

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

761080Orig1s000

PRODUCT QUALITY REVIEW(S)



U.S. FOOD & DRUG
ADMINISTRATION

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

LABELS AND LABELING REVIEW

Date of review:	July 9, 2018
Reviewer:	Vicky Borders-Hemphill, PharmD Labeling Review Specialist Office of Biotechnology Products (OBP)
Through:	Scott Lute, PhD, Product Quality Reviewer OBP/Division of Biotechnology Review and Research II
Application:	BLA 761080
Applicant:	Hospira, Inc., a Pfizer Company
Submission Date:	September 21, 2017
Product:	Nivestym (filgrastim-aafi)
Dosage form(s):	Injection
Strength and Container-Closure:	300 mcg/mL and 480 mcg/1.6 mL single-dose vials and 300 mcg/0.5 mL and 480 mcg/0.8 mL single-dose prefilled syringes
Indication, dose, route, and frequency of administration:	is a leukocyte growth factor indicated to: • Decrease the incidence of infection, as manifested by febrile neutropenia, in patients with nonmyeloid malignancies receiving myelosuppressive anti-cancer drugs associated with a significant incidence of severe neutropenia with fever. • Reduce the time to neutrophil recovery and the duration of fever, following induction or consolidation chemotherapy treatment of patients with acute myeloid leukemia (AML). • Reduce the duration of neutropenia and neutropenia-related clinical sequelae, e.g., febrile neutropenia, in patients with nonmyeloid malignancies undergoing myeloablative chemotherapy followed by bone marrow transplantation (BMT). • Mobilize autologous hematopoietic progenitor cells into the peripheral blood for collection by leukapheresis. • Reduce the incidence and duration of sequelae of severe neutropenia (e.g., fever, infections, oropharyngeal ulcers) in symptomatic patients with congenital neutropenia, cyclic neutropenia, or idiopathic neutropenia
Background and Summary Description:	The Applicant submitted an application for Agency review
Recommendations:	The container labels and carton labeling (submitted on June 13, 2018) and prescribing information, patient information labeling, instructions for use (submitted on July 2, 2018), are acceptable from an OBP labeling perspective.

Materials Considered for this Label and Labeling Review	
Materials Reviewed	Appendix Section
Proposed Labels and Labeling	A
Other	B (n/a)
Relevant Code of Federal Regulations, United States Pharmacopeia (USP) standards, and CDER Labeling Best Practices	C
Acceptable Labels and Labeling	D

n/a = not applicable for this review

DISCUSSION and CONCLUSION

We evaluated the proposed labels and labeling for compliance to the applicable requirements in the Code of Federal Regulations and United States Pharmacopeia (USP) nomenclature (see Appendix C).

The prescribing information, patient information labeling, instructions for use, container labels, and carton labeling were reviewed and found to comply with relevant regulations (21 CFR 610.60 through 21 CFR 610.67; 21 CFR 201.2 through 21 CFR 201.25; 21 CFR 201.50 through 21 CFR 201.57; 21 CFR 201.100) and USP standards.

The container labels and carton labeling (submitted on June 13, 2018) and prescribing information, patient information labeling, instructions for use (submitted on July 2, 2018), are acceptable from an OBP labeling perspective (see Appendix D).

APPENDICES

Appendix A: Proposed Labeling

Prescribing Information/Patient information labeling/Instructions for use (submitted on December 18, 2017 <\\cdsesub1\evsprod\bla761080\0009\m1\us\lab-0937-0-1-pkg-insert-track.doc>)

Container Labels (submitted on September 21, 2017)

Prefilled syringe

(b) (4)

Evaluation Tables: Label^{1,2} and Labeling³ Standards
Container⁴ Label Evaluation

Prefilled Syringe label

<u>Regulations, Guidance and CDER Best Labeling Practices</u>	<u>Conforms</u>
<u>Proper Name</u> (21 CFR 610.60, 21 CFR 201.50, 21 CFR 201.10) <i>for container of a product capable of bearing a full label</i> Comment/Recommendation: <i>partial label</i>	☐ No ☐ Yes ☒ N/A
<u>Manufacturer name, address, and license number</u> (21 CFR 610.60) <i>for container of a product capable of bearing a full label</i> Comment/Recommendation: <i>partial label</i>	☐ No ☐ Yes ☒ N/A
<u>Lot number or other lot identification</u> (21 CFR 610.60, 21 CFR 201.18, 21 CFR 201.100) Comment/Recommendation: <i>partial label</i>	☐ No ☐ Yes ☒ N/A
<u>Expiration date</u> (21 CFR 610.60, 21 CFR 201.17) Comment/Recommendation: <i>partial label</i>	☐ No ☐ Yes ☒ N/A
<u>Multiple dose containers (recommended individual dose)</u> 21 CFR 610.60	☐ No ☐ Yes ☒ N/A
<u>Statement: "Rx only"</u> 21 CFR 610.60 21 CFR 201.100	☐ No ☒ Yes ☐ N/A
<u>Medication Guide</u> 21 CFR 610.60 21 CFR 208.24	☐ No ☐ Yes ☒ N/A
<u>No Package for container</u> 21 CFR 610.60	☐ No ☐ Yes ☒ N/A
<u>Partial label</u> 21 CFR 610.60 21 CFR 201.10	☐ No ☒ Yes ☐ N/A
<u>No container label</u> 21 CFR 610.60	☐ No ☐ Yes ☒ 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.

<u>Ferrule and cap overseal</u>	☐ No ☐ Yes ☒ N/A
<u>Visual inspection</u> 21 CFR 610.60	☐ No ☐ Yes ☒ N/A
<u>NDC numbers</u> 21 CFR 201.2 21 CFR 207.35	☐ No ☒ Yes ☐ N/A
<u>Route of administration</u> 21 CFR 201.5 21 CFR 201.100	☐ No ☒ Yes ☐ N/A
<u>Preparation instructions</u> 21 CFR 201.5	☐ No ☐ Yes ☒ N/A
<u>Package type term</u> 21 CFR 201.5	☐ No ☒ Yes ☐ N/A
<u>Drugs</u> <u>Misleading statements</u> 21 CFR 201.6	☐ No ☒ Yes ☐ N/A
<u>Strength</u> 21 CFR 201.10 21CFR 201.100	☐ No ☒ Yes ☐ N/A
<u>Drugs</u> <u>Prominence of required label statements</u> 21 CFR 201.15	☐ No ☒ Yes ☐ N/A
<u>Spanish-language (Drugs)</u> 21 CFR 201.16	☐ No ☐ Yes ☒ N/A
<u>FD&C Yellow No. 5 and/or FD&C Yellow No. 6</u> 21 CFR 201.20	☐ No ☐ Yes ☒ N/A
<u>Phenylalanine as a component of aspartame</u> 21 CFR 201.21	☐ No ☐ Yes ☒ N/A
<u>Sulfites; required warning statements</u> 21 CFR 201.22	☐ No ☐ Yes ☒ N/A
<u>Bar code label requirements</u> 21 CFR 201.25 21CFR 610.67	☐ No ☒ Yes ☐ N/A
<u>Strategic National Stockpile (exceptions or alternatives to labeling requirements for human drug products)</u> 21 CFR 610.68 21 CFR 201.26	☐ No ☐ Yes ☒ N/A

<u>Net quantity</u> 21 CFR 201.51	☐ No ☒ Yes ☐ N/A
<u>Usual dosage statement</u> 21 CFR 201.55 21 CFR 201.100 Comment/Recommendation: <i>container label lacks space</i>	☐ No ☐ Yes ☒ N/A
<u>Inactive ingredients</u> 21 CFR 201.100	☐ No ☐ Yes ☒ N/A
Comment/Recommendation: <i>container label lacks space</i>	
<u>Storage requirements</u>	☐ No ☐ Yes ☒ N/A
Comment/Recommendation: <i>container label lacks space</i>	
<u>Dispensing container</u> 21 CFR 201.100	☐ No ☐ Yes ☒ N/A

Vial label

Regulations, Guidance and CDER Best Labeling Practices	Conforms
<u>Proper Name</u> (21 CFR 610.60, 21 CFR 201.50, 21 CFR 201.10) <i>for container of a product capable of bearing a full label</i> Comment/Recommendation: <i>partial label</i>	☐ No ☐ Yes ☒ N/A
<u>Manufacturer name, address, and license number</u> (21 CFR 610.60) <i>for container of a product capable of bearing a full label</i> Comment/Recommendation: <i>partial label</i>	☐ No ☐ Yes ☒ N/A
<u>Lot number or other lot identification</u> (21 CFR 610.60, 21 CFR 201.18, 21 CFR 201.100) Comment/Recommendation: <i>partial label</i>	☐ No ☐ Yes ☒ N/A
<u>Expiration date</u> (21 CFR 610.60, 21 CFR 201.17) Comment/Recommendation: <i>partial label</i>	☐ No ☐ Yes ☒ N/A
<u>Multiple dose containers (recommended individual dose)</u> 21 CFR 610.60	☐ No ☐ Yes ☒ N/A
<u>Statement: "Rx only"</u> 21 CFR 610.60 21 CFR 201.100	☐ No ☒ Yes ☐ N/A
<u>Medication Guide</u> 21 CFR 610.60 21 CFR 208.24	☐ No ☐ Yes ☒ N/A

<u>No Package for container</u> 21 CFR 610.60	☐ No ☐ Yes ☒ N/A
<u>Partial label</u> 21 CFR 610.60 21 CFR 201.10 Comment/Recommendation: Ensure the lot number and the expiration date appear on the vial labels <i>Applicant response: The Sponsor ensures that the lot number and the expiration date appear on the vial labels. As indicated in Section 1.14.1.1 Draft Carton and Container Labels, a reserved space, denoted as a black region, has been allocated for the lot number and expiration date on the proposed SDV labels and cartons. The lot number and expiration date are printed on-line during packaging of the drug product in this reserved black region.</i> FDA response: We find the applicant's response acceptable	☐ No ☒ Yes ☐ N/A
<u>No container label</u> 21 CFR 610.60	☐ No ☐ Yes ☒ N/A
<u>Ferrule and cap overseal</u> Comment/Recommendation: FOR VIALS: Confirm there is no text on the ferrule and cap overseal of the vials to comply with a revised United States Pharmacopeia (USP), General Chapters: <7> Labeling (Ferrules and Cap Overseals). <i>Applicant response: The Sponsor confirms that there is no text on the ferrule and cap overseal of the vials, to comply with USP General Chapter <7>.</i> FDA response: We find the applicant's response acceptable	☐ No ☒ Yes ☐ N/A
<u>Visual inspection</u> 21 CFR 610.60 Comment/Recommendation: FOR VIALS: Confirm there is sufficient area on the container to allow for visual inspection when the label is affixed to the vial and indicate where the visual area of inspection is located per 21 CFR 610.60(e). <i>Applicant response: The Sponsor confirms that when the label has been affixed to the vial, a sufficient area of the vial shall remain uncovered for its full length or circumference to permit inspection of the contents per 21 CFR 610.60(e)</i> FDA response: We find the applicant's response acceptable	☐ No ☒ Yes ☐ N/A
<u>NDC numbers</u> 21 CFR 201.2 21 CFR 207.35	☐ No ☒ Yes ☐ N/A

<u>Route of administration</u> 21 CFR 201.5 21 CFR 201.100	☐ No ☐ Yes ☒ N/A
<u>Preparation instructions</u> 21 CFR 201.5	☐ No ☐ Yes ☒ N/A
<u>Package type term</u> 21 CFR 201.5	☐ No ☒ Yes ☐ N/A
<u>Drugs</u> <u>Misleading statements</u> 21 CFR 201.6	☐ No ☐ Yes ☒ N/A
<u>Strength</u> 21 CFR 201.10 21 CFR 201.100 Comment/Recommendation: For the 300 mcg/mL strength, revise the strength presentation to "300 mcg/mL" (see USP General Chapters: <7> Labeling) <i>The applicant revised as requested</i>	☒ No ☐ Yes ☐ N/A
<u>Drugs</u> <u>Prominence of required label statements</u> 21 CFR 201.15	☐ No ☒ Yes ☐ N/A
<u>Spanish-language (Drugs)</u> 21 CFR 201.16	☐ No ☐ Yes ☒ N/A
<u>FD&C Yellow No. 5 and/or FD&C Yellow No. 6</u> 21 CFR 201.20	☐ No ☐ Yes ☒ N/A
<u>Phenylalanine as a component of aspartame</u> 21 CFR 201.21	☐ No ☐ Yes ☒ N/A
<u>Sulfites; required warning statements</u> 21 CFR 201.22	☐ No ☐ Yes ☒ N/A
<u>Bar code label requirements</u> 21 CFR 201.25 21 CFR 610.67	☐ No ☒ Yes ☐ N/A
<u>Strategic National Stockpile (exceptions or alternatives to labeling requirements for human drug products)</u> 21 CFR 610.68 21 CFR 201.26	☐ No ☐ Yes ☒ N/A
<u>Net quantity</u> 21 CFR 201.51	☐ No ☒ Yes ☐ N/A
<u>Usual dosage statement</u> 21 CFR 201.55 21 CFR 201.100	☐ No ☒ Yes ☐ N/A

Inactive ingredients 21 CFR 201.100	☐ No ☐ Yes ☒ N/A
Comment/Recommendation: <i>container label lacks space</i>	
Storage requirements	☐ No ☒ Yes ☐ N/A
Dispensing container 21 CFR 201.100	☐ No ☐ Yes ☒ N/A

Package Label⁵ Evaluation

Prefilled syringe blister

Regulations, Guidance and CDER Best Labeling Practices	Conforms
Proper name (21 CFR 610.61, 21 CFR 201.50, 21 CFR 201.10)	☐ No ☒ Yes ☐ N/A
Manufacturer name, address, and license number 21 CFR 610.61	☐ No ☒ Yes ☐ N/A
Comment/Recommendation: The Applicant may include the Country of Origin per U.S. Customs Border and Protection regulations 19 CFR 134.11, consider revising from "Made in Croatia" to "Product of Croatia" on all carton labeling.	
Applicant's response: <i>The Sponsor appreciates the Agency's recognition of, 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. In line with these regulations, as well as CBP's Informed Compliance Publication (https://www.cbp.gov/trade/rulings/informed-compliancepublications/marketing-country-origin-us-imports), the Sponsor'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 Sponsor 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 filgrastim-aafi carton labeling.</i>	
FDA response: We find the applicant's response acceptable	
Lot number or other lot identification	☐ No

⁵ 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.

21 CFR 610.61	☒ Yes ☐ N/A
<u>Expiration date</u> 21 CFR 610.61 21 CFR 201.17	☐ No ☒ Yes ☐ N/A
<u>Preservative</u> 21 CFR 610.61	☐ No ☒ Yes ☐ N/A
<u>Number of containers</u> 21 CFR 610.61	☐ No ☒ Yes ☐ N/A
<u>Strength/volume</u> 21 CFR 610.61 21 CFR 201.10 21 CFR 201.100	☐ No ☒ Yes ☐ N/A
<u>Storage temperature/requirements</u> 21 CFR 610.61	☐ No ☒ Yes ☐ N/A
<u>Handling: "Do Not Shake", "Do not Freeze" or equivalent</u> (21 CFR 610.61)	☐ No ☒ Yes ☐ N/A
<u>Multiple dose containers (recommended individual dose)</u> 21 CFR 610.61	☐ No ☐ Yes ☒ N/A
<u>Route of administration</u> 21 CFR 610.61 21 CFR 201.5 21 CFR 201.100	☐ No ☒ Yes ☐ N/A
<u>Known sensitizing substances</u> 21 CFR 610.61	☐ No ☐ Yes ☒ N/A
Comment/Recommendation: For the PFS, the prescribing information has the statement <i>"The NIVESTYM syringe plunger stopper and needle cover are not made with natural rubber latex".</i>	
<u>Inactive ingredients</u> 21 CFR 610.61 21 CFR 201.100	☐ No ☒ Yes ☐ N/A
Comment/Recommendation: Remove the statement (b) (4) from the unit carton (blister tray).	
Product Quality confirmed that the applicant includes (b) (4) if needed; however, in all the batch records supplied, no (b) (4)	
Applicant's response: The statement (b) (4) has been removed from the unit carton.	
The applicant revised as requested	

<u>Source of the product</u> 21 CFR 610.61	☐ No ☒ Yes ☐ N/A
<u>Minimum potency of product</u> 21 CFR 610.61	☐ No ☒ Yes ☐ N/A
<u>Rx only</u> 21CFR 610.61 21 CFR 201.100	☐ No ☒ Yes ☐ N/A
<u>Divided manufacturing</u> 21 CFR 610.63	☐ No ☐ Yes ☒ N/A
<u>Distributor</u> 21 CFR 610.64	☐ No ☒ Yes ☐ N/A
<u>Bar code</u> 21 CFR 610.67 21 CFR 201.25	☐ No ☒ Yes ☐ N/A
<u>Strategic National Stockpile (exceptions or alternatives to labeling requirements for human drug products)</u> 21 CFR 610.68 21 CFR 201.26	☐ No ☐ Yes ☒ N/A
<u>NDC numbers</u> 21 CFR 201.2 21 CFR 207.35	☐ No ☒ Yes ☐ N/A
<u>Preparation instructions</u> 21 CFR 201.5	☐ No ☒ Yes ☐ N/A
<u>Package type term</u> 21 CFR 201.5	☐ No ☒ Yes ☐ N/A
<i>Comment/Recommendation:</i> Add the statement "Discard unused portion" following "Single-Dose Prefilled Syringe with Needle guard" The applicant revised as requested	
<u>Drugs</u> <u>Misleading statements</u> 21 CFR 201.6	☐ No ☐ Yes ☒ N/A
<u>Drugs</u> <u>Prominence of required label statements</u> 21 CFR 201.15	☐ No ☒ Yes ☐ N/A
<u>Spanish-language (Drugs)</u> 21 CFR 201.16	☐ No ☐ Yes ☒ N/A
<u>FD&C Yellow No. 5 and/or FD&C Yellow No. 6</u> 21 CFR 201.20	☐ No ☐ Yes ☒ N/A

<u>Phenylalanine as a component of aspartame</u> 21 CFR 201.21	☐ No ☐ Yes ☒ N/A
<u>Sulfites: required warning statements</u> 21 CFR 201.22	☐ No ☐ Yes ☒ N/A
<u>Net quantity</u> 21 CFR 201.51	☐ No ☒ Yes ☐ N/A
<u>Usual dosage statement</u> 21 CFR 201.55 21 CFR 201.100	☐ No ☒ Yes ☐ N/A
<u>Dispensing container</u> 21 CFR 201.100	☐ No ☐ Yes ☒ N/A
<u>Medication Guide</u> 21 CFR 610.60 21 CFR 208.24	☐ No ☐ Yes ☒ N/A

PFS shelf carton

<u>Regulations, Guidance and CDER Best Labeling Practices</u>	<u>Conforms</u>
<u>Proper name</u> (21 CFR 610.61, 21 CFR 201.50, 21 CFR 201.10)	☐ No ☒ Yes ☐ N/A
<u>Manufacturer name, address, and license number</u> 21 CFR 610.61	☐ No ☒ Yes ☐ N/A
<u>Comment/Recommendation:</u> The Applicant may include the Country of Origin per U.S. Customs Border and Protection regulations 19 CFR 134.11, consider revising from "Made in Croatia" to "Product of Croatia" on all carton labeling. <i>Applicant's response:</i> The Sponsor appreciates the Agency's recognition of, 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. In line with these regulations, as well as CBP's Informed Compliance Publication (https://www.cbp.gov/trade/rulings/informed-compliancepublications/marking-country-origin-us-imports), the Sponsor'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 Sponsor 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 filgrastim-aafi carton labeling.	

