	4.9-5.1	4.6-6.4	4.4-5.4	Yes/yes/yes
		8 hours	8.8-11.8	10.4-14.6	9.3-9.8	Yes/yes/yes
		24 hours	10.4-11.0	11.7-12.7	11.1-12.8	Yes/yes/yes
	% Basic Variants	0 hour	<LOQ	<LOQ	<LOQ	Yes/yes/yes
		4 hours	3.0-4.7	2.2-2.6	2.4-4.5	Yes/yes/yes (Note: The basic variants are product related impurities and the reduced level of Basic Variants is acceptable)
		8 hours	7.3-10.5	5.7-10.0	7.9-11.5	Yes/yes/yes (See note above)
		24 hours	20.2-24.0	20.7-21.5	20.0-21.6	Yes/yes/yes

	% Total Charge Variants	0 hour	0.4	<LOQ	<LOQ	Yes/yes/yes
		4 hours	8.0-9.5	6.8-9.0	6.8-9.9	Yes/yes/yes
		8 hours	16.1-22.3	20.2-20.4	17.7-20.8	Yes/yes/yes
		24 hours	31.1-34.4	32.2-32.4	31.1-34.4	Yes/yes/yes
Peptide Mapping	% Q21 Deamidated	0 hour	0.8	<0.5-0.6	0.5	Yes/yes/yes (Note: The deamidated pegfilgrastim is a product related impurity and the reduced level of deamidated pegfilgrastim in PF-06881894 is acceptable)
		24 hours	0.9	<0.5-0.6	0.6	Yes/yes/yes (See note above)
	% D28 Isomerization	0 hour	<0.5	<0.5	<0.5	Yes/yes/yes
		24 hours	1.7-2.5	2.2-2.6	1.7-2.2	Yes/yes/yes
	% W59 Oxidation	0 hour	0.6	0.5-0.7	0.6	Yes/yes/yes
		24 hours	0.9-1.3	0.6-1.0	0.8	Yes/yes/yes
	% W59 Dioxidation	0 hour	<0.5	<0.5	<0.5	Yes/yes/yes
		24 hours	<0.5	<0.5	<0.5	Yes/yes/yes
	% Q91 Deamidated	0 hour	<0.5	<0.5	<0.5	Yes/yes/yes
		24 hours	<0.5	<0.5	<0.5	Yes/yes/yes
	% Q108 Deamidated	0 hour	<0.5	<0.5	<0.5	Yes/yes/yes
		24 hours	<0.5	<0.5	<0.5	Yes/yes/yes
	% W119 Oxidation	0 hour	<0.5	<0.5	<0.5	Yes/yes/yes
		24 hours	<0.5	<0.5	<0.5	Yes/yes/yes
	% M122 Oxidation	0 hour	<0.5	<0.5	<0.5	Yes/yes/yes
		24 hours	<0.5	<0.5	<0.5	Yes/yes/yes
% M127 Oxidation	0 hour	<0.5-0.5	<0.5	<0.5	Yes/yes/yes	
	24 hours	0.5	<0.5	0.5	Yes/yes/yes	
% M138 Oxidation	0 hour	0.5	<0.5-0.5	0.5	Yes/yes/yes	
	24 hours	0.6-0.7	0.5-0.6	0.7	Yes/yes/yes	