FDA response: We find the applicant's response acceptable	
<u>Lot number or other lot identification</u> 21 CFR 610.61	☐ No ☒ Yes ☐ N/A
<u>Expiration date</u> 21 CFR 610.61 21 CFR 201.17	☐ No ☒ Yes ☐ N/A
<u>Preservative</u> 21 CFR 610.61	☐ No ☒ Yes ☐ N/A
<u>Number of containers</u> 21 CFR 610.61	☐ No ☒ Yes ☐ N/A
<u>Strength/volume</u> 21 CFR 610.61 21 CFR 201.10 21 CFR 201.100	☐ No ☒ Yes ☐ N/A
<u>Storage temperature/requirements</u> 21 CFR 610.61	☐ No ☒ Yes ☐ N/A
<u>Handling: "Do Not Shake", "Do not Freeze" or equivalent</u> (21 CFR 610.61)	☐ No ☒ Yes ☐ N/A
<u>Multiple dose containers (recommended individual dose)</u> 21 CFR 610.61	☐ No ☐ Yes ☒ N/A
<u>Route of administration</u> 21CFR 610.61 21 CFR 201.5 21 CFR 201.100	☐ No ☒ Yes ☐ N/A
<u>Known sensitizing substances</u> 21CFR 610.61	☐ No ☐ Yes ☒ N/A
Comment/Recommendation: For the PFS, the prescribing information has the statement "The NIVESTYM syringe plunger stopper and needle cover are not made with natural rubber latex".	
<u>Inactive ingredients</u> 21 CFR 610.61 21 CFR 201.100	☐ No ☒ Yes ☐ N/A
Comment/Recommendation: Remove the statement (b) (4) from the shelf carton labeling Applicant's response: The statement (b) (4) has been removed from the unit carton.	
The applicant revised as requested	
<u>Source of the product</u> 21 CFR 610.61	☐ No ☒ Yes

	☐ N/A ☐ No ☒ Yes ☐ N/A
<u>Minimum potency of product</u> 21 CFR 610.61	☐ No ☒ Yes ☐ N/A
<u>Rx only</u> 21CFR 610.61 21 CFR 201.100	☐ No ☒ Yes ☐ N/A
<u>Divided manufacturing</u> 21 CFR 610.63	☐ No ☐ Yes ☒ N/A
<u>Distributor</u> 21 CFR 610.64	☐ No ☒ Yes ☐ N/A
<u>Bar code</u> 21 CFR 610.67 21 CFR 201.25	☐ No ☒ Yes ☐ N/A
<u>Strategic National Stockpile (exceptions or alternatives to labeling requirements for human drug products)</u> 21 CFR 610.68 21 CFR 201.26	☐ No ☐ Yes ☒ N/A
<u>NDC numbers</u> 21 CFR 201.2 21 CFR 207.35	☐ No ☒ Yes ☐ N/A
<u>Preparation instructions</u> 21 CFR 201.5	☐ No ☒ Yes ☐ N/A
<u>Package type term</u> 21 CFR 201.5	☐ No ☒ Yes ☐ N/A
Comment/Recommendation: Add the statement "Discard unused portion" following "Single-Dose Prefilled Syringe with Needle guard"	
<i>The applicant revised as requested</i>	
<u>Drugs</u> <u>Misleading statements</u> 21 CFR 201.6	☐ No ☐ Yes ☒ N/A
<u>Drugs</u> <u>Prominence of required label statements</u> 21 CFR 201.15	☐ No ☒ Yes ☐ N/A
<u>Spanish-language (Drugs)</u> 21 CFR 201.16	☐ No ☐ Yes ☒ N/A
<u>FD&C Yellow No. 5 and/or FD&C Yellow No. 6</u> 21 CFR 201.20	☐ No ☐ Yes ☒ N/A
<u>Phenylalanine as a component of aspartame</u> 21 CFR 201.21	☐ No ☐ Yes

<u>Sulfites; required warning statements</u> 21 CFR 201.22	☒ N/A ☐ No ☐ Yes
<u>Net quantity</u> 21 CFR 201.51	☒ N/A ☐ No ☒ Yes ☐ N/A
<u>Usual dosage statement</u> 21 CFR 201.55 21 CFR 201.100	☐ No ☒ Yes ☐ N/A
<u>Dispensing container</u> 21 CFR 201.100	☐ No ☐ Yes ☒ N/A
<u>Medication Guide</u> 21 CFR 610.60 21 CFR 208.24	☐ No ☐ Yes ☒ N/A

Vial carton

Regulations, Guidance and CDER Best Labeling Practices	Conforms
<u>Proper name</u> (21 CFR 610.61, 21 CFR 201.50, 21 CFR 201.10)	☐ No ☒ Yes ☐ N/A
<u>Manufacturer name, address, and license number</u> 21 CFR 610.61	☐ No ☒ Yes ☐ N/A
<u>Lot number or other lot identification</u> 21 CFR 610.61	☒ No ☐ Yes ☐ N/A
Comment/Recommendation: Ensure that the lot number and expiration date appear on the vial carton labeling <i>Applicant's response: The Sponsor ensures that the lot number and the expiration date appear on the vial carton labeling. As indicated in Section 1.14.1.1 Draft Carton and Container Labels, a reserved space, denoted as a black region, has been allocated for the lot number and expiration date on the proposed SDV labels and cartons. The lot number and expiration date are printed on-line during packaging of the drug product in this reserved black region.</i> FDA response: We find the applicant's response acceptable	
<u>Expiration date</u> 21 CFR 610.61 21 CFR 201.17	☒ No ☐ Yes ☐ N/A
<u>Preservative</u> 21 CFR 610.61	☐ No ☒ Yes ☐ N/A
<u>Number of containers</u> 21 CFR 610.61	☐ No ☒ Yes ☐ N/A
<u>Strength/volume</u>	☐ No

21 CFR 610.61 21 CFR 201.10 21 CFR 201.100	☒ Yes ☐ N/A
<u>Storage temperature/requirements</u> 21 CFR 610.61	☐ No ☒ Yes ☐ N/A
<u>Handling: "Do Not Shake", "Do not Freeze" or equivalent</u> (21 CFR 610.61)	☐ No ☒ Yes ☐ N/A
<u>Multiple dose containers (recommended individual dose)</u> 21 CFR 610.61	☐ No ☐ Yes ☒ N/A
<u>Route of administration</u> 21CFR 610.61 21 CFR 201.5 21 CFR 201.100	☐ No ☒ Yes ☐ N/A
<u>Known sensitizing substances</u> 21CFR 610.61	☐ No ☐ Yes ☒ N/A
<u>Inactive ingredients</u> 21 CFR 610.61 21 CFR 201.100 <u>Comment/Recommendation:</u> Remove the statement (b) (4) from the inactive ingredients list.	☐ No ☒ Yes ☐ N/A
<i>The applicant revised as requested</i>	
<u>Source of the product</u> 21 CFR 610.61	☐ No ☒ Yes ☐ N/A
<u>Minimum potency of product</u> 21 CFR 610.61	☐ No ☒ Yes ☐ N/A
<u>Rx only</u> 21CFR 610.61 21 CFR 201.100	☐ No ☒ Yes ☐ N/A
<u>Divided manufacturing</u> 21 CFR 610.63	☐ No ☐ Yes ☒ N/A
<u>Distributor</u> 21 CFR 610.64	☐ No ☒ Yes ☐ N/A
<u>Bar code</u> 21 CFR 610.67 21 CFR 201.25	☐ No ☒ Yes ☐ N/A
<u>Strategic National Stockpile (exceptions or alternatives to labeling requirements for human drug products)</u> 21 CFR 610.68	☐ No ☐ Yes ☒ N/A

21 CFR 201.26	
<u>NDC numbers</u>	☐ No
21 CFR 201.2	☒ Yes
21 CFR 207.35	☐ N/A
<u>Preparation instructions</u>	☐ No
21 CFR 201.5	☒ Yes
	☐ N/A
<u>Package type term</u>	☐ No
21 CFR 201.5	☒ Yes
	☐ N/A
<u>Drugs</u>	☐ No
<u>Misleading statements</u>	☐ Yes
21 CFR 201.6	☒ N/A
<u>Drugs</u>	☐ No
<u>Prominence of required label statements</u>	☒ Yes
21 CFR 201.15	☐ N/A
<u>Spanish-language (Drugs)</u>	☐ No
21 CFR 201.16	☐ Yes
	☒ N/A
<u>FD&C Yellow No. 5 and/or FD&C Yellow No. 6</u>	☐ No
21 CFR 201.20	☐ Yes
	☒ N/A
<u>Phenylalanine as a component of aspartame</u>	☐ No
21 CFR 201.21	☐ Yes
	☒ N/A
<u>Sulfites; required warning statements</u>	☐ No
21 CFR 201.22	☐ Yes
	☒ N/A
<u>Net quantity</u>	☐ No
21 CFR 201.51	☒ Yes
	☐ N/A
<u>Usual dosage statement</u>	☐ No
21 CFR 201.55	☒ Yes
21 CFR 201.100	☐ N/A
<u>Dispensing container</u>	☐ No
21 CFR 201.100	☐ Yes
	☒ N/A
<u>Medication Guide</u> 21 CFR 610.60	☐ No
21 CFR 208.24	☐ Yes
	☒ N/A

Prescribing Information and Patient Labeling Evaluation

Regulations	Conforms
PRESCRIBING INFORMATION	
Highlights of prescribing information	
PRODUCT TITLE 21 CFR 201.57(a)(2)	☐ No ☒ Yes ☐ N/A
DOSAGE AND ADMINISTRATION 21 CFR 201.57(a)(7)	☐ No ☒ Yes ☐ N/A
DOSAGE FORMS AND STRENGTHS 21 CFR 201.57(a)(8)	☒ No ☐ Yes ☐ N/A
Comment/Recommendation: We deleted information ("...syringe plunger stopper and needle cover are not made with natural rubber latex" and "with BD Ultrasafe Plus Passive Needle Guard") that is not required to appear under this section of the labeling per 21 CFR 201.57(a)(8). We note that this information is already included in the more appropriate place, section 16 of the FPI. <i>The applicant revised as requested</i>	
Full Prescribing Information	
2 DOSAGE AND ADMINISTRATION 21 CFR 201.57(c)(3)	☐ No ☒ Yes ☐ N/A
3 DOSAGE FORMS AND STRENGTHS 21 CFR 201.57(c)(4)	☒ No ☐ Yes ☐ N/A
Comment/Recommendation: We added identifying characteristics per 21 CFR 201.57(c)(4). <i>The applicant revised as requested.</i> We deleted information that is not required to appear under this section of the labeling per 21 CFR 201.57(c)(4). We note that this information is already included in the more appropriate place, section 16 of the FPI. <i>The applicant revised as requested</i>	
6.2 IMMUNOGENICITY Draft Guidance for Industry: Labeling for Biosimilar Products	☒ No ☐ Yes ☐ N/A
Comment/Recommendation: <i>Ensure the format is consistent with Labeling Review Tool regarding the standard statement in the LRT: Include the following standard statement or appropriate modification at the beginning of the Immunogenicity subsection preceding the immunogenicity data (for biosimilar products see draft Guidance for Industry: Labeling for Biosimilar Products):</i> <i>The applicant revised as requested</i>	
11 DESCRIPTION (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))	☒ No ☐ Yes ☐ N/A
Comment/Recommendation: We added the route of administration per 21 CFR 201.57(c)(12)	

<i>The applicant revised as requested</i>	
<u>16 HOW SUPPLIED/ STORAGE AND HANDLING</u> 21 CFR 201.57(c)(17)	☒ No ☐ Yes ☐ N/A
Comment/Recommendation: We added the dosage form and identifying characteristics per 21 CFR 201.57(c)(17). <i>The applicant revised as requested</i>	
<u>MANUFACTURER INFORMATION</u> 21 CFR 610.61, 21 CFR 610.64	☐ No ☒ Yes ☐ N/A
<u>INSTRUCTIONS FOR USE AND PATIENT INFORMATION</u>	
<u>TITLE (NAMES AND DOSAGE FORM)</u>	☐ No ☒ Yes ☐ N/A
<u>STORAGE AND HANDLING</u>	☐ No ☒ Yes ☐ N/A
Comment/Recommendation: <i>See section 16 for the PI.</i>	
<u>INGREDIENTS</u>	☐ No ☒ Yes ☐ N/A
Comment/Recommendation: <i>List inactive ingredients in alphabetical order per USP <1091> Labeling of Inactive Ingredients.</i>	
<u>MANUFACTURER INFORMATION</u> 21 CFR 610.61, 21 CFR 610.64	☒ No ☐ Yes ☐ N/A
Comment/Recommendation: For patient information labeling: we added the licensed applicant/manufacture information per 21 CFR 610.61(b) <i>The applicant revised as requested</i> For IFU: we added the clarifying statement "Manufactured by" <i>The applicant revised as requested</i>	

APPENDIX D. Acceptable Labels and Labeling

Prescribing Information (submitted on July 2, 2018 via email see below)

Instructions for Use (submitted on July 2, 2018 via email see below)

Patient Information (submitted on July 2, 2018 via email see below)



Vicky
Borders-Hemphill

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Scott
Lute

Digitally signed by Scott Lute
Date: 7/09/2018 12:40:52PM
GUID: 508da6d8000263fdf930d372c65435cd

Center for Drug Evaluation and Research
Office of Pharmaceutical Quality
Office of Process and Facilities
Division of Microbiology Assessment
WO Building 22
10903 New Hampshire Ave.
Silver Spring, MD 20993

PRODUCT QUALITY MICROBIOLOGY REVIEW AND EVALUATION

To: Administrative File, **STN 761080**
From: Maxwell Van Tassell, Ph.D., DMA Branch IV
Through: Reyes Candau-Chacon, Ph.D., Acting Quality Assessment Lead, DMA Branch IV
Subject: Addendum to Review Memo for New 351(k) Biologics License Application (BLA)
US License: 1974
Applicant: Hospira, Inc.
Product: Nivestym (PF-06881893)
Indication: Decrease the incidence of infection, as manifested by febrile neutropenia, in patients with nonmyeloid malignancies receiving myelosuppressive anti-cancer drugs associated with a significant incidence of severe neutropenia with fever
Dosage: Solution for injection, intravenous and subcutaneous
300 mcg/0.5 & 1.0 mL; 480 mcg/0.8 & 1.6 mL
Facilities: Hospira Zagreb d.o.o.
Prudnička cesta 60, Prigorje Brdovečko, Croatia
(FEI: 3010630287)
Receipt Date: 09/21/2017
Action Date: 07/21/2018

Recommendation for Approvability: The drug substance part of this BLA is recommended for approval from a product quality microbiology perspective with the following post-market commitment:

- To qualify an in-process endotoxin test method with an increased method sensitivity to allow for the establishment of (b) (4) in-process action limits. The in-process limits (b) (4) after manufacture of 30 drug substance batches. The readjusted in process endotoxin limits will be reported per 21 CFR 601.12.

Review Addendum

In an information request issued on June 11, 2018, the agency requested that the applicant (b) (4) endotoxin action limits (b) (4) (b) (4) than the Drug Substance release specification (b) (4) EU/mL).

Conclusion

- I. The drug substance section of this BLA, as amended, was reviewed from a product quality microbiology perspective and is recommended for approval with the following post-market commitment:
 - a. To qualify an in-process endotoxin test method with an increased method sensitivity to allow for the establishment of (b) (4) in-process action limits. The in-process limits (b) (4) after manufacture of 30 drug substance batches. The readjusted in process endotoxin limits will be reported per 21 CFR 601.12.
- II. Information and data in this submission not related to microbial control of the drug substance should be reviewed by the OBP reviewer.
- III. A pre-license inspection was conducted at Hospira Zagreb d.o.o., Croatia, from February 12th – 20th by OPF/DMA, OPF/DIA, and OBP. An eight-item Form FDA 483 was issued. Refer to Panorama for compliance status of the facilities.



Maxwell
Van Tassell

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Reyes
Candau-Chacon

Digitally signed by Reyes Candau-Chacon
Date: 6/21/2018 09:27:33AM
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DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service
Food and Drug Administration
Center for Drug Evaluation and Research
WO Bldg. 51, 10903 New Hampshire Ave.
Silver Spring, MD 20993

To: Administrative File, STN 761080/0
From: Marion Michaelis, CSO, CDER/OPQ/OPF/DIA
Endorsement: Peter Qiu, Ph.D., Branch Chief, CDER/OPQ/OPF/DIA
Subject: Original BLA
US License: Pending
Applicant: Hospira, Inc., a Pfizer Company
Mfg Facility: **Drug Substance:** Hospira Zagreb d.o.o, Croatia
FEI: 3010630287
Drug Product (Pre-filled Syringe): Hospira, Zagreb d.o.o, Croatia
FEI: 3010630287
Drug Product (Single Dose Vial): Hospira, Inc., McPherson, Kansas
Product: Nivestym™ (Filgrastim), Established Name: PF-06881893, a proposed biosimilar to Neupogen
Dosage: Solution for Injection (intravenous and subcutaneous), 300 mcg/0.5 & 1.0 mL; 480 mcg/0.8 & 1.6 mL
Indication: Decrease the incidence of infection, as manifested by febrile neutropenia, in patients with nonmyeloid malignancies receiving myelosuppressive anti-cancer drugs associated with a significant incidence of severe neutropenia with fever
Due Date: 9/21/2018

RECOMMENDATION: Approval is recommended from a facility perspective.

SUMMARY

Hospira, Inc., a Pfizer Company (Hospira) submitted this new Biologics License Application (BLA) dated September 21, 2017 for PF-06881893. The commercial PF-06881893 drug product has been developed as a proposed biosimilar to the reference product, Neupogen®. As part of this BLA there was one pre-license (PLI) inspection conducted which was the following:

- Hospira, Zagreb d.o.o (Hospira) (FEI: 3010630287) – Drug Substance (DS) and Drug Product (DP) Manufacturing Inspection Conducted 2/12-20/2018 - Outcome Classified as VAI (CMS #220200)

The PLI inspection conducted at Hospira, Zagreb for DS and DP manufacturing during the review of this application was a first-time inspection at this facility and resulted in an eight item 483.

The scope of this review is primarily for equipment and facilities. Items documented as “reviewed on inspection” in this memo are discussed in the Establishment Inspection Report (EIR) associated with the individual PLI for this application.

From a facility perspective approval is recommended.

REVIEW:

Drug Substance Manufacturing

3.2.S.2.1 DS Manufacturers

Hospira is proposing the following manufacturing and testing facilities for PF-06881893 drug substance:

SITE INFORMATION	FUNCTION	FEI NUMBER
Hospira Zagreb d.o.o Prudnička cesta 60 Prigorje Brdovečko Croatia, 10291	Profile CBI, SVS and IDD -Manufacture PF-06881893 DS -In-process control testing -Release testing of DS -Stability testing of (b) (4) -Stability testing of PF-06881893 DS	3010630287

Inspection History:

Hospira Zagreb d.o.o (FEI:3010630287) – Manufacturing and Testing

An inspection was conducted 2/12-20/2018 by CDER/OPF. This inspection was a PLI for this application for PF-06881893 DS and DP (syringes) manufacturing and first time inspection of this Hospira Zagreb, Croatia, facility. The inspection was conducted in accordance with CP 7346.832, Pre-Approval Inspections, ICH Q7, Good Manufacturing Practice Guidance for Active Pharmaceutical Ingredients and sections of CP 7356.002M, Inspections of Licensed Therapeutic Drug Products. Coverage during the inspection included facilities, equipment and processes, procedures and conformance to the application. The profile codes covered were CBI and SVS. The inspection resulted in the issuance of an 8-item FDA Form 483. A summary of the eight observations was the following; (b) (4)

(b) (4) is inadequate to prevent contamination of the sterile product, sterilization validation process was inadequate, procedures for (b) (4) were inadequate, (b) (4) was not supported by media fills, (b) (4) lacked adequate instruction for classification of rejects and procedures for (b) (4) were inadequate. The initial field recommendation was VAI. The EIR, associated exhibits, FDA Form 483, and the firm's response are included in CMS Work #220200. The compliance review indicates that the firm's proposed corrections to the 483 observations were adequate and the final classification is VAI.

The pre-filled syringe associated with this application is considered a combination product and an IDD profile code was assigned. The CDRH consult memo from OC dated 5/21/2018 indicates that an inspection is not required at this site at this time since no manufacturing activity for the combination product occurs at this facility. IDD profile should be included on the next inspection at this facility.

3.2. S.2.2 Manufacturing

Manufacturing of DS occurs at Hospira, Croatia facility. The PF-06881893 DS manufacturing process (b) (4)

Drug Product Manufacturing

3.2.P.3.1. DP Manufacturers

Hospira is proposing the following manufacturing and testing facilities for PF-06881893 DP:

BLA 761080/0 - PF-06881893
Hospira, Inc., a Pfizer Company

SITE INFORMATION	FUNCTION	FEI NUMBER
Hospira Zagreb d.o.o Prudnička cesta 60 Prigorje Brdovečko Croatia, 10291	Profile SVS, CBI and IDD • Manufacture of PF-06881893 DP -Pre-filled Syringes • In-process control testing of DP • Release testing of DP • Stability testing of DP • Stability storage of DP • PFS assembly • Primary packaging of DP • Secondary packaging and labeling of DP • QA release of final combination product • Storage of final combination product • Receipt and inspection of PFS components	3010630287
Hospira, Inc. 1776 N Centennial Drive Pherson, KS 67460	Profile SVS • Manufacture of PF-06881893 DP – Single Dose Vials • Acceptance testing of drug substance (appearance, identity, and bacterial endotoxins) • In-process control testing of DP • Release testing of DP (sterility and bacterial endotoxins) • Stability testing of DP (sterility and bacterial endotoxins) • Stability storage of DP (for sterility and bacterial endotoxin) • Secondary packaging and labeling of DP • Storage of DP • QA Release of DP	1925262
(b) (4)		
Hospira Inc., 375 North Field Drive Lake Forest, IL 60045	Profile IDD Design center for PF-06881893 Prefilled Syringe (b) (4)	3004591926

Inspection History:

Hospira Zagreb d.o.o (FEI:3010630287) – Manufacturing and Testing
See DS manufacturing section for inspection history of this facility.

Hospira, Inc (FEI: 1925262) – Manufacturing and Testing

The most recent inspection on 2/15/2018 was a pre-approval inspection for an NDA. This inspection focused on the (b) (4) delivery device portion of the NDA product. There was no 483 issued at the close of the inspection, however a withhold recommendation was put forward for the NDA. The withhold was recommended since there was incomplete qualification of their packaging and assembly line for the

(b) (4) device associated with the NDA product. Profiles SVS and IDD were covered. This withhold recommendation does not have any impact on this BLA and was application specific to the NDA. Prior to this on 10/27/2017 a Warning Letter follow-up and cGMP inspection took place. The inspection resulted in a VAI classification with the issuance of a 10 item 483 and profile SVL was covered along with several other profiles. The compliance review under Work ID#191705 indicates that corrections were adequately provided to uphold a VAI recommendation. Prior to this on 6/8/2016 a surveillance and pre-approval inspection took place. The inspection was classified as OAI with the issuance of a 14 item 483. A Warning Letter dated 2/14/2017 was issued. A warning letter follow-up inspection took place 10/27/2017 and was classified as VAI.

An inspection waiver memo was documented for this single dose vial drug product manufacturing site and is uploaded in Panorama.

(b) (4)
The most recent inspection (b) (4) was a surveillance inspection. The inspection was classified as NAI and profile CTL was covered. Prior to this on (b) (4) a surveillance inspection was conducted. The inspection was classified as NAI. Prior to this on (b) (4) a PDUFA pre-approval inspection took place and the inspection was classified as NAI.

Hospira Inc. (FEI:3004591926) - Design center for PF-06881893 Prefilled Syringe (b) (4)
The most recent inspection on 4/13/2015 was a surveillance inspection covering clinical research studies. The inspection was classified as VAI with the issuance of a two item 483. Prior to this on 12/9/2014 a surveillance inspection took place that covered testing. The inspection was classified as NAI. Prior to this on 9/9/2013 a surveillance inspection took place. This inspection was classified as NAI. FACTS firm profile data indicates that profiles SPD, CTX, SVL, LVP, SNI, SVS, SVT, CTL and BTP have previously been covered before. EIRs reviewed indicate that Hospira has been covered for drugs and medical devices and currently markets 510K approved electronic infusion pumps, which are medical devices. A post approval surveillance request will be placed to cover IDD profile code.

The CDRH consult memo from OC dated 5/21/2018 indicates that an inspection is not required at this site at this time since no manufacturing activity for the combination product occurs at this facility. The IDD profile code should be included on the next inspection at this facility.

3.2.P.3. Overview of PF-06881893 DP Manufacturing Pre-filled Syringe Operations Conducted at Hospira, Zagreb

(b) (4)

Combination Product – Pre-Filled Syringe

A consult review was requested from CDRH/OC for the device constituent of this combination product which consists of a pre-filled syringe designed to deliver PF-06881893 DP for injection. CDRH/OC evaluated the applicant's compliance with applicable Quality System Requirements for the approvability of this application. The consult request included an evaluation of the facilities involved in any aspect of the device component. I reviewed the consult memo from CDRH dated 5/21/2018 (uploaded in Panorama) which included the facility device inspection recommendations. The CDRH reviewer indicated that no pre-approval inspections were needed

CONCLUSION:

Adequate information of the facilities and equipment were provided for PF-06881893 DS and DP manufacturing. Additionally, all facilities associated with manufacturing are in a favorable compliance status and therefore approval is recommended from a facility perspective.

Marion
Michaelis -S

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ou=FDA, ou=People,
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cn=Marion Michaelis -S
Date: 2018.06.20 08:28:42 -04'00'

Marion Michaelis
Consumer Safety Officer
CDER/OPQ/OPF/DIA/B1

Zhihao
Qiu -S

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Zhihao (Peter) Qiu, Ph.D.
Branch Chief
CDER/OPQ/OPF/DIA/B1



Office of Pharmaceutical Quality Integrated Review
BLA 761080 Nivestym (filgrastim-aafi)
June 18, 2018

Application Team Lead: Howard Anderson, PhD, OBP/DBRRII
Tertiary Reviewer: Xianghong (Emily) Jing, PhD, OBP/DBRRII

OPQ Recommendation: OPQ recommends approval of this BLA for Nivestym 300 mcg/0.5 ml and 480 mcg/0.8 ml in a single-dose pre-filled syringe (PFS), and Nivestym 300 mcg/1.0 ml and 480 mcg/1.6 ml in a single-dose vial (SDV).

Justification

The results reviewed by OBP support a determination that Nivestym is highly similar to US-licensed Neupogen. The manufacture of Nivestym drug substance (DS) and drug product (DP) is validated and well controlled and produces a product that is pure and potent. Real time stability results are provided to support the 18 month SDV strengths expiry and 30 month PFS strengths expiry. The BLA is recommended for approval from a sterility assurance and microbiology product quality perspective. The DIA reviewer is recommending approval of the commercial manufacture of drug substance and PFS drug product produced at Hospira Zagreb (Pfizer), and SDV produced at Hospira MaPherson, KS. The OBP drug substance and analytical similarity, drug product quality, immunogenicity assay, and DMA DS and DP microbiological, technical reviews are located as separate documents in Panorama. The DIA technical review is being finalized, however DIA provided a statement that the BLA is recommended for approval from a facility perspective. The DIA technical review is pending to incorporate a CDRH consult request.

The immunogenicity assays used to evaluate anti-drug antibodies (ADA) are adequately validated and patients anti-drug antibody (ADA) rates are similar for Nivestym and US-licensed Neupogen.

Drug Name/Dosage Form	Nivestym (PF-06881893)/Solution in Pre-Filled Syringe (PFS) and Single Dose Vial (SDV)
Strength/Potency	PFS 300 mcg/0.5 ml and 480 mcg/ 0.8 ml, SDV 300 mcg/0.5ml and 480 mcg/1.6 ml
Route of Administration	Intravenous and Subcutaneous
Rx/OTC Dispensed	Rx
Indication	Nivestym is indicated for the decrease of incidence of infection, as manifested by febrile neutropenia, in patients with non-myeloid malignancies receiving myelosuppressive anti-cancer drugs associated with a significant incidence of severe neutropenia.



BLA 761080 Nivestym (filgrastim-aafi) QUALITY REVIEW



Applicant/Sponsor	Hospira, Inc, a Pfizer Company
US agent, if applicable	NA

Product Overview

PF-06881893 is a recombinant human N-methionyl granulocyte colony stimulating factor protein that is manufactured in *Escherichia coli* (*E. coli*). PF-06881893 is a 175-amino acid, single-chain, non-glycosylated protein (18,799 Da). The primary structure of PF-06881893 is identical to natural G-CSF, except for an additional methionine residue at the N-terminus. G-CSF is a growth factor produced by monocytes, fibroblasts, and endothelial cells. It regulates the maturation, proliferation, and differentiation of the precursor cells of neutrophilic granulocytes. rmetHuG-CSF or filgrastim is used for the treatment of neutropenia in patients and stimulates the production of white blood cells by binding and activating G-CSF receptor found on leukocytes.

Quality Review Team and Signature Approval Section

OBP Product Quality, DMA Microbiological, DIA Facility, Immunogenicity Assay technical reviews, and the OBP labeling review are archived in Panorama and contain primary and secondary review sign off. Provided below are the OPQ staff involved in the review of this BLA. Electronic signatures for Dr. Howard Anderson, the OPQ application team lead (ATL), and Dr. Xianghong (Emily) Jing, the tertiary reviewer, are located at the end of this document. The CMC statistical review is located in DARRTS.

DISCIPLINE	REVIEWER	OFFICE/DIVISION
Business Regulatory Process Manager	Kelly Ballard	OPRO
OBP Label Review	Vicky Borders-Hemphill	OBP
DS Quality and Analytical Similarity	Malini Wileman	OBP/DBRR II
Drug Product Quality	Scott Lute	OBP/DBRR II
Immunogenicity Assays	Scott Lute	OBP/DBRR II
Immunogenicity Assays Team Lead	Harold Dickensheets	OBP/DBRR II
Facilities	Marion Michaelis	OPF/DIA/IABI
Microbiology DS	Maxwell Van Tassell	OPF/DMA/MABIV
Microbiology DP	Aimee Cunningham	OPF/DMA/MABIV
Application Team Lead	Howard Anderson	OBP/DBRR II
Facilities Team Lead	Zhihao Peter Qiu	OPF/DIA/IABI
Microbiology Team Lead	Maria Reyes Candau-Chacon	OPF/DMA/BIV
Tertiary OBP Reviewer	Xianghong (Emily) Jing	OBP/DBRR II



BLA 761080 Nivestym (filgrastim-aafi) QUALITY REVIEW



Multidisciplinary 761080 Review Team

DISCIPLINE	REVIEWER	OFFICE/DIVISION
RPM	Wanda Nguyen	OHOP
Cross-disciplinary Team Lead	Donna Przepiorka	OHOP
Medical Officer	Lea Cunningham	OHOP
Clinical Pharmacology	Theingi Thway	CDER
Statistics	Lola Luo / Tianhua Wang	CDER/OB
CMC Statistics	Yuan Li Shen	OMPT/CDER/OTS/OB/DBV

Quality Review Data Summary

1. LEGAL BASIS FOR SUBMISSION: 351(k)

2. RELATED/SUPPORTING DOCUMENTS

A. BLA 761080 OPQ Amendments Reviewed

Submissions Reviewed	Amendment Submission Date
OBP	
Seq 0011	12/22/2017
Seq 0013	1/12/2018
Seq 0019	3/19/2018
Seq 0024	5/2/2018
Seq 0026	5/17/2018
Seq 0034	6/11/2018
DMA DP	
Seq 0021	03/19/2018
Seq 0023	03/30/2018
Seq 0025	05/17/2018
Seq 0027	05/22/2018
Seq 0028	05/30/2018
Seq 0030	05/31/2018
Seq 0033	06/08/2018
DMA DS	
Seq 0018	03/07/2018
Seq 0025	05/17/2018
Seq 0028	05/30/2018
Seq 0032	06/07/2018

B. DMFs:

DMF Number	Holder	Item referenced	Letter of cross-reference	Comments
(b) (4)			Yes	Adequate

(b) (4)

Yes	Not Reviewed/Adequate Information provided in the BLA
Yes	Adequate
Yes	Not Reviewed/Adequate Information provided in the BLA
Yes	Not Reviewed/Adequate Information provided in the BLA
Yes	Adequate
Yes	Not reviewed
Yes	Not reviewed

3. NOMENCLATURE

- A. Names
- i. Proprietary Name: Nivestym™
 - ii. Trade Name: Nivestym
 - iii. Non-Proprietary/USAN: Filgrastim-aafi
 - iv. INN Name: Filgrastim-aafi
 - v. Company Code: PF-06881893
 - vi. OBP systematic name: RPROT P09919 (CSF3_HUMAN) Granulocyte colony stimulating factor (PF06881893)
- B. Pharmacologic category: Therapeutic recombinant protein

4. CONSULTS: There was a consult from the OND RPM to CDRH ODE and OC for the pre-filled syringe.

Integrated Review

I. Recommendations

A. Recommendation and Conclusion on Approvability

The results reviewed by OBP support a determination that Nivestym is highly similar to US-licensed Neupogen. The manufacture of Nivestym drug substance (DS) and drug product (DP) is validated, well

controlled and produces a product that is pure and potent. Real time stability results are provided to support the 18 month SDV strength expiry and 30-month expiry for the two PFS syringe strengths. The BLA is recommended for approval from a sterility assurance and microbiology product quality perspective. The DIA reviewer is recommending approval of the commercial manufacture of drug substance and PFS drug product produced at Hospira Zagreb (Pfizer), and SDV produced at Hospira McPherson, KS. The OBP drug substance and analytical similarity, drug product quality, immunogenicity assay, DMA DS and DP microbiological, DIA facility technical, and OBP labeling reviews are located as separate documents in Panorama.

The immunogenicity assays used to evaluate anti-drug antibodies (ADA) are adequately validated and patients ADA rates are similar for Nivestym and US-licensed Neupogen.

B. Phase 4 Post-Marketing Commitment (PMC) Recommendations (draft language)

DMA (draft language)

1. (b) (4) drug substance endotoxin in-process limits and conduct additional drug substance in-process endotoxin method qualification.

C. Approval action letter language

- Manufacturing location

- o Drug substance – Hospira Zagreb d.o.o., 10291 Prigorje Brdovecko, Croatia (FEI 3010630287)
- o PFS Drug product – Hospira Zagreb d.o.o., 10291 Prigorje Brdovecko, Croatia (FEI 3010630287)
- o SDV Drug Product – Hospira, Inc, 1776 N Centennial Drive, McPherson, KS (FEI 1925262)

- Fill size and dosage form

- o PFS 300 mcg/0.5ml and 480 mcg/0.8ml
- o SDV 300 mcg/1.0 ml and 480 mcg/1.6 ml

- Dating period

- o PFS Drug product – Request for 30 months at $5^{\circ}\text{C} \pm 3^{\circ}\text{C}$ is granted. BLA contains three years of real-time stability results for 30 months for 3 PPQ lots.
- o SDV Drug product – Request for 18 months at $5^{\circ}\text{C} \pm 3^{\circ}\text{C}$ is granted. BLA contains 18 months of real-time stability results for 3 PPQ lots.
- o Drug substance – Request for (b) (4) months stored at (b) (4) $^{\circ}\text{C}$ is granted. (b) (4)

- A request for a categorical exclusion is granted for the need to prepare an environmental assessment in accordance with 21 CFR 25.31 (c). The request is provided in BLA 761080 section 1.12.14. The estimated concentration of substance at the point of entry into the aquatic environment will be below 1 part per billion. A claim that no known extraordinary circumstances as per 21 CFR 25.15(d) is provided.
- Nivestym is exempt from lot release and the sponsor is not currently required to submit samples of future lots of Nivestym to the Center for Drug Evaluation and Research (CDER) for release by the Director, under 21 CFR 610.2.

II. Analytical Similarity

The testing plan as well as the lots used in each assay were prospectively defined by the sponsor. A total of 50 DP lots of US-licensed Neupogen were purchased over time for the analytical similarity assessment. All lots used were within the manufacturers assigned expiry dates. The PF-06881893 lots tested in the analytical similarity assessment include the engineering, clinical, and process performance qualification (PPQ) lots. A total of 26 DP lots (16 PFS and 10 SDV) manufactured from 14 DS lots were included in the similarity assessment. All lots were manufactured using the proposed commercial manufacturing process, and were tested for analytical similarity using a subset of PF-06881893 characterization and release methods. All the three US-licensed Neupogen lots used in the comparative non-clinical (lot 1047463) and clinical (lots 1050859 and 1060852) studies are included in the analytical similarity assessment. Each Nivestym drug product lot evaluated was derived from an independent drug substance lot.

The Table 1 below summarizes the analytical similarity study results. In summary, the potency parameter assessed by the Tier 1 statistical equivalence method met the equivalence acceptance criterion, and all lots assessed by Tier 2 statistical evaluation meet the acceptance criterion that 90% of the lots are within mean $\pm$ three standard deviations. All Nivestym parameters met Tier 3 graphical visual assessment. Minor differences were noted in the Tier 2 statistical attributes for the following:

1. 1 lot out of 12 lots was outside of the quality range because it had a normalized protein concentration of 104.3%, a value just above the upper quality range limit of 103.2% for protein concentration. However, with a normalized deliverable content of 105.6%, the lot is within the normalized deliverable content limits.
2. 1 Lot out of 12 lots was just outside the quality range at 1% Met127 levels, a value just above the upper quality range limit of 0.9% for the RP-HPLC method. In a related Tier 3 method using Glu-C peptide mapping there was not difference in Met 127 content. The residual uncertainty raised by this results is mitigated by the results of the functional potency assay, showing no meaningful differences in potency between Nivestym and US-licensed Neupogen.

Table 1		
Tier 1-Equivalence Testing		
Assay	Assay Parameter	Demonstration of similarity (lots)
Potency	Cell Based Proliferation Assay	Yes (14 Nivestym/15 US Licensed Neupogen)
Tier 2 Statistical Quality Range (3 standard Deviations)		
Assay	Assay Parameter	Demonstration of similarity
UV-Vis spectroscopy	Protein Concentration/Strength	Yes (13/14 lots Nivestym Yes / 24 lots Neupogen)
ELISA based Competitive Receptor Binding Assay (CRBA)	Receptor Binding Assay	Yes (11 Nivestym/18 US licensed Neupogen)
Surface Plasmon Resonance (SPR)	Receptor Binding Affinity (K_D)	Yes (11 Nivestym /17 US licensed Neupogen)
RP-HPLC	Total Product Related Substance and Oxidized Met 122, Met 138, reduced)	Yes (12 Nivestym / 24 US licensed Neupogen)
RP-HPLC	Met 127 Oxidation	Yes (11/12 Nivestym Lots/ 27 US licensed Neupogen Lots)
RP-HPLC	Deamidated	Yes (22 Nivestym/27 US licensed Neupogen)
Cation Exchange Chromatography (CEX)	Total Product Charge Variants	Yes (11 Nivestym/18 US licensed Neupogen)
Differential Scanning Calorimetry (DSC)	Tertiary Structure	Yes (11 Nivestym/18 US licensed Neupogen)
Tier 3 Visual Assessment of graphical displays		
SPR	Receptor Binding Kinetics (K_{on} , K_{off} , K_D)	Yes (11 Nivestym / 17 US licensed Neupogen)
CEX	Total Charge Variants (Acidic Variants peaks 1 and 2)	Yes (14 Nivestym / 38 Neupogen)
CEX	Deamidated Peaks 1 and 2	Yes 22 (Nivestym/27 US licensed Neupogen)
Glu-C Peptide Mapping	Site-specific deamidation of Gln12, Gln21, Gln68, Gln71, Gln91, Gln108, Gln120, Gln132, Gln159, and Gln174	Yes (11 Nivestym/11 U/S Licensed Neupogen)
RP-UPLC-MS	Intact Molecular Weight	Yes (2 Nivestym/2 US licensed Neupogen)
Glu-C Peptide Mapping	Isomerization	Yes (11 Nivestym/12 US licensed Neupogen)
Glu-C Peptide Mapping/RP-UPLC-MS	Amino acid Sequence Confirmation	Yes (11 Nivestym/11 US licensed Neupogen)
Ellman's Test	Free Thiol	Yes (7 Nivestym/7 US licensed Neupogen)
Far-UV Circular Dichroism (CD)	Secondary Structure	Yes (16 Nivestym/23 US licensed Neupogen)
Intrinsic fluorescence	Tertiary Structure	Yes (16 Nivestym/23 US licensed Neupogen)
Hydrogen Deuterium exchange	Secondary and Tertiary Structure	Yes (3 Nivestym/4 US licensed Neupogen)
SV-AUC	Tertiary Structure, High Molecular Weight Product Related Aggregates	Yes (16 Nivestym/32 US licensed Neupogen)
Nuclear Magnetic Resonance	Tertiary Structure	Yes (2 Nivestym/2 US licensed Neupogen)

X-ray crystallography	Tertiary Structure	Yes
SEC	High Molecular Weight Substances	Yes (26 Nivestym/38 US licensed Neupogen)
SDS-PAGE	Product Variants	Yes (16 Nivestym/18 US licensed Neupogen)
CE-SDS	Product Variants	Yes (12 Nivestym/10 US licensed Neupogen)
Glu-C Peptide Mapping	Met 1, 127, 138, Trp 59, 119 oxidations, Deamidation Gln 21, 71, 91, 108, 132, Aspartate Isomerization, N-terminal truncations, fMet1, Asp110 and related substances	Yes (11 Nivestym/11 US licensed Neupogen)
RP-HPLC	Succinimide, reduced species	Yes (22 Nivestym/ 23 of 27 US Licensed Neupogen below LOQ)
RP-HPLC	Cys-37-Cys43 and Cys 65-Cys 75	Yes (22 Nivestym/27 US Licensed Neupogen)
No Tier Assignment (18 Lots Nivestym and 5 lots US-licensed Neupogen)		
HCP, HCD, Osmolarity, Polysorbate 80, Appearance, Color Clarity, Visible Particles, Sub Visible Particles		

III. Summary of Quality Assessments

A. CQA Identification, Risk and Lifecycle Knowledge Management

Table 1 below provides a summary of critical quality attributes and their control strategy that are relevant to both drug substance and drug product. Additional information is located in primary DS and DP Product Quality, DS and DP Microbiology Control, and Facility technical reviews in Panorama.