Light Stress	RP-HPLC	% (RRT 0.87)	0	<LOQ	<LOD	<LOQ-0.2	Yes/yes/yes
			0.2X	0.4-0.5	0.3-0.4	0.3	Yes/yes/yes
			0.4X	1.8-3.9	2.9-5.2	3.2-4.0	Yes/yes/yes
			0.6X	4.4-5.5	3.3-4.5	1.8-2.6	Yes/yes/yes
		% (RRT 0.98)	0	0.7-0.8	0.5-0.7	1.0-1.1	Yes/yes/yes
			0.2X	5.1-5.3	2.8-4.4	4.2	Yes/yes/yes (Note: The oxidized species is a product related impurity and the reduced level of oxidation in PF-06881894 is acceptable)
			0.4X	3.0-3.4	4.0	3.1-3.2	Yes/yes/yes
			0.6X	ND-5.2	ND	ND-3.2	Yes/yes/yes
		% (RRT 1.05)	0	0.5-0.6	ND	0.6-0.7	Yes/yes/yes
			0.2X	0.9-1.1	0.8-1.1	0.8-1.4	Yes/yes/yes
			0.4X	ND	ND	ND	Yes/yes/yes
			0.6X	ND	ND- <LOQ	ND	Yes/yes/yes
		% (RRT 1.07)	0	0.6-0.8	<LOQ	0.6	Yes/yes/yes (See note above)
			0.2X	1.1	0.6-1.0	0.4-0.6	Yes/yes/yes (See note above)
			0.4X	<LOQ-0.6	ND- <LOQ	<LOQ	Yes/yes/yes
			0.6X	<LOQ	0.5-0.7	0.4-0.6	Yes/yes/yes
		% (RRT 1.13)	0	<LOD -<LOQ	<LOD	<LOQ	Yes/yes/yes
			0.2X	<LOQ	<LOQ-0.3	<LOQ	Yes/yes/yes
		0.4X	<LOD -<LOQ	<LOD -<LOQ	<LOQ	Yes/yes/yes	
		0.6X	<LOQ	<LOQ	<LOQ	Yes/yes/yes	
		% Total Related Protein	0	2.9-3.2	0.5-0.7	4.4-4.5	Yes/yes/yes (Note: The Total Related Protein is a product related impurity and the reduced level of Total Related Protein in PF-06881894 is acceptable)
			0.2X	11.2-12.4	7.5-12.5	9.1-9.4	Yes/yes/yes
			0.4X	94.5-96.2	94.1-94.4	91.8-95.6	Yes/yes/yes
			0.6X	93.5-99.2	92.6-93.2	95.3-95.9	Yes/yes/yes
	SEC-HPLC	% Aggregates	0	0.2	0.1-0.3	0.2	Yes/yes/yes
			0.2X	0.9-1.0	0.7-1.6	0.7	Yes/yes/yes
			0.4X	30.8-34.1	27.4-34.4	32.3-32.9	Yes/yes/yes
			0.6X	36.1-36.5	33.0-36.5	28.7-30.1	Yes/yes/yes

		% HMWS A	0	0.1	<LOQ	0.1	Yes/yes/yes
			0.2X	0.2	0.1-0.2	0.2	Yes/yes/yes
			0.4X	<LOD	<LOD	<LOD	Yes/yes/yes
			0.6X	<LOD	<LOD	<LOD	Yes/yes/yes
		% HMWS B	0	1.2-1.4	0.3-0.5	1.2	Yes/yes/yes (Note: The HMWS is product related impurity and the reduced level of HMWS is acceptable)
			0.2X	1.6-1.8	0.7-1.0	1.6	Yes/yes/yes (See note above)
			0.4X	4.7-4.8	4.2-4.4	4.7-4.8	Yes/yes/yes
			0.6X	5.7-5.9	5.2-5.3	5.5	Yes/yes/yes
		% Des-Pegylated species	0	0.1	<LOQ-0.1	0.1	Yes/yes/yes
			0.2X	0.2	0.1-0.3	0.1	Yes/yes/yes
			0.4X	0.3	0.2-0.4	0.3	Yes/yes/yes
			0.6X	0.3-0.4	0.2-0.3	0.3-0.4	Yes/yes/yes
		% Total Size Variants	0	1.7-1.9	0.4-0.9	1.7	Yes/yes/yes (Note: The Total Size Variants are product related impurities and the reduced level of Total Size Variants is acceptable)
			0.2X	3.1	1.6-3.1	2.6	Yes/yes/yes (See note above)
			0.4X	35.8-39.1	31.8-39.2	37.2-37.9	Yes/yes/yes
			0.6X	42.4-42.5	38.6-42.0	34.5-35.9	Yes/yes/yes
	Peptide Mapping	% Q21 Deamidated	0	0.8-0.9	<0.5-0.6	0.5-0.6	Yes/yes/yes (Note: The deamidated species are product related impurities and the reduced level of deamidated species is acceptable)
			0.2X	0.7-0.8	<0.5-0.6	0.5-0.6	Yes/yes/yes (See note above)
			0.6X	0.8-0.9	<0.5-0.6	0.6	Yes/yes/yes (See note above)
		% D28 Isomerization	0	<0.5	<0.5	<0.5	Yes/yes/yes
			0.2X	<0.5	<0.5	<0.5	Yes/yes/yes
			0.6X	0.8-0.9	0.6-0.8	0.5-0.8	Yes/yes/yes