Table 1: DS and DP CQA Identification, Risk and Lifecycle Knowledge Management

CQA	Risk	Origin	Control Strategy	Comment
Strength	Efficacy/Safety	Protein in vial can be impacted by deviations in DP manufacturing	(b) (4)	
Potency-	Efficacy	Nivestym structure is impacted by bioreactor conditions		Stability indicating
Charge Variants	Immunogenicity	Can result from slight from variations in manufacturing conditions, heat exposure can increase attribute		Stability indicating
High Molecular Weight (HMW) Impurities	Immunogenicity, Loss of efficacy due to decrease in potency	Heat exposure can increase attribute		Stability indicating

Low Molecular Weight (LMW) Impurities	Loss of efficacy due to decrease in potency	Host cell proteins can contain proteases that degrade the product	(b) (4)	Stability indicating
Oxidation and Deamidation	Increase in Immunogenicity	Increase in ADA can reduce potency and PK/PD		Not stability indicating
Appearance, Physical Form Clarity, Color	Safety and Efficacy	Manufacturing process and formulation		None
Identity	Safety and Efficacy	NA		None

B. Nivestym Drug Substance Quality Summary

Table 2 below is a summary of critical quality attributes and their control strategy that are relevant to drug substance. For additional information see primary Product Quality and DS Microbiology Controls technical reviews in Panorama.

Table 2. CQA Identification, Risk and Live Knowledge Management

CQA	Risk	Origin	Control Strategy	Comment
Bioburden	Safety, purity, and efficacy (degradation or modification of the product by contaminating microorganisms)	Raw materials or contamination during manufacturing	(b) (4)	None
Endotoxin	Safety and purity	Raw materials or contamination during manufacturing		PMC to (b) (4) in-process limits and method qualification
Host cell protein	Safety and immunogenicity	Process-related impurity from the production cells		None

Host cell DNA	Safety	Process-related impurity from the production cells	(b) (4)	None
Leachable Impurities	Safety	Process-related impurities, and drug substance container closure system		None

1. Description

Nivestym is a recombinant N-methionyl granulocyte-colony stimulating factor (G-CSF) that is manufactured by E. coli fermentation. PF-06881893 is a 175-amino acid, single-chain, non-glycosylated protein (18,799 Da). The primary structure of PF-06881893 is identical to natural G-CSF, except for an additional methionine residue at the N-terminus of PF-06881893. It has an α -helical structure, a 4 helical-bundle motif and contains an N-terminal methionine and 2 intramolecular disulfide bonds: Cys37-Cys43 and Cys65-Cys75. PF-06881893 also contains a free cysteine at position 18 (Cys18).

2. Mechanism of Action (MOA)

G-CSF is produced naturally by endothelium, macrophages, and several other immune cells. Critical quality attributes include Nivestym binding cell surface receptors to initiate signal transduction resulting in cell surface proliferation. Nivestym stimulates bone marrow to produce granulocytes and stem cells that are released into the bloodstream. Nivestym stimulates the survival, proliferation, differentiation and function of neutrophil precursors and mature neutrophils.

3. Potency

A cell based potency assay quantifies biological activity by measuring the ability of Nivestym to stimulate proliferation of NFS-60 cells. Cell proliferation is determined by measuring Adenosine Tri-Phosphate (ATP) mixed with a light emitting reagent. The amount of light is proportional to the amount of ATP, which is in turn proportional to the number of cells. The raw data for luminescence values from microplate data acquisition software is imported to a software program to perform a dose response parallel line analysis for the test sample and reference standard to confirm method suitability. Potency is calculated relative to the reference standard using the same software. The method is consistent with the filgrastim clinical mechanism.

4. Reference Standard (RS)

(b) (4)

5. Manufacturing Process Summary

(b) (4)

**6. Container closure**

(b) (4)

**7. Dating period and storage conditions**

(b) (4)

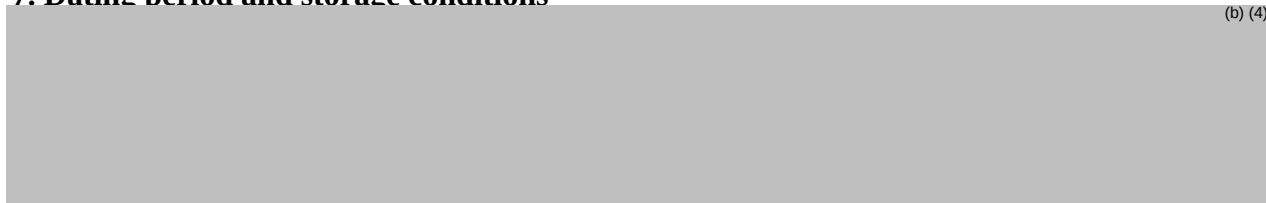
**C. Nivestym Drug Product Quality Summary**

Table 3 provides a summary of the identification, risk, and lifecycle knowledge management for drug product CQAs that derive from the drug product manufacturing process and general drug product attributes. For additional information see primary Product Quality and DP Microbiology Controls technical reviews in Panorama.

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

CQA	Risk	Origin	Control Strategy	Comment
Sterility (contaminant)	Safety (Infection), Purity and Efficacy (degradation or modification of products by contaminating microorganisms)	Contamination may be introduced throughout manufacturing process	(b) (4)	None
Endotoxin (contaminant)	Safety, purity, and immunogenicity	Raw materials; contamination may be introduced throughout the DP manufacturing process		Provides a 2-fold safety factor.
Particulate Matter	Blood vessel occlusion and Immunogenicity	Product or process related impurity		None
Container closure integrity	Safety	Container closure breaches during storage. May be impacted by storage conditions.		None
Identity	Safety and efficacy	NA		None
Appearance Physical Form, Clarity, Color	Safety and efficacy	Manufacturing		None
pH, Osmolality, and extractable volume	General product quality attributes	Manufacturing		None

1. Potency and Strength

Potency is defined relative to a qualified reference standard using a cell based bioassay. The cell based assay acceptance criteria for the DS and DP are (b) (4)% (Relative to RS) and on stability. Nivestym is intended to be marketed (b) (4) mg/0.8 ml liquid in a prefilled syringe. Nivestym strength is 300 mcg/0.5 ml and 480 mcg/0.8 ml in a single-dose pre-filled syringe (PFS), and Nivestym 300 mcg/1.0 ml and 480 mcg/1.6 ml in a single-dose vial (SDV).

2. Summary of Product Design

Nivestym is a sterile 600 mcg/ml filgrastim-aafi liquid filled single use prefill syringes or vials.

3. List of Excipients

Nivestym excipients include sorbitol as a (b) (4), sodium and acetate as a (b) (4), polysorbate 80 as a (b) (4). All excipients are compendial grade. Additional excipient information is in the Product Quality primary technical review in Panorama.

4. Reference material(s)

The DP reference standard is the Nivestym (filgrastim-aafi) drug substance. There is no formal Nivestym DP reference standard. Nivestym RS information is in the DS Product Quality primary technical review in panorama.

5. Manufacturing Process

(b) (4)

6. Container Closure (CC)

Nivestym primary CC consists of a glass syringe barrel with a graduation label and 27-gauge 0.5-inch staked needle with a rigid needle shield, a plunger stopper, and plunger rod with a thumb pad, and needle guard.

(b) (4)

Nivestym SDV CC consists of a 2-ml glass vial, (b) (4) stopper, and aluminum seal with a tamper-evident Flip-Off cap.

7. DP Stability

The sponsor requested for a 30 month PFS expiry at $5^{\circ}\text{C} \pm 3^{\circ}\text{C}$ and provided three years of real-time results for 30 months for 3 PPQ lots and supporting stability results. The sponsor requested an 18 month SDV expiry for SDV stored at $5^{\circ}\text{C} \pm 3^{\circ}\text{C}$ and provided real-time results for 18 months for 3 PPQ lots and supporting stability results.

D. Novel Approaches/Precedents: none**E. Product Quality Labeling Recommendations**

Nivestym (filgrastim-aafi) is a proposed biosimilar to Neupogen (filgrastim) and the labeling indicates that:

Store NEUPOGEN at 2° to 8°C (36° to 46°F) in the carton to protect from light. Do not leave NEUPOGEN in direct sunlight. Avoid freezing; if frozen, thaw in the refrigerator before administration. Discard NEUPOGEN if frozen more than once. Avoid shaking. Transport via a pneumatic tube has not been studied

F. Establishment Information

Overall Facility Recommendation: Approval				
Drug Substance				
Site Name	Address	FEI Number	Responsibility	Final Recommendation
Hospira Zagreb d.o.o.	10291 Prigorje Brdovecko, Croatia, 10291	3010630287	• Manufacture of drug substance • In-process, release and stability testing of drug substance	Approval based on acceptable inspection outcome

Drug Product				
Site Name	Address	FEI Number	Responsibility	Final Recommendation
Hospira Zagreb d.o.o.	10291 Prigorje Brdovecko, Croatia, 10291	3010630287	• Manufacture of drug product pre-filled syringes • In-process, release and stability testing of drug product • Packaging, labeling and storage	Approval based on acceptable inspection outcome
Hospira, Inc.	1776 N Centennial Drive McPherson, KS, 67460	1925262	• Manufacture of drug product single dose vials • In-process, release and stability testing of drug product • Packaging, labeling and storage	Approval based on acceptable profile

(b) (4)	• Stability Testing – Container Closure Integrity	Approval based on acceptable profile
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G. Facilities

A pre-license inspection of drug substance and drug product (pre-filled syringe) manufacturing and testing, Hospira Zagreb d.o.o., Croatia facility was conducted February 12-20, 2018 by CDER (DMA, DIA and OBP). The inspection in support of this application covered Quality, Production, Facilities and Equipment, Laboratory Control, and Material systems. The inspection was classified as “Voluntary Action Indicated”. The facility was approved based on the inspectional assessment. Other manufacturing and testing facilities associated with this application were approved based on acceptable profile and compliance status.

For additional information see the Facilities Technical Review: Division of Inspectional Assessment.

H. Lifecycle Knowledge Management

1. Drug Substance

- i. **Protocols approved:** Annual stability protocol, qualification of new working reference standard, and purification column expiry.
- ii. **Outstanding review issues/residual:** None
- iii. **Future inspection points to consider:** None

2. Drug Product

- i. **Protocols approved:** Annual stability protocol and (b) (4).
- ii. **Outstanding review issues/residual:** None
- iii. **Future inspection points to consider:** None

Quality Assessment Summary Tables

Table 1: Noteworthy Elements of the Application

#	Checklist		Yes	No	N/A
Product Type					
1.	Recombinant Product		X		
2.	Naturally Derived Product			X	
3.	Botanical			X	
4.	Human Cell Substrate/Source Material			X	
5.	Non-Human Primate Cell Substrate/Source Material			X	
6.	Non- Primate Mammalian Cell Substrate/Source Material		X		
7.	Non-Mammalian Cell Substrate/Source Material		X		
8.	Transgenic Animal Sourced			X	
9.	Transgenic Plant Sourced			X	
10.	New Molecular Entity			X	
11.	PEPFAR Drug			X	
12.	PET Drug			X	
13.	Sterile Drug Product		X		
14.	Other				
Regulatory Considerations					
15.	Citizen Petition and/or Controlled Correspondence Linked to the Application (#)			X	
16.	Comparability Protocol(s)			X	
17.	End of Phase II/Pre-BLA Agreements			X	
18.	SPOTS (Special Products On-line Tracking System			X	
19.	USAN Name Assigned		X		
20.	Other				
Quality Considerations					
21.	Drug Substance Overage			X	
22.	Design Space	Formulation	X		
23.		Process		X	
24.		Analytical Methods		X	
25.		Other			
26.	Other QbD Elements			X	
27.	Real Time Release Testing (RTRT)			X	
28.	Parametric Release in lieu of Sterility Testing			X	
29.	Alternative Microbiological Test Methods			X	
30.	Process Analytical Technology in Commercial Production			X	
31.	Non-compendial Analytical Procedures	Drug Product	X		
32.		Excipients		X	
33.		Drug Substance	X		
34.	Excipients	Human or Animal Origin		X	
35.		Novel		X	
36.	Nanomaterials			X	
37.	Genotoxic Impurities or Structural Alerts			X	
38.	Continuous Manufacturing			X	
39.	Use of Models for Release			X	
40.	Other				



Howard
Anderson

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Center for Drug Evaluation and Research
Office of Pharmaceutical Quality
Office of Process and Facilities
Division of Microbiology Assessment
WO Building 22
10903 New Hampshire Ave.
Silver Spring, MD 20993

PRODUCT QUALITY MICROBIOLOGY REVIEW AND EVALUATION

To: Administrative File, **STN 761080**
From: Maxwell Van Tassell, Ph.D., DMA Branch IV
Through: Reyes Candau-Chacon, Ph.D., Acting Quality Assessment Lead, DMA Branch IV
Subject: New 351(k) Biologics License Application (BLA)
US License: 1974
Applicant: Hospira, Inc.
Product: Nivestym (PF-06881893)
Indication: Decrease the incidence of infection, as manifested by febrile neutropenia, in patients with nonmyeloid malignancies receiving myelosuppressive anti-cancer drugs associated with a significant incidence of severe neutropenia with fever
Dosage: Solution for injection, intravenous and subcutaneous
300 mcg/0.5 & 1.0 mL; 480 mcg/0.8 & 1.6 mL
Facilities: Hospira Zagreb d.o.o.
Prudnička cesta 60, Prigorje Brdovečko, Croatia
(FEI: 3010630287)
Receipt Date: 09/21/2017
Action Date: 07/21/2018

Recommendation for Approvability: The drug substance part of this BLA is recommended for approval from a product quality microbiology perspective, pending (b) (4) endotoxin in-process action limits.

Review Summary

Hospira, Inc. submitted 351(k) BLA 761080 in eCTD on September 21, 2017 to obtain approval of Nivestym, a biosimilar to the US-licensed reference product Neupogen®. Nivestym is a recombinant methionyl granulocyte-colony stimulating factor produced in *Escherichia coli* for the treatment of neutropenia.

This review contains the assessment of the microbial quality attributes of the PF-06881893 drug substance (DS) from a microbiological quality perspective. For review of drug product microbiology aspects of the application, please see review by Aimee Cunningham, Ph.D.

Drug Substance Quality Microbiology Information Reviewed

Sequence number	Date	Description
eCTD 0001	09/21/2017	Original 351(k) BLA submission
eCTD 0018	03/07/2018	Response to information request
eCTD 0025	05/17/2018	Response to information request
eCTD 0028	05/30/2018	Response to information request
eCTD 0032	06/07/2018	Response to information request

Review Assessment**3.2.S DRUG SUBSTANCE****3.2.S.1 GENERAL INFORMATION**

The Nivestym drug substance (DS), PF-06881893, is a recombinant 175 amino acid, single chain, α -helical protein comprising a methionyl human granulocyte-colony stimulating factor. The PF-06881893 protein is approximately 18.8 kDa and contains two intramolecular disulfide bonds, a single free thiol group, and a four-helical-bundle motif. Biological activity is based on the binding of high-affinity receptors on the surface of developing and mature neutrophils in bone marrow, resulting in cell proliferation.

3.2.S.2 MANUFACTURE**3.2.S.2.1 Manufacturer(s)**

The following facilities are used for the manufacture and testing of the PF-06881893 drug substance (DS), from Table 3.2.S.2.1-1 from the submission:

Table 3.2.S.2.1-1: Manufacturers

Name and Address of Manufacturing Site	Responsibilities
Hospira Zagreb d.o.o. ^a Prudnička cesta 60, 10291 Prigorje Brdovečko, Croatia FEI 3010630287	- Preparation and testing of cell banks - Manufacture PF-06881893 DS - In-process control testing - Release testing of DS - Stability testing of (b) (4) - Stability testing of PF-06881893 DS
(b) (4)	- Quality control testing of master cell bank and working cell bank - Cell banks remote backup storage location
	Quality control testing of master cell bank and working cell bank
	Quality control testing of master cell bank and working cell bank
	Cell banks remote backup storage location

^a Hospira is a wholly owned subsidiary of Pfizer, Inc.

Conclusion

- I. The drug substance section of this BLA, as amended, was reviewed from a product quality microbiology perspective and is recommended for approval pending (b) (4) in-process endotoxin limits, which will be reviewed in an addendum to this review memo.
- II. Information and data in this submission not related to microbial control of the drug substance should be reviewed by the appropriate division.
- III. A pre-license inspection was conducted at Hospira Zagreb d.o.o., Croatia, from February 12th – 20th by OPF/DMA, OPF/DIA, and OBP. An eight-item Form FDA 483 was issued. Refer to Panorama for compliance status of the facilities.



Maxwell
Van Tassell

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Reyes
Candau-Chacon

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OFFICE OF DEVICE EVALUATION

DIVISION OF ANESTHESIOLOGY, GENERAL HOSPITAL,
RESPIRATORY, INFECTION CONTROL, AND DENTAL DEVICES

GENERAL HOSPITAL DEVICES BRANCH INTERCENTER CONSULT MEMORANDUM



Date	June 11, 2018
To	Wanda Nguyen, Pharmacist, CDER/OND/OHOP/DHP
Requesting Division	Division of Hematology Products
From	Kenya Brothers / John McMichael CDRH/ODE/DAGRID/GHDB
Through (Team Lead)	John McMichael, ICC Team Lead CDRH/ODE/DAGRID/GHDB
Through (Branch Chief)	CAPT Alan Stevens CDRH/ODE/DAGRRID/GHDB
Subject	Consult for Submission # BLA 761080 ICCR2017-01810 ICC1700856
Recommendation	CDRH/ODE Recommends that the Device Constituent Parts of the Combination Product are APPROVABLE.