		% W59 Oxidation	0	0.7	0.7-1.1	0.7-0.8	Yes/yes/yes
			0.2X	2.5-2.6	1.7-3.4	1.8	Yes/yes/yes (Note: The difference is due to method variability)
			0.6X	43.9-44.7	39.9-41.2	36.3-38.2	Yes/yes/yes
		% W59 Dioxidation	0	<0.5	<0.5-0.5	<0.5	Yes/yes/yes
			0.2X	1.3-1.4	1.1-1.8	1.0-1.1	Yes/yes/yes
			0.6X	36.9-37.6	34.5	30.5-32.8	Yes/yes/yes
		% Q91 Deamidated	0	<0.5	<0.5	<0.5	Yes/yes/yes
			0.2X	<0.5	<0.5	<0.5	Yes/yes/yes
			0.6X	<0.5	ND- <0.5	<0.5	Yes/yes/yes
		% Q108 Deamidated	0	<0.5	<0.5	<0.5	Yes/yes/yes
			0.2X	<0.5	<0.5	<0.5	Yes/yes/yes
			0.6X	6.1-6.6	1.9-4.1	2.4-2.7	Yes/yes/yes (Note: The deamidated species are product related impurities and the reduced level of deamidated species is acceptable)
		% W119 Oxidation	0	<0.5	<0.5	<0.5	Yes/yes/yes
			0.2X	0.6	<0.5-1.1	<0.5	Yes/yes/yes
			0.6X	10.3-10.4	5.5-16.7	7.2-8.1	Yes/yes/yes
		% M122 Oxidation	0	<0.5	<0.5	<0.5	Yes/yes/yes
			0.2X	1.3	0.8-1.4	1.0-1.1	Yes/yes/yes
			0.6X	67.4-68.4	62.7-64.5	58.6-58.9	Yes/yes/yes
		% M127 Oxidation	0	<0.5-0.5	0.5	0.6-0.7	Yes/yes/yes
			0.2X	4.2-4.9	2.9-5.1	3.5-3.6	Yes/yes/yes
			0.6X	90.2-90.9	87.4-89.2	84.1-86.4	Yes/yes/yes
		% M138 Oxidation	0	<0.5-0.5	0.5-0.8	0.7-0.8	Yes/yes/yes
			0.2X	3.9-4.2	2.9-4.9	3.1-3.2	Yes/yes/yes
			0.6X	88.4-89.1	86.0-87.0	80.4-83.1	Yes/yes/yes
High pH	RP-HPLC	% (RRT 0.87)	0	<LOQ	<LOQ	<LOQ	Yes/yes/yes

	2 days	<LOQ	<LOQ	<LOQ	Yes/yes/yes
	4 days	<LOQ	<LOQ	<LOQ	Yes/yes/yes
	7 days	<LOQ	<LOQ	<LOQ	Yes/yes/yes
% (RRT 0.98)	0	1.4	ND	1.4	Yes/yes/yes
	2 days	1.5-1.6	ND	1.4	Yes/yes/yes
	4 days	1.6	ND	1.3-1.4	Yes/yes/yes
	7 days	1.6-1.7	ND	1.4-1.7	Yes/yes/yes
% (RRT 1.05)	0	1.0-1.1	0.4-0.8	1.2-1.3	Yes/yes/yes (Note: The oxidized species are product related impurities and the reduced level of oxidized species is acceptable)
	2 days	1.6	1.3-1.5	1.9-2.0	Yes/yes/yes (See note above)
	4 days	2.0-2.1	1.7-2.0	2.2-2.5	Yes/yes/yes (See note above)
	7 days	2.5-2.6	2.2	3.0-3.1	Yes/yes/yes (See note above)
% (RRT 1.07)	0	0.8	<LOQ-0.5	0.7-0.8	Yes/yes/yes
	2 days	1.0-1.1	0.5-0.7	0.8-0.9	Yes/yes/yes (See note above)
	4 days	1.2-1.4	0.6-0.8	1.3-1.6	Yes/yes/yes (See note above)
	7 days	1.7-2.0	0.9-1.5	0.8-1.5	Yes/yes/yes (See note above)
% (RRT 1.13)	0	0.9	0.4-1.0	0.9-1.0	Yes/yes/yes
	2 days	3.4-4.1	3.2-3.9	5.0-5.9	Yes/yes/yes
	4 days	7.0	5.5-6.4	6.3-7.1	Yes/yes/yes (See note above)
	7 days	9.9-10.5	8.9-9.5	9.2-11.7	Yes/yes/yes (See note above)
% Total Related Protein	0	5.3-5.6	2.0-4.0	5.6-6.0	Yes/yes/yes (Note: The Total Size Variants are product related impurities and the reduced level of Total Size Variants is acceptable)
	2 days	11.9-12.1	7.9-9.4	12.9-13.7	Yes/yes/yes (See note above)
	4 days	16.7-17.1	11.5-13.8	17.0-19.0	Yes/yes/yes (See note above)

			7 days	21.9-22.8	16.5-18.3	23.3-25.6	Yes/yes/yes (See note above)
	SEC-HPLC	% Aggregates	0	3.6	1.9-3.1	3.3-4.0	Yes/yes/yes (Note: The aggregate is a product related impurity and the reduced level of aggregate is acceptable)
			2 days	8.9-9.5	5.5-7.1	9.8-11.1	Yes/yes/yes (See note above)
			4 days	12.8-13.2	7.9-9.8	13.1-15.1	Yes/yes/yes (See note above)
			7 days	17.5-18.0	11.2-13.1	17.1-21.0	Yes/yes/yes (See note above)
		% HMWS A	0	0.5-0.6	0.3-0.5	0.7	Yes/yes/yes (Note: The HMW is a product related impurity and the reduced level of HMW is acceptable)
			2 days	1.2-1.3	0.9-1.1	1.3-1.4	Yes/yes/yes (See note above)
			4 days	1.5	1.2-1.3	1.6	Yes/yes/yes (See note above)
			7 days	1.8-1.9	1.5-1.7	1.4-1.8	Yes/yes/yes (See note above)
		% HMWS B	0	2.2-2.6	1.6-2.0	2.2-2.5	Yes/yes/yes (See note above)
			2 days	4.7-4.8	3.9-4.5	4.2-4.4	Yes/yes/yes (See note above)
			4 days	6.0-6.2	5.7-6.1	6.0-6.3	Yes/yes/yes (See note above)
			7 days	7.8-8.2	8.2-8.3	8.2-8.7	Yes/yes/yes
		% Des-Pegylated species	0	0.1	<LOQ-0.1	0.1	Yes/yes/yes
			2 days	0.1	<LOQ-0.1	0.1	Yes/yes/yes
			4 days	0.1	0.1	0.1	Yes/yes/yes
			7 days	0.1	0.1	0.1	Yes/yes/yes
		% Total Size Variants	0	6.5-6.9	3.7-5.6	6.5-7.0	Yes/yes/yes (Note: The Total Size Variants are product related impurities and the reduced level of Total Size Variants is acceptable)