Digital Signature Concurrence Table

Reviewer	John C. Mcmichael -S 2018.06.11 15:00:56
Team Lead	-04'00'
Branch Chief	Alan M. Stevens -S Digitally signed by Alan M. Stevens -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=1300189211, cn=Alan M. Stevens -S Date: 2018.06.12 15:01:13 -04'00'

1. Submission Overview

Table 1. Submission Information	
ICCR # (Lead)	ICCR2017-01810
ICCR SharePoint Link	http://sharepoint.fda.gov/orgs/OSMP/ocp/ICRR/Lists/ICRR%20Forms/DispForm.aspx?ID=1989
ICC tracking # (Lead)	ICC1700856
Submission Number	BLA 761080
Sponsor	Hospira, Inc.
Drug/Biologic	PF-06881893 PFS
Indications for Use	To decrease the incidence of infection, as manifested by febrile neutropenia, in patients with nonmyeloid malignancies receiving myelosuppressive anti-cancer drugs associated with a significant incidence of severe neutropenia
Device Constituent	Pre-filled Syringe
Related Files	IND 10991

Table 2. Review Team	
CDER/CBER Lead Review Division	Division of Hematology Products
Submission RPM	Nahleen Lopez
Lead Device Reviewer	Kenya Brothers

Table 3. Important Dates	
1 st round of Information Requests	November 28, 2017
Final Lead Device Review Memo Due	June 4, 2018
Interim Due Dates	Meeting Date
Filing	November 3, 2017
Mid-Cycle	February 15, 2018
Wrap-up	June 7, 2018

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2. PURPOSE/BACKGROUND

2.1. Scope

Hospira, Inc. submitted this Biologics Licensing Application (BLA) for PF-06881893 to propose their drug product as a biosimilar to Neupogen®.

On October 30, 2017, CDER made the following request:

Please review the device for the PFS combination presentation

This review is limited to the performance of the syringe – it will not cover review of the drug product (including leachables and extractables from the primary container closure into the drug product), manufacturing facility or the primary container closure (single dose vial).

Note: Reviewer comments are indicated in *italics* and text copied directly from the submission is indicated by Calibri 10pt font.

2.2. Prior Interactions

To my knowledge, CDRH has had no prior interactions with the sponsor related to this submission.

2.2.1. Related Files

IND 10991 – PF-06881893 (submitted September 4, 2015)

2.3. Indications for Use

Combination Product	Indications for Use
PF-06881893	To decrease the incidence of infection, as manifested by febrile neutropenia, in patients with nonmyeloid malignancies receiving myelosuppressive anti-cancer drugs associated with a significant incidence of severe neutropenia.
Pre-filled Syringe	Delivery of the drug product

3. ADMINISTRATIVE

3.1. Documents Reviewed

Document Title	Date - Version
Syringe (71001391) 300mcg/0.5 PFS	April 5, 2017
Carton (71001411) 300mcg/1ml 1pk Unit PFS	April 5, 2017
Carton (71001419) 300mcg/1ml 1pk Shelf PFS	April 5, 2017
Carton (71001420) 300mcg/ml 10pk PFS	April 5, 2017
lab-0933-0-1-pkg-insert-clean	July 2017
Container Closure System – PFS	August 31, 2017
Response to 08 Dec 2017 FDA Request for Information	December 20, 2017
Device - Design Verification	December 20, 2017

4. DEVICE DESCRIPTION AND PERFORMANCE REQUIREMENTS

The following information was taken from “Container Closure System – PFS” under Sequence 0001 in GSR, Section 3.2.P.7:

The device constituent part is a single dose pre-filled syringe (PFS) that is supplied in two different strengths – 300mcg/0.5ml or 480mcg/0.8ml (equivalent to 600mcg filtrate per ml of solution). The sponsor asserts the same device constituents are used in each PFS presentation; however, the plunger is colored differently to indicate the strength of the solution ((b) (4) – 300mcg/05ml; (b) (4) – 480mcg/0.8ml).

Figure 3.2.P.7-1. Ready to Use PF-06881893 Prefilled Syringe Configurations (b) (4)



The PFS consists of the following components:

Table 3.2.P.7-1. Container Closure System Components for PF-06881893 PFS

Syringe Components	Component Description	Materials of Construction	Material Code	Supplier	References
Product Contacting Components					
(b) (4) Syringe System	<u>Glass Barrel:</u> transparent (clear) 1 mL long syringe barrel <u>Glass conformity:</u> in compliance with USP <660> EP 3.2.1, and JP P 7.01.	USP (b) (4) Glass	(b) (4)		
	<u>Needle:</u> 27-gauge ½ inch 5 (b) (4) staked stainless steel needle <u>Needle conformity:</u> conforms to (b) (4)	Stainless steel needle			

Syringe Components	Component Description	Materials of Construction	Material Code	Supplier	References
	<u>Rigid Needle Shield:</u> (b) (4)				DMF (b) (4) DMF (b) (4)
(b) (4) Plunger Stopper	<u>Plunger stopper:</u> (b) (4) <u>Plunger stopper conformity:</u> meets the identification tests such as (b) (4)	(b) (4)	(b) (4)	(b) (4)	DMF (b) (4) DMF (b) (4)
Non-Product Contacting Components					
Plunger Rod (b) (4)	(b) (4)				
Plunger Rod (b) (4)	(b) (4)				
Needle Guard with Spring	BD Ultrasafe Plus Passive Needle Guard (b) (4)	(b) (4)			

The various components of the PFS are depicted below.

Figure 3.2.P.7-2. PF-06881893 Prefilled Syringe Components
 (b) (4)



Device Characteristic	Description / Specification
Syringe Name	Nivestym™
Syringe Platform Name	(b) (4)
Priming Dose / Volume	N/A
Dose accuracy	NLT (b) (4) mL NLT (b) (4) mL
Injection Time	N/A
Injection Site	• Front of thigh • Abdomen • Upper outer arm • Upper outer buttocks
Injection tissue and depth of injection	Subcutaneous
Audible / visual feedback	Clear medication window
Cap Removal Force	NMT (b) (4) N
Breakloose / Glide Force	NMT (b) (4) N
Visibility of medication container	Clear inspection window
Needle Specifications • Length(s) • Gauge(s) • Connection type - o ISO 11608-2:2012 - o Prestaked	• ½" • 27G • Staked
Type of Use (e.g. single use, disposable, reusable, other)	Single use

Device Characteristic	Description / Specification
Intended user (e.g., self-administration, professional use, user characteristics and / or disease state that impact device use)	Self-administration
Method of actuation	Depress plunger
Automated Functions	N/A
Residual Medication	N/A
Drug Container Type	PFS
Dose Units of Measure (e.g., mL, Units, mg, increments, etc.)	ml
Environments of use	Home use
Storage conditions and expiry	Store refrigerated b/w 36°F – 46°F; do not freeze
Graduation marks / fill lines	Graduation marks
Preparation and administration (describe all that are applicable) - Warm to room temp prior to injection - Assembling components - Prime steps - Setting dose - Skin preparation steps (e.g., pinch skin, inject through clothing, etc.) - Changing / disposing needles - Etc.	- Warm to room temp prior to injection - Choose injection site (front of thigh, abdomen, outer upper arm, upper outer buttocks – different site for each injection) and clean skin with alcohol wipe - Push plunger rod until stopper lines up with correct dose (unless prescribed full dose, then just use) - Pinch skin - Insert needle straight up and down or at 45° angle and push down on plunger rod as far as it will go – hold in place for 5s - Dispose of syringe in sharps container
Safety Features Needle safety	Rigid needle shield Passive needle guard
Material composition of PFS	<u>Drug Product Contacting</u> (b) (4) glass - Stainless steel needle (b) (4) needle shield (b) (4) stopper (b) (4) <u>Non-Drug Product Contacting</u> (b) (4) plunger rod (b) (4) (b) (4) passive needle guard with (b) (4) spring

5. DESIGN CONTROL REVIEW

5.1. Design Review Summary

6.3.2. *Biocompatibility*

The following information was taken from “Container Closure System – PFS” under Sequence 0001 in GSR, Section 3.2.P.2.4.4.1:

The sponsor performed Biocompatibility testing on the following components of the PFS:

(b) (4)

The subject device is categorized as an externally communicating device with indirect contact with the blood path. The sponsor considered the contact to be prolonged (>24h to 30d) due to the necessity of repeat exposure of the patient to the device. The testing was reported to have been done in accordance with ISO 10993-1:2009, an FDA-recognized consensus standard. FDA’s Guidance document entitled, “Use of International Standard ISO 10993-1, ‘Biological evaluation of medical devices - Part 1: Evaluation and testing within a risk management process’” recommends that the following Biocompatibility tests be performed for devices in this category:

- Cytotoxicity
- Sensitization
- Irritation or Intracutaneous Reactivity

- Acute Systemic Toxicity
- Material Mediated Pyrogenicity
- Subacute/Subchronic Toxicity
- Hemocompatibility

The sponsor performed the recommended testing on all patient-contacting components of the device (Table 3.2.P.2.4-7). They reported only conducting Cytotoxicity testing on the non-patient contacting components (plunger rod, needle safety device) based on previous experience with FDA (e.g., NDA 202515 – Morphine Sulfate Injection, NDA 021171 – Demerol). The results of Biocompatibility testing are summarized in the table below.

Table 3.2.P.2.4-8. Biocompatibility Test Results for DP Contacting Syringe Components

Biological Safety Endpoint	Test Method	Acceptance Criteria	Test Results	Pass/ Fail
Cytotoxicity	ISO MEM Elution Assay with L929 Mouse Fibroblast Cells	If cell reactivity grade is 0 – 2, test article is considered non-cytotoxic.	Cell reactivity grade is 0 at 24, 48, and 72 + 4 hours - non-cytotoxic.	Pass
Irritation	Intracutaneous Irritation	The test passes if the difference between the test article and the control mean score is 1.0 or less.	Comparative dermal observation scores for normal saline and sesame oil extracts were 0.0 and 0.0 respectively.	Pass
Pyrogenicity	Materials Mediated Rabbit Pyrogen	Test fails if any of the 3 animals had an individual temperature increase $\leq 0.5^{\circ}\text{C}$.	The temperature increases for the 3 animals were 0.1°C , 0.3°C , and 0.0°C respectively.	Pass
Sensitization	Guinea Pig Maximization Sensitization	Individual test animal scores of '1' or greater indicate sensitization (fail), provided scores of less than '1' are observed in the controls.	Both normal saline & sesame oil test article extracts were observed with a sensitization response of "0".	Pass
Acute Systemic Toxicity	Acute Systemic Injection	The test fails if two or more mice die or show clinical signs of toxicity, or if 3 or more animals had a body weight loss of more than 10%.	Abnormal clinical signs, body weight loss, or fatality observed in 0/10 animals.	Pass

Biological Safety Endpoint	Test Method	Acceptance Criteria	Test Results	Pass/ Fail
Subchronic Toxicity	Subchronic (14 Day) IV Toxicity in Rats (14 Dose Method)	The test fails if more than one rat dies or shows clinical signs of toxicity, if mean body weight loss is observed, or if hematology, clinical chemistry, or histopathology show a pattern of toxicity.	0/10 rats died or showed clinical signs of toxicity, mean body weight loss, or a pattern of toxicity in hematology, clinical chemistry, or histopathology.	Pass
Hemocompatibility	ASTM Hemolysis - Direct Contact Method	Test passes if Hemolytic Index equals 0-5% (> 5% = Fail).	Hemolytic Index is 0.0% - non-hemolytic.	Pass
	ASTM Hemolysis – Extract Method		Hemolytic Index is 0.0% - non-hemolytic.	Pass

Table 3.2.P.2.4-9. Cytotoxicity Test Results for Non-Drug Product Contacting Syringe Components

Test Method	Acceptance Criteria	Test Results	Pass/ Fail
ISO MEM Elution Assay with L929 Mouse Fibroblast Cells	If cell reactivity grade is 0 – 2, test article is considered non-cytotoxic. If cell reactivity grade is 3-4, test article is considered cytotoxic.	Safety Device: Cell reactivity grade is 0 at 24, 48, and 72 ± 4 hours - non-cytotoxic.	Pass
		(b) (4) Plunger Rod: Cell reactivity grade is 0 at 24, 48, and 72 ± 4 hours - non-cytotoxic.	Pass
		(b) (4) Plunger Rod: Cell reactivity grade is 0 at 24, 48, and 72 ± 4 hours - non-cytotoxic.	Pass

6.3.3. Sterility

The sponsor performed Container Closure Integrity testing to assess the ability of the PFS to remain sterile after manufacture (all patient-contacting materials supplied sterile and drug product is added to the syringe using aseptic techniques). To perform the microbial ingress testing, the syringe was filled with growth media and sealed prior to being submerged in “a concentrated solution of highly motile microorganisms under specified conditions.” The microorganisms used were the bacterium *Brevundimonas diminuta* (ATCC 19146) at a concentration of greater than 1×10^5 CFU/ml. The turbidity (microbial growth) of the syringes was assessed after an incubation period of at least seven days at 30° – 35° C. to conduct the dye ingress test, final, finished products were submerged in a vacuum chamber (500mbar) containing methylene blue dye in water for 30min. The test articles were then inspected for color change using UV-Vis spectroscopy. The results of these two studies are summarized in the following table.

Table 3.2.P.2.5-1. Sensitivity Assessment of CCI Methods Using Syringes with Laser Drilled Holes

CCI Methods		Sensitivity Assessment Results		
		Negative Control	Positive Control	Test Samples
			Defect Size: 5 Microns Effective Circular Diameter	
Microbial Ingress Test	Exposed to Vacuum Conditions	NT	Fail (n=3)	Pass (n=25) ^a
	Not Exposed to Vacuum Conditions	Pass (n=3)	Fail (n=3)	Pass (n=25) ^a
Dye Ingress Test (UV-Vis)	Exposed to Vacuum Conditions	NT	Fail 0.2797 AU 0.2546 AU 0.2462 AU	Pass (n=5) ^b
	Not Exposed to Vacuum Conditions	Pass (n=1)	NT	NT

a. Samples were media-filled syringes from media fill SM 03/2016.

b. Results are from intermediate precision samples tested during method validation.

NT, Not Tested

Reviewer Comments

It does not appear that the sponsor performed any of the design verification testing identified in the Design Verification Review table. The only mechanical performance tests that appear to have been conducted are the Extractable Volume and Break Loose/Extraction Force testing, which are proposed as lot release tests.

The sponsor reports conducting Biocompatibility testing in accordance with ISO 10993-1, which is appropriate. they stated that Implantation and Genotoxicity testing were not conducted due to the limited duration of each exposure. This practice is acceptable, as these tests are not recommended for a device with this type of patient contact. They did not provide any test reports; therefore, I am unable to determine if the extractions were performed correctly (correct dilution ratio, sufficient exposure time/temp, etc.).

The sponsor reports conducting Container Closure Integrity testing to demonstrate that the syringe can maintain a sterile barrier at shelf life. All test articles passed the testing; but there were no test reports provided nor was there a justification for the sample size used. Moreover, the sponsor did not indicate if the test articles had been subjected to simulated shipping/distribution prior to testing.

REVIEWER UPDATE POST-INFORMATION REQUEST:

The Sponsor provided complete design verification testing on all essential performance requirements of the device constituent (See Section 11).

*The Design Verification/Validation are **SUFFICIENT**.*

7. DISCIPLINE SPECIFIC SUB-CONSULTED REVIEW

No consults were obtained in the completion of this review.

8. RISK ANALYSIS

8.1. Risk Analysis Attributes

Risk Analysis Attributes	Yes	No	N/A
Risk analysis conducted on the combination product	✓		
Hazards adequately identified (e.g. FMEA, FTA, post-market data, etc.)	✓		
Mitigations are adequate to reduce risk to health	✓		
Version history demonstrates risk management throughout design / development activities	✓		

8.2. Summary of Risk Analysis

Reviewer Comments

The sponsor provided the results of a Risk Analysis in Sequence 0001, Section 3.2.P.2.3 for the combination product manufacturing process.

In response to an information request (See Section 11) the Sponsor provided a device based risk analysis for the combination product.

9. LABELING

The following information was taken from Draft Carton and Container Labels under Sequence 0001 in GSR, Section 1.14.1.1:

Syringe Label:



- **The Sponsor provided complete verification test reports for dose accuracy, breakloose / glide force, injection depth / needle length, cap removal force, and visual/audible feedback.**
 - **The complete test reports were included in the BLA application for reference and included all referenced information from the information request of the consultant reviewer.**
- **The Sponsor provided adequate biocompatibility testing to support the contact type / duration associated with the combination product.**
- **The Sponsor provided information to confirm adherence to the 2017 cGMP FDA Guidance document by detailing the streamlined approach in compliance with 21 CFR 820 (device QSRs).**
- **The information provided by the Sponsor adequately addresses all outstanding information requests by the consultant reviewer.**

12.RECOMMENDATION

CDRH/ODE recommends Approval of the device constituent parts of the combination product.

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

QUYEN B TRAN
06/12/2018

Center for Drug Evaluation and Research
Office of Pharmaceutical Quality
Office of Process and Facilities
Division of Microbiology Assessment
WO Building 22
10903 New Hampshire Ave.
Silver Spring, MD 20993

PRODUCT QUALITY MICROBIOLOGY REVIEW AND EVALUATION

Reviewer: Aimee L. Cunningham, Ph.D., M.P.H.
Acting Quality Assessment Lead: Reyes Candau-Chacon, Ph.D.

BLA: 761080/0
Applicant: Hospira, Inc.
US License Number: 1974
Submission Reviewed: Original BLA
Product: PF-06881893 (filgrastim/Nivestym™, a Neupogen biosimilar)
Indication: decrease the incidence of infection, as manifested by febrile neutropenia, in patients with non-myeloid malignancies receiving myelosuppressive anti-cancer drugs associated with a significant incidence of severe neutropenia with fever
Dosage Form: solution for injection (i.v. or s.c.)
Manufacturing Sites: Hospira, Inc. in McPherson, KS (FEI: 1925262) and Hospira Zagreb in Zagreb, Croatia (FEI: 3010630287)
FDA Receipt Date: 09/21/2017
Action Date: 07/21/2018

Conclusion and Approvability Recommendation

The drug product section of this BLA was reviewed from a sterility assurance and quality microbiology standpoint and is recommended for approval.

Product Quality Microbiology Assessment: Drug Product

Drug Product Quality Microbiology Information Reviewed

Sequence number	Date	Description
0001	09/21/2017	Original BLA
0021	03/19/2018	IR response
0023	03/30/2018	IR response
0025	05/17/2018	IR response
0027	05/22/2018	Sponsor's informal meeting minutes (T-con)
0028	05/30/2018	Update BLA from multiple IRs

Sequence number	Date	Description
0030	05/31/2018	IR response
0033	06/08/2018	IR response

The following DMFs were referenced in this BLA submission for sterilization validation information:

DMF #	Content	Date Reviewed	Finding	Document Name
(b) (4)	(b) (4)	04/05/2018	Adequate	(b) (4) M05R01.docx
		06/13/2017	Adequate	M45R01.doc
		08/02/2017	Adequate	M48R01.doc
		06/05/2018	Adequate	M55R01.docx
		05/08/2018	Adequate	M14R01.docx

Module 3.2

PRE-FILLED SYRINGE (PFS)

P.1 Description and Composition of the Drug Product

PF-06881893 is a sterile, colorless, aqueous solution containing no preservatives. It is intended for single use by subcutaneous injection. Drug product (DP) contains PF-06881893 drug substance (DS), and the following excipients: sorbitol, (b) (4) polysorbate 80, and water for injection (WFI). (b) (4)

(b) (4) There are two fill presentations (300 mcg/0.5 mL and 480 mcg/0.8 mL); both are supplied in a 1 mL syringe. The below table (adapted from the submission) describes the composition of PF-06881893.

Component	mg per 0.5 mL (300 mcg)	mg per 0.8 mL (480 mcg)	Function	Ref. to Standard
PF-06881893 DS	0.3	0.48	Active ingredient	In house specification
Sorbitol	25	40	(b) (4)	NF
Sodium	0.0175	0.028	(b) (4)	(b) (4)
Acetate	0.295	0.472	(b) (4)	(b) (4)
Polysorbate 80	0.02	0.032	(b) (4)	NF, in house
(b) (4)				
Water for Injection	qs to 0.5 mL	qs to 0.8 mL	(b) (4)	USP

SATISFACTORY

P.2 Pharmaceutical Development

(b) (4)

P.7 Container Closure System

The container closure system for the PFS presentation is a single-dose, ready-to-use syringe supplied in two dose strengths. Different color plunger rods and graduation labels are used to distinguish the two dosages ((b) (4) 300 mcg/0.5 mL and (b) (4) 480 mcg/0.8 mL). Individual components of the PFS are documented in Figure 3.2.P.7-2 and shown below.