		2 days	15.1-15.5	10.2-12.7	15.6-16.8	Yes/yes/yes (See note above)
		4 days	20.7-20.9	14.9-17.3	21.0-22.9	Yes/yes/yes (See note above)
		7 days	27.6-27.8	21.0-23.2	27.6-30.8	Yes/yes/yes (See note above)
IC-HPLC	% Acidic Variants	0	2.3-3.5	0.5-1.1	2.3-2.4	Yes/yes/yes (Note: The acid variants are product related impurities and the reduced level of acid variants is acceptable)
		2 days	2.7-3.1	2.4-3.0	3.2-4.0	Yes/yes/yes (See note above)
		4 days	4.0	2.4-3.1	4.1-4.2	Yes/yes/yes (See note above)
		7 days	5.7-6.2	5.2-5.8	5.9-6.5	Yes/yes/yes
	% Basic Variants	0	1.6-2.3	0.9-1.8	1.5-2.4	Yes/yes/yes (Note: The basic variants are product related impurities and the reduced level of basic variants is acceptable)
		2 days	14.5-14.7	8.0-9.4	13.0-13.6	Yes/yes/yes (See note above)
		4 days	19.0-19.3	13.0-14.8	17.9-20.9	Yes/yes/yes (See note above)
		7 days	22.3-22.8	17.4-18.4	21.7-25.9	Yes/yes/yes (See note above)
	% Total Charge Variants	0	4.6-5.1	1.3-2.9	3.9-4.7	Yes/yes/yes (Note: The total charge variants are product related impurities and the reduced level of total charge variants is acceptable)
		2 days	17.2-17.8	10.5-12.5	16.2-17.6	Yes/yes/yes (See note above)
		4 days	23.0-23.3	15.3-17.9	22.1-25.0	Yes/yes/yes (See note above)
		7 days	28.5-28.6	22.6-24.2	28.2-31.8	Yes/yes/yes (See note above)
Peptide Mapping	% Q21 Deamidated	0	0.7-0.8	<0.5-0.6	0.5	Yes/yes/yes
		7days	0.7-0.8	<0.5-0.5	0.5	Yes/yes/yes

	% D28 Isomerization	0	<0.5	<0.5	<0.5	Yes/yes/yes
		7days	<0.5	<0.5	<0.5	Yes/yes/yes
	% W59 Oxidation	0	0.6	0.6-0.8	0.6	Yes/yes/yes
		7days	0.7-0.8	0.6-0.8	0.6-0.7	Yes/yes/yes
	% W59 Dioxidation	0	<0.5	<0.5	<0.5	Yes/yes/yes
		7days	<0.5	<0.5-0.5	<0.5	Yes/yes/yes
	% Q91 Deamidated	0	ND	ND	ND	Yes/yes/yes
		7days	ND	ND	ND	Yes/yes/yes
	% Q108 Deamidated	0	<0.5	<0.5	<0.5	Yes/yes/yes
		7days	<0.5	<0.5	<0.5	Yes/yes/yes
	% W119 Oxidation	0	<0.5	<0.5	<0.5	Yes/yes/yes
		7days	<0.5	<0.5	<0.5	Yes/yes/yes
	% M122 Oxidation	0	<0.5	<0.5	<0.5	Yes/yes/yes
		7days	<0.5	<0.5	<0.5	Yes/yes/yes
	% M127 Oxidation	0	<0.5-0.5	<0.5	<0.5	Yes/yes/yes
		7days	0.5	<0.5-0.5	0.5	Yes/yes/yes
	% M138 Oxidation	0	<0.5-0.5	<0.5	<0.5-0.5	Yes/yes/yes
		7days	0.5	<0.5-0.5	0.5	



Xu
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