Figure 3.2.P.7-2. PF-06881893 Prefilled Syringe Components



All components are provided ready-to-use by (b) (4) descriptions of each component, its material of construction, code, supplier, and reference are provided in Table 3.2.P.7-1. Specifications and COAs for all components are provided in the BLA. (b) (4)

SATISFACTORY

P.8 Stability

DP PFS stability samples will be stored horizontally at 2-8°C. Microbial control will be assessed at the following timepoints: sterility at release and 36 months, endotoxin at release and annually until 36 months, and CCIT annually until 36 months. At least one commercial scale lot of PFS will be placed onto stability assessment annually, alternating between the two dosages each year. Testing beyond the stability time point listed may be allowed to generate data in support of extension of shelf life (an extension of shelf life would be report in an AR). Any OOS results on stability samples will be reported to the Agency. The initial shelf life of PF-06881893 DP is 30 months at 2-8°C for the PFS presentation. All available stability data for long term (5°C), accelerated (25°C/60% RH) and in-use (25°C post long-term storage) conditions meet the specifications for sterility, endotoxin, and CCIT.

Reviewer's Note: An IR was sent on 05/10/2018 to request (b) (4)

(b) (4) In responses submitted 05/17/2018 (eCTD sequence 0025), the Sponsor acknowledged the Agency's recommendation (b) (4)

SATISFACTORY

SINGLE DOSE VIAL (SDV)

P.1 Description and Composition of the Drug Product

PF-06881893 is a sterile, colorless aqueous solution without preservatives intended for single use by either intravenous or subcutaneous injection. There are two dosages: 300 mcg (1 mL fill volume) or 480 mcg (1.6 mL fill volume); both presentations are supplied in a 2 mL vial. PF-06881893 DP contains DS and the following excipients: sorbitol, (b) (4), polysorbate 80, and WFI. (b) (4)

The following table has been adapted from the submission and describes the composition of PF-06881893.

Component	mg per 0.5 mL (300 mcg)	mg per 0.8 mL (480 mcg)	Function	Ref. to Standard
PF-06881893 DS	0.3	0.48	Active ingredient (b) (4)	In house specification
Sorbitol	50	80		NF (b) (4)
Sodium	0.035	0.056		
Acetate	0.59	0.94		
Polysorbate 80	0.04	0.064		
Water for Injection	qs to 1 mL	qs to 1.6 mL	(b) (4)	USP

SATISFACTORY

P.2 Pharmaceutical Development

(b) (4)

P.7 Container Closure System

The container closure system for the SDV is comprised of a 2 mL (b) (4) glass vial, (b) (4) stopper, and aluminum seal with flip-off cap. Vials and stoppers are identical for both dosages. The seals are identically dimensionally and functionally and do not have product contact. They differ in color to identify the dosage ((b) (4) 300 mcg, (b) (4) 480 mcg). Dimensions and specifications are provided for vials, stoppers, and seals. LOAs are provided for vials and stoppers (DMFs (b) (4) and (b) (4) respectively). Secondary packaging is a carton box with a package insert. A total of 10 vials are packaged per carton regardless of dosage.

SATISFACTORY

P.8 Stability

DP SDV will be stored inverted at 2-8°C. Microbial control will be assessed at the following timepoints: sterility at release and 36 months, endotoxin at release and annually until 36 months, and CCIT annually until 36 months. At least one commercial scale lot of SDV will be placed onto stability assessment annually, alternating between the two dosages each year. Testing beyond the stability time point listed may be allowed to generate data in support of extension of shelf life (an extension of shelf life would be report in an AR). Any OOS results on stability samples will be reported to the Agency. The initial shelf life of PF-06881893 DP is (b) (4) at 2-8°C for the SDV presentation based on currently available stability data. Shelf life may be extended if stability data continue to meet specifications. All available stability data for long term (5°C) and accelerated (25°C/60% RH) storage conditions meet the specifications for sterility, endotoxin, and CCIT.

SATISFACTORY

CGMP Status

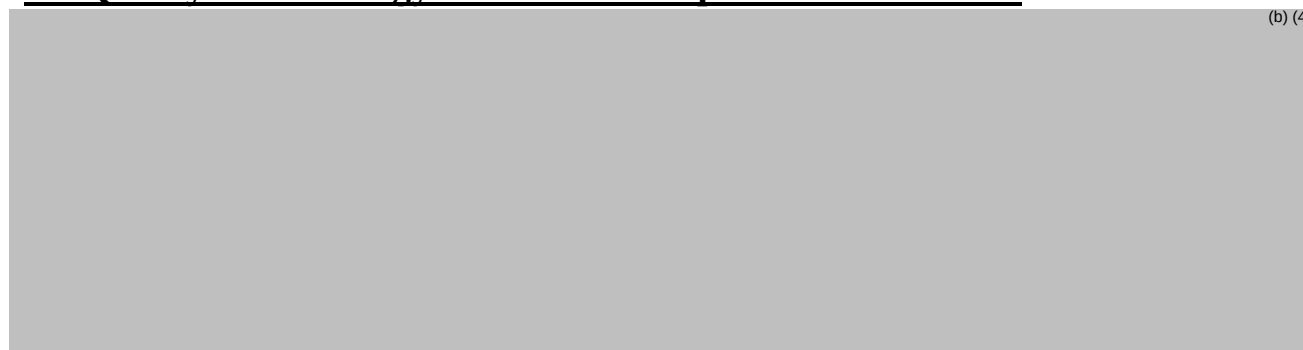
The assessment of manufacturing facilities is documented in Panorama.

Conclusion

- I. The BLA was reviewed from a sterility assurance and quality microbiology perspective and is recommended for approval.
- II. Product quality aspects other than microbiology should be reviewed by OBP and CDRH.
- III. No inspection follow-up items were identified.

DP Quality Microbiology Information Requests Sent and Date

(b) (4)





Aimee
Cunningham

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Reyes
Candau-Chacon

Digitally signed by Reyes Candau-Chacon

Date: 6/11/2018 12:16:39PM

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DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service
Food and Drug Administration
Center for Devices and Radiological Health
Office of Compliance, Division of Manufacturing & Quality
Respiratory, ENT, General Hospital, and Ophthalmic Device Branch

Date: May 21, 2018

To: Office of Combination Products at combination@fda.gov

Regulatory Business Program Manager (RBPM)/Regulatory Program
Manager (RPM): Name, Office, E-mail: Kelly Ballard, CDER, OPQ, OPRO

Kelly.ballard@fda.hhs.gov

Office of Combination Products at combination@fda.gov

CDER/OPQ/OPF: Juandria.Williams@fda.hhs.gov

Through: Nazia Rahman, Lead, DMQ, OC, CDRH, WO-66, Rm 3458, Email:

Nazia.Rahman@fda.hhs.gov

Nazia Rahman -S
2018.06.08 15:18:37 -04'00'

From: Crystal Lewis, REGO Branch, DMQ, OC, CDRH

Applicant: Hospira, Inc.
275 North Field Drive
Lake Forest, IL 60045

FEI# 3004621470

Application # BLA 761080

Consult # ICC# 1800076

Product Name: PF-06881893

Pre-Approval Inspection: No

Documentation Review: No Additional Information Required

Final Recommendation: APPROVABLE

The Office of Compliance at CDRH received a consult request from CDER to evaluate the applicant's compliance with applicable Quality System Requirements for the approvability of BLA 761080.

PRODUCT DESCRIPTION

The Hospira Zagreb Filgrastim PF-06881893 drug product (DP) is proposed as a biosimilar to the US licensed Neupogen reference product. PF-06881893 is intended for the treatment of the following:

- Febrile neutropenia in patients with nonmyeloid malignancies who are receiving myelosuppressive anti-cancer drugs
- Reduced neutrophil recovery time and reduced duration of fever after chemotherapy for the treatment of patients with acute myeloid leukemia
- Reduced neutropenia related consequences in patients with nonmyeloid malignancies undergoing myeloablative chemotherapy followed by bone marrow transplantation
- Assembly of autologous hematopoietic progenitor cells into peripheral blood for leukapheresis collection
- Reduced incidence and duration of severe neutropenia in symptomatic patients with congenital neutropenia, cyclic neutropenia or idiopathic neutropenia

(b) (4)

PF-06881893 DP is provided in a 300mcg/0.5mL (600mcg/mL) and a 480 mcg/0.8mL (600mcg/mL) dosage strengths in a prefilled syringe (PFS) presentation for subcutaneous injection (SC). The DP is also available in a 300mcg/1.0mL and a 480mcg/1.6mL (300 mcg/mL) dose strength in a single dose vial (SDV) presentation for SC injection and intravenous (IV) infusion.

Figure 1. Ready to Use PF-06881893 Prefilled Syringe Configurations

(b) (4)



REGULATORY HISTORY

The following facilities were identified as being subject to applicable Quality System Requirements under 21 CFR part 820 and 21 CFR Part 4.4:

1. Hospira Inc.
275 North Field Drive
Lake Forest, IL 60045
FEI # 3004621470

Responsibility – This manufacturer is the applicant. Also, this is the correspondence address for the firm. No inspectional history was available for the applicant.

Inspection Recommendation:

An inspection is not required because:

- No manufacturing activity for the combination product occurs at this site.
2. Hospira Zagreb d.o.o
Prudnicka cesta 60
Prigorje Brdovecko, Croatia (HRV) 10291
FEI # 3010630287

Responsibility – This facility is responsible for the preparation and testing of cell banks, manufacture of PF-06881893 Drug Substance (DS) and Drug Product (DP) prefilled syringes (PFS), in-process control testing DS and DP PFS, release testing of DS and DP PFS. Also, this facility is responsible for stability storage and testing of DP PFS, PFS assembly, packaging and

labeling of DP PFS, release of final combination product, storage of final combination product and receipt/inspection of PFS components.

Inspectional History – An analysis of the firm’s inspection history over the past 2 years showed that an inspection was conducted 02/12/2018 to 02/20/2018. The inspection covered drugs and medical devices and was classified VAI.

Inspection Recommendation:

An inspection is not required because:

- A recent medical device inspection of the firm was acceptable.

DOCUMENTATION REVIEW

The application was searched for documents pertaining to applicable 21 CFR part 820 regulations for this combination product.

Management Control, 21 CFR 820.20

(b) (4)



The documentation review of the application for compliance with the Quality System Requirements showed no deficiencies.

Crystal Lewis
-S

Crystal Lewis

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Prepared: CLewis: 05/21/18

Reviewed: FMLast name: Month/Day/Year

CTS No.: ICC800076

BLA 761080

Memorandum of Review:

Submission Tracking Number (STN):	BLA 761080
Subject:	Immunogenicity Review
Stamp Date:	11/03/2017
Review/Revision Date:	05/01/2018
Primary Reviewer:	Scott Lute
Secondary Reviewer:	Harold Dickensheets
RBPM:	Kelly Ballard
Consults:	N/A
Applicant:	Hospira
Product:	PF06881893-a proposed biosimilar to filgrastim
Indication:	Same as filgrastim
GRMP Date:	6/12/2018
BsUFA Date	7/21/2018

Recommendation: This BLA is recommended for approval from the anti-drug antibody (ADA) assays perspective.

Executive Summary:

The assays submitted to support the immunogenicity portion of the PF-06881893 biosimilar were adequately validated. The sponsor performed three clinical studies: ZIN-FIL-1502, ZIN-FIL-1501, and C1121012. Each study used a screening and confirmatory assay for anti-drug antibodies (ADA) to PF-06881893 or US-licensed Neupogen. The sponsor then used a cell-based assay receptor signaling assay for detection of neutralizing antibodies (NAb). Finally, the sponsor measured the isotypes of ADAs using a SPR assay. The validation reports for all three assays were reviewed and found to be acceptable. The screening and confirmatory assay had adequate sensitivity and drug tolerance; however, we recommend the sponsor use the theoretical 1% false positive rate (FPR) for the confirmatory assay instead of the proposed 0.1% FPR. Analysis of sponsor data using confirmatory cut points derived from the two FPR demonstrated minimal differences in ADA incidence rates between subjects treated with PF-06881893 or US-licensed Neupogen, when compared at either the 1% or 0.1% FPR. The NAb assay is adequate to make determinations of neutralizing activity and the SPR assay is able to detect ADA isotypes in positive samples. The immunogenicity results obtained from analysis of data from the clinical trials (7.4% Hospira vs. 4.9% Neupogen using CCP defined at the 0.1% FPR, and 9.1% Hospira vs. 8.9% Neupogen at the 1% FPR) indicate no significant difference in ADA incidence or the presence of neutralizing ADA between the subjects treated with PF-06881893 and those treated with US-licensed Neupogen. The lack of differences in potential immunogenicity-related clinical signals between subjects treated with the PF-06881893 or US-licensed Neupogen filgrastim further supports their similarity.

Review-Immunogenicity

Introduction

The PF-06881893 development program included 3 comparative clinical studies in healthy subjects to demonstrate biosimilarity to US-licensed Neupogen.

- ZIN-FIL-1502: a single-dose crossover PK/PD equivalence study
- ZIN-FIL-1501: a multiple-dose crossover design PK/PD equivalence study
- C1121012: a multiple-dose, non-inferiority, parallel-design, comparative immunogenicity and safety study

The sponsor used a typical 3-tiered approach to assessing immunogenicity of PF-06881893 and US-Neupogen in each of these trials. An electrochemiluminescence (ECL) assay was developed and used to test samples for the presence of anti-drug antibodies (ADA) against filgrastim. Samples testing positive in the screening (Tier 1) assay were further tested in a confirmatory ECL assay (Tier 2). Confirmed ADA positive samples were then characterized in Tier 3 testing, which included a cell-based neutralizing antibody (NAb), SPR-based isotype determination, and ECL antibody titer assay (same format as the screening assay).

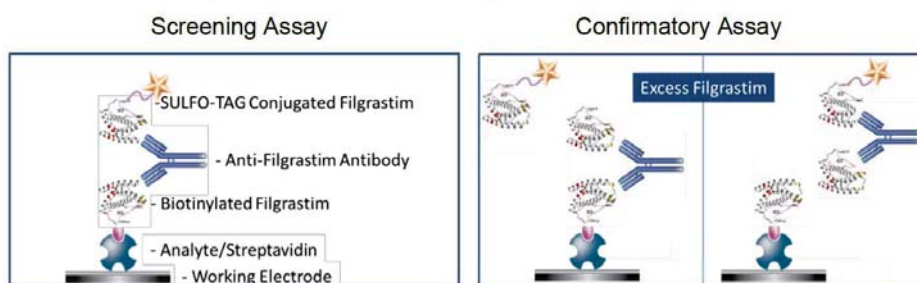
In support of the ADA and NAb assays, the sponsor submitted validation reports and follow-up partial validation reports (to align with the 2016 FDA Guidance on immunogenicity) assessing the quality of the immunogenicity assays. A review of the assay validation reports is below.

5.3.1.4 Reports of Bioanalytical and Analytical Methods for Human Studies

Screening and Confirmatory ADA Assay Overview

The analysis of ADA against filgrastim in human serum used a homogenous ECL-based Meso Scale discovery (MSD) bridging assay (Figure 1). ADAs were detected in the

Figure 1. Schematic Representation of the Homogeneous ECL-based Bridging Assay for Detection of Anti-filgrastim Antibodies



presence of soluble Hospira filgrastim labeled with biotin or ruthenium/sulfo-tagged for capture and detection, respectively. Unlabeled Hospira filgrastim was used as the competitive ligand in the confirmatory assay. A polyclonal goat anti-human G-CSF antibody (b) (4) spiked into human serum served as a positive control and

normal healthy human serum pool (from 10 individuals) screened for ADAs was used as a negative control. The following are considered critical reagents:

- Filgrastim drug substance (2.5mg/mL)
- Biotinylated filgrastim
- Sulfo-tagged/Ruthenium labeled filgrastim
- Human G-CSF antibody (b) (4)
- Negative control: serum pool from 10 individual normal healthy human sera (purchased from (b) (4))

The positive controls for the ADA screening assay are as follows:

High positive control (HPC): 70,000 ng/mL human G-CSF antibody

Low positive control (LPC2): 8 ng/mL human G-CSF antibody

Monitoring LPCs: LPC1 12 ng/mL, LPC3 6 ng/mL

The in-study acceptance criteria for the controls were:

- The mean results of the 3 NC duplicates must be negative
- The validated LPC2 must be positive
- Normalized ($[RLU \text{ of PC}] / [RLU \text{ of NC}]$) must be within the following limits

Table 3. Summarized In-study Acceptance Criteria for Controls in ADA Analysis

Limit Type	NC (Mean RLU)	Normalized HPC	Normalized LPC2
Upper Limit	117	9,323.3	2.88
Lower Limit	64	2,280.9	1.47

The development and validation studies for the ADA assay demonstrated the ability to detect ADA for Hospira filgrastim and US Neupogen, determined the minimal required dilution, stability, and robustness of the assay and its components (see table below).

Assay Parameter	(b) (4) 2015-B-06-05	(b) (4) 16-074-020-REP
	Validation	Supplemental Validation
	Used for Clinical Study: ZIN-FIL-1501 and ZIN-FIL-1502	Used for Clinical Study: C1121012
Assay Ability to Detect ADA to: -PF-06881893 -Neupogen -Lenograstim	PF-06881893 Yes Neupogen Yes Lenograstim Yes	PF-06881893 Yes Neupogen Yes Lenograstim Not Retested
Minimum Required Dilution (MRD)	2 (50% serum)	Not Retested
Freeze/Thaw Stability (4 cycles)	Acceptable	Not Retested
Short-term Stability at +4°C, nominal (7 days)	Acceptable	Not Retested
Short-term Stability at Room Temperature (24 h)	Acceptable	Not Retested
Robustness ^a -Solution Phase Incubation Time -Solid Phase Incubation Time	4 hours to 2 nights at +4°C, nominal 45 to 75 minutes	Not Retested

The MRD was determined by measuring different concentration levels of anti-human G-CSF antibodies spike in 100%, 50%, and 10% matrix (normal human serum pool). The dilution with the highest signal-to-noise ratio and lowest background signal was determined to be the MRD for the ADA assay. See table 5 below for the signal-to-noise ratio analysis.

Table 5: Signal-to-noise ratio for the selection of the MRD.

	ADA spiked into NHS pool [ng/mL]						
	+ 70,000	+ 12	+ 24	+ 0	+ 70,000	+ 12	+ 24
	Mean Signal (RLU)				Signal-to-noise ratio		
100%	440,987	246	500	130	3,392	2	4
50%	531,774	171	321	107	4,970	2	3
10%	244,405	137	148	94	2,600	1	2

Reviewer's Comment: The MRD analysis tested dilutions of 0, 1:2, and 1:10 and the MRD of 1:2 (50% dilution) provided the best signal to noise ratio. Additionally, the impact of hemolysis was tested and shown to have no significant impact on assay capability (data not included in this review). The stability and robustness studies are suitable. The assay demonstrated the ability to detect ADA for both Hospira filgrastim and US-Neupogen. These parameters are acceptable.

ADA Assay Validation

The ADA assay was originally validated in 2015; however, the FDA guidance on immunogenicity assays was updated in 2016 and the ADA assay was re-validated according to the new guidelines. The updated validation included re-analysis of the cut points, sensitivity, drug tolerance, matrix interference, assay precision, and assay controls. A summary of the original and updated validation results is found in Table 4 below.

Table 4. Summary of ECL Assay Validation Results

Assay Parameter	(b) (4) 2015-B-06-05	(b) (4) -16-074-020-REP
	Validation Used for Clinical Study: ZIN-FIL-1501 and ZIN-FIL-1502	Supplemental Validation Used for Clinical Study: C1121012
Screening Cut Point Type	Floating	In-study Floating
Screening Correction Factor (SCF)	1.625	1.054 (in-study)
Confirmatory Cut Point (99.9%)	24.3%	16.7% (in-study)
Confirmatory Cut Point (99.0%)	18.1%	12.8% (in-study)
Titer Cut Point Type	Floating	In-study Floating
Titer Correction Factor (TCF)	1.625	1.085 (in-study)
Sensitivity (Screening Assay)	6.8 ng/mL	2.2 ng/mL (0.53 – 3.91 ng/mL)
Sensitivity (Confirmatory Assay)	Not Tested	PF-06881893: 4.04 – 8.11 ng/mL Neupogen: 3.85 ng/mL
Confirmatory Competing Drug Concentration	60,000 ng/mL	Not Retested
HPC Concentration in NHS pool (Assay Acceptance)	70,000 ng/mL	70,000 ng/mL
LPC1 Concentration in NHS pool (Assay Monitoring)	Not Tested	12 ng/mL
LPC2 Concentration in NHS pool (Assay Acceptance)	8 ng/mL	8 ng/mL
LPC3 Concentration in NHS pool (Assay Monitoring)	Not Tested	6 ng/mL
Drug Tolerance Screening Assay (6 ng/mL to 250 ng/mL Antibody)	70,000 ng/mL Antibody PF-06881893: >60 µg/mL Neupogen: >60 µg/mL 250 ng/mL Antibody: PF-06881893: 5 µg/mL Neupogen: 2.5 µg/mL	PF-06881893 and Neupogen 70,000 ng/mL Not retested 250 ng/mL: ~ 200 µg/mL 12 ng/mL: > 5 µg/mL 8 ng/mL: > 0.5 µg/mL 6 ng/mL: > 0.5 µg/mL
Drug Tolerance Confirmatory Assay (250 ng/mL Antibody)	Not Tested	PF-06881893: 50 µg/mL Neupogen: 12.5 µg/mL
Drug Tolerance Confirmatory Assay (12 ng/mL Antibody)		PF-06881893: 5 µg/mL Neupogen: 0.6 µg/mL
Drug Tolerance Confirmatory Assay (8 ng/mL Antibody)		PF-06881893: >0.5 µg/mL Neupogen: >0.5 µg/mL
Drug Tolerance Confirmatory Assay (6 ng/mL Antibody)		PF-06881893: >0.5 µg/mL Neupogen: >0.5 µg/mL
Matrix Interference – 30 Individual Screening Assay	Not Tested	12 ng/mL 30 of 30 Positive 8 ng/mL 30 of 30 Positive 6 ng/mL 30 of 30 Positive
Matrix Interference – 30 Individual Confirmatory Assay	Not Tested	12 ng/mL 29 of 29 Positive ^b 8 ng/mL 23 of 29 Positive ^{b,c} 6 ng/mL 23 of 29 Positive ^b
Intra-assay Precision	70,000 ng/mL 4.2% CV	Not Retested
	8 ng/mL 5.7% CV	Not Retested
Inter-assay Precision Screening Assay (normalized)	HPC (70,000 ng/mL) 6.1% CV	HPC (70,000 ng/mL) 19.0% CV
	LPC 1 (8 ng/mL) 6.6% CV	LPC 2 (8 ng/mL) 14.0% CV
Inter-assay Precision-Confirmatory Assay (Sensitivity Runs, N=12)	HPC (70,000 ng/mL) 6.1% CV	HPC (70,000 ng/mL) 0.7% CV
	LPC 1 (8 ng/mL) 6.6% CV	LPC 2 (8 ng/mL) 17.3% CV
Inter-assay Precision-Confirmatory Assay (PF-06881893, N=6)	Not Applicable	HPC (70,000 ng/mL) 0.8% CV
	Not Applicable	LPC 2 (8 ng/mL) 10.2% CV
Inter-assay Precision-Confirmatory Assay (Neupogen, N=6)	Not Applicable	HPC (70,000 ng/mL) 1.0% CV
	Not Applicable	LPC 2 (8 ng/mL) 16.4% CV
High Dose Hook Effect	100 µg/mL (samples positive up to 400 µg/mL)	Not Retested

Reviewer's Comment: *The validation summary table indicates that the sponsor has addressed concerns relating to the new 2016 FDA guidance. More detailed review of select assay parameters is below.*

Cut-Point Determination (Table 4 above)

Screening and titering cut-point determination

- Original validation

The assay cut point determination was originally run (2015 report) by testing 51 normal samples in 2 runs by 2 analysts (classic approach). The data was not normally distributed even after log transformation. After outlier exclusion, normality was still not achieved. The sponsor indicated that the results of the normal blood donors were in the range of the instrument background noise and the classic approach to screening cut point determination was inappropriate.

The sponsor decided to use the “spike approach” where a low level (8 ng/mL ADA) was spiked into the normal samples to achieve a signal above the instrument noise. The spiked sample:NC ratio was then used to determine the screening correction factor (CF). For the spike approach, the 5% false positive rate, CF was calculated to be 1.625. Since this calculation was based off a known sample, the titer CF was also set at 1.625.

The original validation cut point (CF of 1.625) was used for clinical studies ZIN-FIL-1501 (N=350) and ZIN-FIL-1502 (N=141); however, the number of screened positive samples was lower than expected. As a result, pre-dose samples (60 and 24, respectively) from each trial were used to determine an in-study cut point.

- ZIN-FIL-1501: in-study FCP based on 5% false positive, CF=1.167
- ZIN-FIL-1502: fixed cut point of 87 RLU

- Re-validation

For re-validation, in compliance with the 2016 FDA guidance, the sponsor used 51 randomly selected pre-dose serum samples from study C1121012 (234 subjects, N=1493), in six independent assay runs performed by two analysts over at least a three-day period to determine an in-study cut point and correction factor. It was determined that the 5% false positive (95% CI) for the pre-dose samples had a CF of 1.054 [CF=Mean norm. pre-dose signals w/o outliers + (1.645 x SD)], the titer assay CF was based on 1% false positive (99% CI), and had a CF of 1.085.

For both assay validations, it was determined, based on ANOVA, that a floating cut point (FCP) is appropriate.

$$\text{FCP} = \text{CF} \times \text{NC}_{\text{plate}}$$

$$\text{FCP} = \text{CF} \times \text{NC}_{\text{IS}} \text{ (in-study)}$$

- Clinical Report Analysis

For all clinical study reports, the original validation cut point (CF=1.625) was considered not appropriate, as it led to too few screened positive samples. In-study cut points or

correction factors were determined to allow for a higher incidence of screened positive samples. Below is a table comparing the cut points from the 3 clinical studies.

Study Report	In-Study CF or CP	Sample (N)	Screened Positive
ZIN-FIL-1501	CF= 1.167	350	1.7%
ZIN-FIL-1502	87 RLU	141	13.5%
C1121012	CF= 1.054	1493	15.7%

The titering assay results based on the in-study cut point resulted in low titer ADAs with a high of 16. A table below summarizes the titering results from the 3 clinical trials.

Study Report	In-Study Cut point	Sample (N)	Titer range
ZIN-FIL-1501	111 RLU	1	4
ZIN-FIL-1502	130-132 RLU	15	<2 - 16
C1121012	78-82 RLU	68	<2 - 16

Reviewer's Comment: The FDA agreed that the spike approach was an acceptable alternative to the classic approach. The difference in calculation between the classic approach and the spike approach is that the classic approach uses the 95th percentile of an unknown sample, while the spike approach uses the 5th percentile of a known sample. For the follow up validation, the CF is significantly lower (1.625 original vs 1.054 in-study), this makes the 8 ng/mL LPC further away from the in-study cut point.

- CF 1.625 RLU comparisons (NC ~145 RLU, LPC ~190 RLU)
- In-study CF 1.054 RLU comparisons (NC~91 RLU, LPC ~190 RLU)

Confirmatory cut-points

- Original validation

The confirmatory cut-point (CCP) determination tested the same 51 normal samples in the presence of 60,000 ng/mL of unlabeled Hospira filgrastim as determined during development. The % inhibition of the signal was used to determine true positive serum samples.

$$\% \text{ signal inhibition} = 100 \times [1 - (\text{study spiked sample} / \text{unspiked sample})]$$

For the CCP, the %inhibition was $3.09 \times \text{SD} + \text{mean} (\% \text{inhibition})$, the 99.9th percentile of $2.33 \times \text{SD} + \text{mean} (\% \text{inhibition})$, the 99th percentile (24.3% and 18.1%, respectively).

$$\text{CCP} = 18.1\% \text{ or } (1\% \text{ false positive})$$

The original validation confirmatory cut point (18.1% inhibition) was used for clinical studies ZIN-FIL-1501 (N=350) and ZIN-FIL-1502 (N=141). The results showed that 1 sample from ZIN-FIL-1501 was confirmed positive and 15 samples from ZIN-FIL-1502

were confirmed positive. If the 0.1% false positive cut point were used, the confirmed positive would be 0 and 8 samples, respectively.

- **Re-validation**

The CCP for the re-validation was performed according to the 2016 FDA guidance by using the same 51 pre-dose serum samples used in the screening study for clinical study C1121012, assayed in the absence or presence of added Hospira filgrastim at the same concentration as used previously for pre-study validation of CCP. Samples were tested in three runs by two analysts over a three-day period. Confirmatory cut point was calculated using a parametric approach after removal of outliers. The CCP testing also included evaluation of the incidence of false positives. The corresponding CCPs were, 12.8% based on the 99th percentile and 16.7% based on the 99.9th percentile. After evaluation of the false positive rates, it was determined that the 99.9th percentile CCP gave a rate closer to the true 1% false positive rate. The 99th percentile was 4-8 times higher.

CCP = 16.7% inhibition or (0.1% false positive)

- **Clinical Report Analysis**

The confirmatory cut point from the original validation report and the updated validation report have similar values (18.1% vs. 16.7%, respectively). The sponsor claims that the difference would have no impact on the clinical report results. When the updated CCP of 16.7% is used to analyze clinical study reports ZIN-FIL-1501 and ZIN-FIL-1502, the number of confirmed positives samples changes from 1 to 2 (Report 1501) and 15 to 16 (Report 1502). The sponsor states that these minimal differences support the use of 16.7% inhibition as the updated CCP.

Using the 16.7% inhibition cut point, clinical study C1121012 had 48 out of 1493 samples confirmed positive (3.2% rate). If the lower cut point of 12.8% inhibition were used, the positive number increases to 68 and the rate increases to 4.6%.

Reviewer's Comment: *With the re-validation studies, the sponsor uses the 0.1% false positive CCP. While the FDA recommends the use of a 1% false positive rate for confirmatory cut-points, the sponsor has demonstrated in-study 1% false positive CCP led to a 4-8% rate of false positives, while the 0.1% false positive rate led to a 1.7-4% rate of false positives. Additionally, the difference between the original CCP and the updated CCP is minimal and clinical report analysis demonstrated that the lower % inhibition only increased the confirmed positive rate by 1 sample in earlier clinical reports. However, this difference in CCP usage impacts evaluation of the C1121012 study more significantly, causing an additional ~20 samples to be reflexed to NAb testing. A CCP of 16.7% (0.1% false positive) is acceptable; however, out of an abundance of caution the Agency recommends the use of a 1% false-positive-based CCP.*

Screening and Confirmatory Sensitivity (Table 4 above)

The sensitivity is the lowest concentration of the polyclonal anti-human G-CSF antibody preparation that consistently produces a positive result ($\geq$ in-study FCP). Thus, the measure of sensitivity is linked to the CF for the screening assay. The original validation measured 2-fold serial dilutions (5 concentrations total) of a 12 ng/mL ADA control. The re-validation started with an 8 ng/mL concentration and aligned with the FDA 2016 guidance. For the re-validation, there were two sets of sensitivity runs with set 1 having the higher value.

- Set 1: Hospira filgrastim only in confirmatory assay
- Set 2: Hospira filgrastim and US-Neupogen for parallel, comparative testing in confirmatory assay

For the confirmatory assay, both in-study cut points of 12.8% inhibition (99th percentile) and 16.7% inhibition (99.9th percentile) were analyzed; however only the 16.7% inhibition values were used as it was the in-study FCP. The confirmatory sensitivity values for both the Hospira filgrastim and US-Neupogen are similar (4.04 vs. 3.85), the higher value of 8.11 ng/mL for Hospira filgrastim is from Set 1.

Reviewer's Comment: *The screening sensitivity was improved from 6.8 ng/mL to 2.2 ng/mL for the re-validation studies and demonstrated comparable sensitivity for Hospira filgrastim and US-Neupogen. Despite the improved sensitivity and lower FCP, the LPC remains as 8 ng/mL. Demonstration that the monitoring LPC of 6 ng/mL shows high variability and is not consistently detected indicates that the 8 ng/mL LPC appears to be the best option.*

Drug Tolerance (Table 4 above)

Both the original validation and the re-validation studies demonstrate similar drug tolerance levels for Hospira filgrastim and US-Neupogen. For the screening assay, drug tolerance levels for both drugs were $\sim 200,000$ ng/mL for the ADA reference antibody at 250 ng/mL and >500 ng/mL drug tolerance at the LPC antibody level of 8 ng/mL.

For the confirmation assay, differences were observed between the drug tolerance levels of Hospira filgrastim and US-Neupogen at the 250 ng/mL and 12 ng/mL antibody levels (one dilution factor). For the low-level antibody (8 ng/mL and 6 ng/mL), the drug tolerance levels could not be reliably determined but the drug tolerance was > 500 ng/mL. The sponsor claims the differences in drug tolerance (250 ng/mL: 50 μ g/mL Hospira, 12.5 μ g/mL Neupogen; 12 ng/mL: 5 μ g/mL Hospira, 0.6 μ g/mL Neupogen) could be due to assay variability or the (b) (4)

Despite the differences and the lack of reliability for the low-level antibody samples, the observed drug tolerances are well above the expected $C_{\max}$ levels for single and multiple doses.

Single dose $C_{\max} \sim 16$ ng/mL

Multiple dose C_{max} ~30 ng/mL

Filgrastim has a half-life of 2.8 to 4 hours depending on dosing conditions and the sampling schedule is no less than 10 days from dosing for clinical study samples.

Reviewer's Comment: *An IR was sent to the sponsor on 12/13/2017 in reference to the reported differences in drug tolerance being due to the excipient levels, and asking for more supportive information. The sponsor responded on 01/02/2018, and acknowledged that they have no supportive evidence directly linking the differences to the excipient levels, then pointed out that the minimal drug tolerance levels reported (500 ng/mL) are 15-30-fold higher than the C_{max} observed in clinical studies. The drug tolerance levels are acceptable.*

High Dose Hook Effect and Matrix interference (Table 4 above)

Prior to setting the HPC, the sponsor demonstrated that there was a high dose hook effect with the polyclonal anti-human G-CSF antibody. Maximal signal was observed at 100,000 ng/mL, whereas signal was reduced at higher concentrations. Therefore, the HPC was set to 70,000 ng/mL.

The sponsor tested for matrix interference by spiking 30 normal human sera matrices with the same concentration levels as the LPCs. All NHS spiked samples produced an assay signal above the in-study FCP. A second assessment used 30 randomly selected pre-dose samples from Clinical Study C1121012. The pre-dose samples were also spiked at the three LPC levels. The sponsor claims that the results demonstrate that there is an absence of matrix interference for the ADA assay.

Reviewer's Comment: *The sponsor states that despite the confirmatory assay only reporting 23 of 29 samples as positive, it consistently detected 8 ng/mL. They state that the 6 samples that failed to reach the in-study FCP were all from one plate that was positive at the 6 ng/mL level. From a risk standpoint, if the same samples were positive at a lower concentration level, the risk of interference at the 8 ng/mL is reduced. The demonstration of a high dose hook effect and absence of a matrix effect is acceptable.*

Screening and Confirmatory Assay Precision

The original assay validation demonstrated high intra- and inter-assay precision with %CV values <10% for the screening and confirmatory assays, meeting the acceptance criterion of ≤20%. For the re-validation study, the intra-assay precision was not performed. For inter-assay precision, twelve valid sensitivity runs (Set 1 and Set2, run 3 months apart), were used (See Table 10 below)

Table 10. Summary of Inter-Assay Precision

Assay Parameter	Statistical Parameter	HPC	HPC/ NC	12LPC	12LPC/ NC	8LPC	8LPC/ NC	6LPC	6LPC/ NC	NC
RLU	Mean RLU	231109.7	2758.8	191.6	2.3	165.3	2.0	135.8	1.6	83.2
	Standard Deviation	54652.6	525.0	27.9	0.3	27.4	0.3	14.8	0.2	6.3
	%CV	23.6	19.0	14.6	14.9	16.6	14.0	10.9	9.4	7.6
%Inhibition Filgrastim Hospira Set 1 &2, N=12	Mean %Inhibition	97.7	N/A	54.0	N/A	43.2	N/A	31.5	N/A	-3.3
	Standard Deviation	0.7	N/A	14.0	N/A	7.5	N/A	6.6	N/A	6.2
	%CV	0.7	N/A	25.8	N/A	17.3	N/A	20.8	N/A	-191.1
%Inhibition Neupogen Set 2, N=6	Mean %Inhibition	97.7	N/A	47.0	N/A	46.4	N/A	32.8	N/A	-8.6
	Standard Deviation	1.0	N/A	8.3	N/A	7.6	N/A	7.0	N/A	5.1
	%CV	1.0	N/A	17.7	N/A	16.4	N/A	21.2	N/A	-59.9
%Inhibition Filgrastim Hospira Set 2, N=6	Mean %Inhibition	97.5	N/A	46.9	N/A	47.3	N/A	32.3	N/A	-5.8
	Standard Deviation	0.8	N/A	7.7	N/A	4.8	N/A	6.1	N/A	6.3
	%CV	0.8	N/A	16.4	N/A	10.2	N/A	18.7	N/A	-108.7

The re-validation ADA assay with the lower in-study FCP has an increase in the %CV for both the HPC and LPC. The screening assay precision met the acceptance criterion for the positive controls. For the confirmatory assay, the HPC and LPC2 met the ≤20% acceptance criterion for both the combined sets and for Set 2 alone. However, the monitoring LPCs (LPC1 and LPC3) failed precision for the combined sets (25.8% and 20.8% respectively). When the data sets are separated into Set 1 and Set 2, the inter-assay precision for LPC1 meets the acceptance criterion, while LPC3 fails for Hospira filgrastim and passes for US. Neupogen. According to the SOP, LPC1 and LPC3 are only used for monitoring the assay and to ensure that validated LPC2 (8 ng/mL) is still appropriate after the in-study FCP is determined.

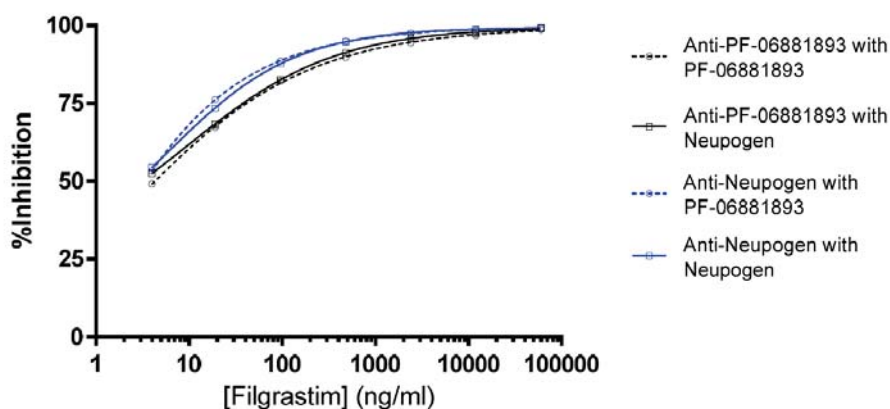
Reviewer's Comment: *While there was an increase in the %CV for the re-validation ADA assay, both the screening and confirmatory assays met the acceptance criterion. Having the monitoring LPCs fail the precision check should have no impact on the assay validation as long as no decision is made using these LPCs. The assay SOP states that the HPC and LPC2 values, not the monitoring LPCs (LPC1 and LPC3), are used for assay acceptance. The assay precision is considered valid.*

Comparative Analysis

The antigen-antibody binding was evaluated to demonstrate the ability of the ADA assay to detect both Hospira filgrastim and US-Neupogen. Rabbits were immunized with both

US-Neupogen and Hospira Filgrastim. The results showed minimal differences in antibody binding between the drug products in a range of 4 ng/mL to 70,000 ng/mL.

Figure 3. Comparative Inhibition of Anti-PF-06881893 and Anti-Neupogen Antibody Signals by PF-06881893 and Neupogen



The ability of the ADA assay to detect antibodies to both Hospira filgrastim and US-Neupogen is critical to demonstrate biosimilarity. The updated validated assay was used to analyze the non-inferiority, parallel-design, comparative immunogenicity study, C1121012. The results of the immunogenicity assay are below.

Table 1. Proportion and 90% CI of Subjects with Negative Baseline ADA and Confirmed Post-dose Positive ADA and NAb at any Visit in Study C1121012

	PF-06881893 5 mcg/kg (N=128)		US-licensed Neupogen 5 mcg/kg (N=127)		Risk Difference (%)	90% CI (Lower-Upper in %)
	N*	n (%)	N*	n (%)		
Proportion with positive ADA	121	9 (7.4)	123	6 (4.9)	2.560	(-2.717, 8.096)
Proportion with positive NAb	121	0	123	0	0	-

Source: Module 5.3.5.4 C1121012 Study Report Body Table 7

Chan and Zhang¹¹ exact method was used to calculate CIs for the PF-06881893 - US-licensed Neupogen difference in proportions.

Subjects with missing baseline values were not included in calculation of the primary and secondary endpoints.

Post-baseline percentages were based on the number of subjects with negative baseline ADA.

Non-inferiority was established when upper limit of the 90% CI was ≤ 0.10 (equivalent to 10 in percentage).

Abbreviations: ADA = anti-drug antibody; CI = confidence interval; N = number of subjects in each group;

N* = number of subjects included in analysis for immunogenicity; n = number of subjects with negative baseline ADA and postdose positive ADA test result; NAb = neutralizing antibody.

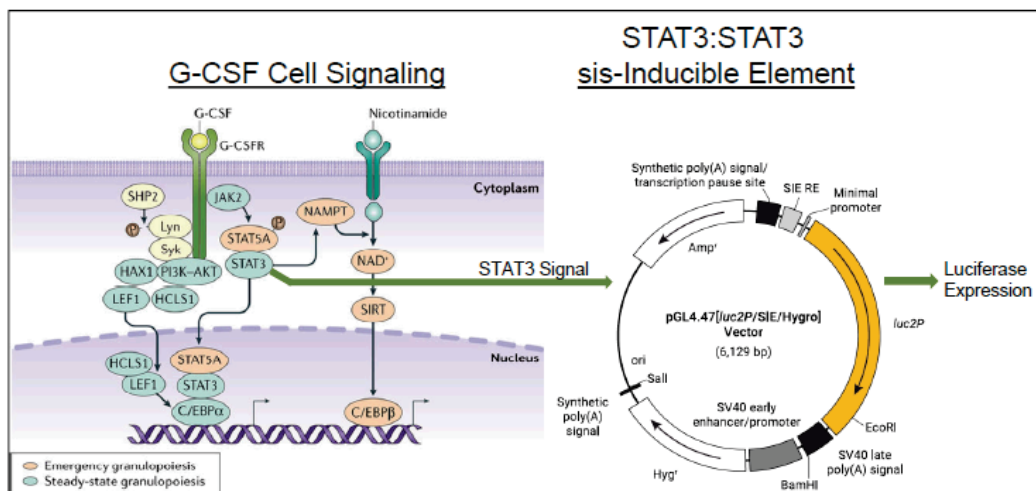
Reviewer's Comment: The comparative analysis data confirms that the ADA assay is capable of detecting binding ADAs to both drugs and is suitable for its intended use. The results of the Clinical study C1121012 demonstrate comparable incidences of confirmed ADA positive patient samples (7.4% Hospira vs. 4.9% Neupogen using CCP defined at the 0.1% FPR, and 9.1% Hospira vs. 8.9% Neupogen at the 1% FPR). This is acceptable.

Reviewer's Comment: The overall validation data for the ADA assay (screening and confirmatory) demonstrates that the assay is capable of reliably detecting ADAs (against both Hospira filgrastim and US-Neupogen) in patient serum samples and that the current use of an in-study screening cut point is more appropriate than the original validated cut point. The assay is acceptable.

NAb Assay Overview and Validation

The NAb Assay Overview

Figure 4. Overview of the NAb Assay



G-CSF cell signaling image modified from Skokowa et. al.;⁴ Vector image modified from Promega Corporation⁵

The NAb assay is a cell-based receptor signaling assay designed to detect the presence of neutralizing antibodies against filgrastim in human serum (figure 4). Engineered GloResponse™ SIE-luc2P stable U937 cells are stimulated with 0.5 ng/mL of Hospira filgrastim (PF-06881893). Binding of filgrastim to the receptor on the U937 cells induces the G-CSF receptor signaling pathway which causes the transfected GloResponse™ plasmid to express firefly luciferase. After addition of the ONE-Glo™ reagent, the luciferase activity is assessed by measurement of the relative light units (RLU). The RLU signal is proportional to the intensity of the G-CSF receptor signaling. NAb, if present in the serum sample, inhibits the G-CSF mediated luciferase activity. The readout of the assay is %inhibition (%Inh).

$$\% \text{ inhibition: } 100 - \frac{RLU \text{ sample} - RLU \text{ BL1}}{RLU \text{ BL2} - RLU \text{ BL1}} \times 100$$

Brief protocol summary

- 5 x 10⁴ GloResponse™ stable U937 cells/well were prepared
- Serum samples were heat-inactivated before use at 56°C for 30-45 minutes

- A mixture of final 0.5 ng/mL filgrastim and positive controls anti-hG-CSF antibodies (b) (4) were added to the cells per well and incubated for 6 hours ± minutes in the presence of 5% human serum

Critical Reagents

- Filgrastim, 2.6 ng/mL, Hospira
- Goat polyclonal antibody against human G-CSF (b) (4)
- NC-1: negative control serum-pool from 10 healthy humans, purchased from (b) (4)
- NC-2: negative control serum pool from 25 treatment naïve C1121012 clinical study samples, (b) (4)
- GloResponse™SIE-lic2P stable U937 cells (Promega cell line ID: CS185302a)

Controls

- Positive Controls (diluted in NC-1)
 - HPC 50 µg/mL
 - LPC1b 4 µg/mL
 - LPC2 3.5 µg/mL (monitoring only)
 - LPC3 4.5 µg/mL (monitoring only)
- Negative Controls
 - NC-1 (commercial HV sera pool, see above); used to generate:
 - BL1-1 (no filgrastim = 100%inh)
 - BL2-1 (no anti-hG-CSF = 0%inh)
 - NC-2 (in-study naïve sera pool, see above); used to generate:
 - BL1-2 (no filgrastim = 100%inh)
 - BL2-2 (no anti-hG-CSF = 0%inh)

MRD: 20 (5% serum)

In-study acceptance criteria:

- The NC should be negative
- The validated LPC1b positive control should be positive

The MRD was determined during the development phase and was not re-evaluated for the validation runs. The figure 1 below demonstrates that, in the presence of approximately EC35 of filgrastim, 2% serum and 5% serum have comparable signal to noise ratio. 5% was selected as the best serum dilution for the assay.

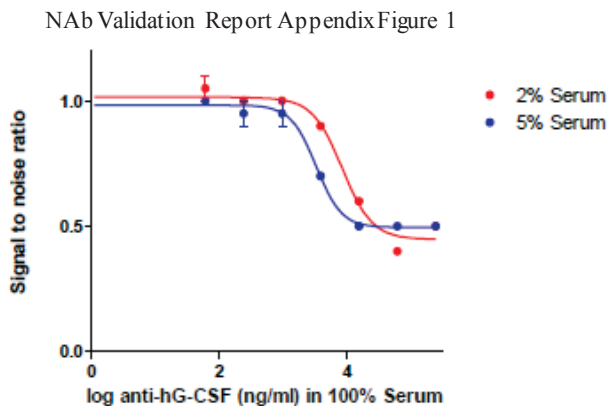
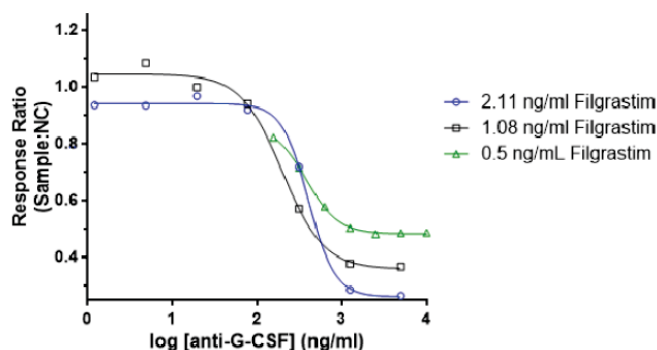


Figure 2. Antibody Dose Curve for NAb Assay



Source: BIO Filgrastim-000881

As a further study, using the MRD of 5% serum, the sponsor tested lower levels of filgrastim for stimulation of the cells and found that an EC20 (0.5ng/mL) gave the best dynamic range and signal to noise ratio for the assay (Figure 2 above) (see Assay Validation: Cell Stimulation section below).

Stability and Robustness:

The stability studies subjected the positive controls to storage up to room temperature overnight and repeated freeze/thaw cycles. Robustness studies included testing different starting concentrations of cells as well as different incubation times. All the parameters tested met the acceptance criteria.

Validation parameter	Acceptance criterion	Result
Freeze-thaw stability	80 – 120 % recovery to standard condition	Passed up to 4 FT cycles*
Bench top stability	80 – 120 % recovery to standard condition	Passed, bench top overnight
Robustness (assay time)	80 – 120 % recovery to standard condition	5 - 7 hrs
Robustness (number of cells)	80 – 120 % recovery to standard condition	4E4 – 6E4 cells/well

Reviewer's Comment: The MRD was determined from testing 2%, 5%, and 10% serum concentrations in the presence of ~EC35 of filgrastim. It was determined that 5% serum gave the best combination of signal to noise ratio and dynamic range. Subsequent testing for cell stimulation with 5% serum MRD determined that an EC20 of filgrastim provided

a better signal to noise ratio and dynamic range than ~EC35. The stability and robustness data support the validation ranges of the assay. These are acceptable.

Assay Validation

The NAb assay was originally validated in 2015 in report (b) (4)-2015-B-09-07. After feedback from the FDA and to meet the updated 2016 FDA guidance on Immunogenicity assays, the assay was re-validated in report (b) (4)-16-074-021-REP. The updated validation included the use of a pool of 25 treatment naïve serum samples from clinical study C1121012 as an additional negative control, determining an in-study cut point, and including two additional LPCs to monitor assay performance. A summary of the validation results of the two exercises is below (table 6)

Table 6. Summary of NAb Assay Validation Results

Assay Parameter	(b) (4)-2015-B-09-07	(b) (4)-16-074-021-REP
	Validation	Supplemental Validation
	Used for Clinical Study: ZIN-FIL-1501 and ZIN-FIL-1502	Used for Clinical Study: C1121012
In-study Cut Point	51.0% Inhibition	48.4% Inhibition
Sensitivity	PF-06881893 3.32 µg/mL	PF-06881893 448.03 ng/mL
	Neupogen Not Tested	Neupogen 409.84 ng/mL
Low Positive Controls	4 µg/mL and 10 µg/mL	3.5 µg/mL, 4 µg/mL, and 4.5 µg/mL
High Positive Control	50 µg/mL	50 µg/mL
Drug Tolerance Normal Human Sera (PF-06881893)	HPC (50 µg/mL) 100 ng/mL	HPC (50 µg/mL) 167 ng/mL
	LPC (10 µg/mL) 10 ng/mL	Not Retested
	Not Tested	LPC (4.5 µg/mL) 6.2 ng/mL
	LPC (4 µg/mL) 1 ng/mL	LPC (4 µg/mL) 6.2 ng/mL
	Not Tested	LPC (3.5 µg/mL) 6.2 ng/mL
Drug Tolerance Normal Human Sera (Neupogen)	Not Tested	HPC (50 µg/mL) 500 ng/mL
		LPC (4.5 µg/mL) 18.5 ng/mL
		LPC (4 µg/mL) 18.5 ng/mL
		LPC (3.5 µg/mL) 6.2 ng/mL
Analytical Specificity (Influence of GM-CSF)	HPC (50 µg/mL) 200 pg/mL	Not Retested
	LPC (10 µg/mL) 200 pg/mL	
	LPC (4 µg/mL) 50 pg/mL	
Analytical Specificity (Sample Matrix Interference)	Not Tested	Testing without PF-06881893 to assess matrix interference per sample
Intra-assay Variation	Passed (maximum 5.4 % CV)	Not Retested
Inter-assay Variation	Passed (maximum 7.2 % CV)	Not Retested
Robustness		
Cell Density	4 – 6 x 10 ⁴ cells/well	Not Retested
Incubation Time	5 – 7 hours	
Minimum Required Dilution (MRD)	20 (5% serum)	Not Retested
Short-term Sample Stability at Room Temperature (overnight)	Acceptable	Not Retested
Freeze/Thaw Stability (4 cycles)	Acceptable	Not Retested

Reviewer's Comment: *The validation summary table indicates that the sponsor has addressed concerns relating to the new 2016 FDA guidance. More detailed review of select assay parameters is below.*

Assay Validation

- Cell Stimulation

The cell-based NAb assay relies on stimulating the cells with G-CSF to activate the cell-signaling pathway and produce luminescence. The FDA recommends using a concentration of stimulant near the effective concentration 50 (EC₅₀) based on the linear portion of a drug dose-response curve. Additionally, for a biosimilar product, it must be demonstrated that the innovator and biosimilar have similar dose-response curves in order to support the use of a single assay platform (See Figure 6 and Table 7 below).

Figure 6. Comparative Dose-Response Curves for PF-06881893 and Neupogen

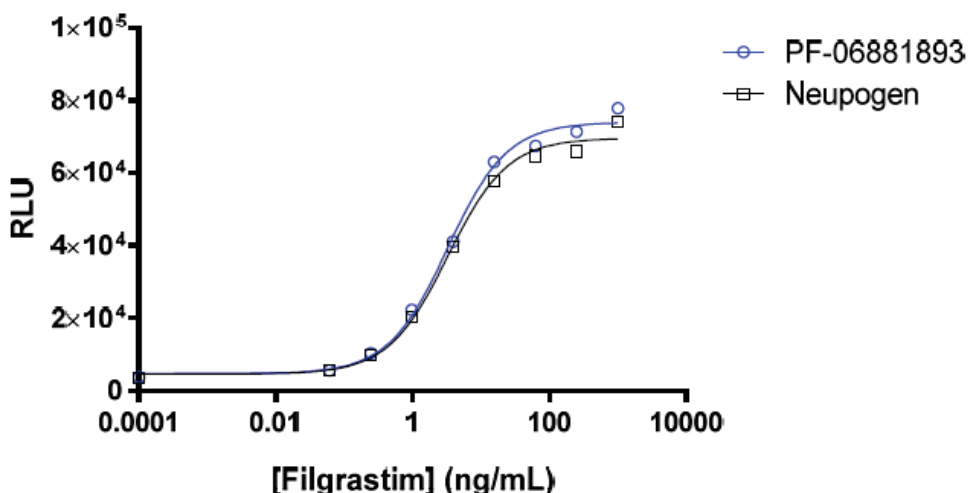


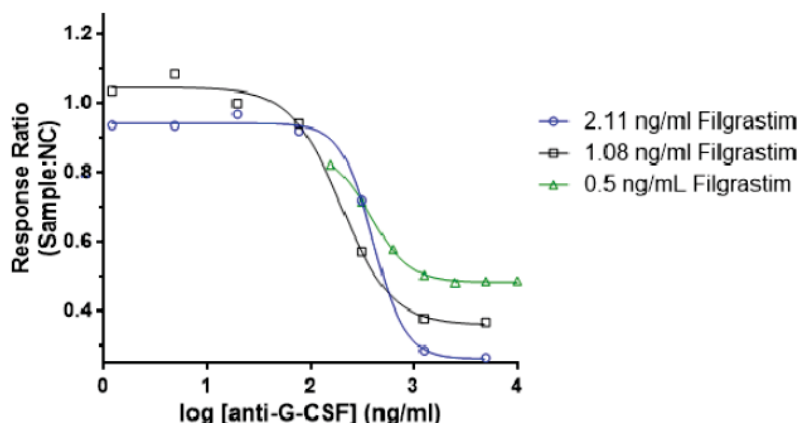
Table 7. Calculated EC₅₀ for PF-06881893 and Neupogen

EC ₅₀ (ng/mL)		Overall Mean	Standard Deviation	%CV
Filgrastim Hospira	Neupogen			
3.269	3.280	3.3	0.0	0.3

The data demonstrates that the assay performs similarly for both Hospira filgrastim and US-Neupogen with similar EC₅₀ values of 3.269 and 3.280, respectively. However, the concentration of stimulant chosen for the assay was 0.5 ng/mL, a value closer to the EC₂₀ value and at the lower end of linearity on the dose-response curve.

Reviewer's Comment: The use of a value closer the EC₂₀ and near the lower end of linearity on the dose-response curve required further justification. An IR was sent to the sponsor 12/13/2017 and a response was received 01/02/2018. The sponsor stated that they observed a correlation between increased filgrastim concentration and lower dynamic range. They tested concentrations of 2.11 ng/mL, 1.08 ng/mL, and 0.5 ng/mL and found that the best balance of dynamic range and signal to noise ratio was with 0.5ng/mL (see Figure 2, below, from IR response).

Figure 2. Antibody Dose Curve for NAb Assay



Source: BIO Filgrastim-000881

The use of filgrastim at EC_{20} [0.5ng/mL] for the NAb assay was selected as it was able reproducibly meet positive control acceptance criteria, provides a wider dynamic range, and produced an assay readout that was sensitive to inhibition. This is an acceptable concentration of filgrastim.

Cut point determination

• Original Validation

The cut point for the original validation was determined after analysis of sera from 51 normal humans. (2 assays by 1 analyst). The normal human sera samples were highly variable with the two runs having standard deviations of 15-16% inhibition. After averaging the two runs, the 99th percentile cut point was determined to be 51% inhibition.

The 51% inhibition cut point was used to analyze study ZIN-FIL-1501 and ZIN-FIL-1502. The results are as follows:

Study Report	ADA Positive	NAb (pos/neg)	Highest %inhibition
ZIN-FIL-1501	1	Neg	2.4%
ZIN-FIL-1502	15	All Neg	22.4%

• Re-validation

The re-validation study involved determining an in-study cut point as part of the C1121012 study phase and following the updated 2016 FDA guidance on Immunogenicity Assays. 30 randomly selected pre-dose samples were heat-inactivated and run in 6 independent assays by 2 analysts over at least 3 days. The result was a fixed in-study 99% cut point of 48.4% inhibition.

Study C1121012 analyzed samples (n=136) from 15 patients confirmed positive for ADA and it was determined that all the samples were negative for neutralizing antibodies.

Reviewer's Comment: *The in-study cut point and the originally validated cut point are in close proximity and analysis of the study reports indicated that the lower fixed in-study cut point of 48.4% inhibition had no impact on the original analysis of the ZIN-FIL-1501 and ZIN-FIL-1502 study reports. The in-study cut point of 48.4% was determined based on the new FDA guidance and evaluated by using 30 individual pre-dose samples from Study C1121012. The cut point is valid and acceptable.*

Sensitivity and Controls

- Original Validation

Assay sensitivity is the lowest concentration at which the positive control (PC) antibody preparation consistently produces a positive result. For the original validation, anti-hG-CSF antibodies were prepared in a range of 150-0.15 µg/mL (4-fold dilution series spanning the cut point) in the presence of 0.5 ng/mL filgrastim. The assay sensitivity was then based on a 5% false positive rate and calculated to be 3.32 µg/mL.

Assay sensitivity = mean of interpolated conc. of each dilution curve at $CP + (t_{0.05df} * SD)$

The low positive controls were also calculated based on the interpolation of the dilution curve but with a 1% false positive rate. LPC1 was 5 µg/mL, but further testing revealed that the % inhibition values were too high so it was lowered and LPC1b is 4 µg/mL. The original LPC2 is simply doubling the original low positive control, 10 µg/mL.

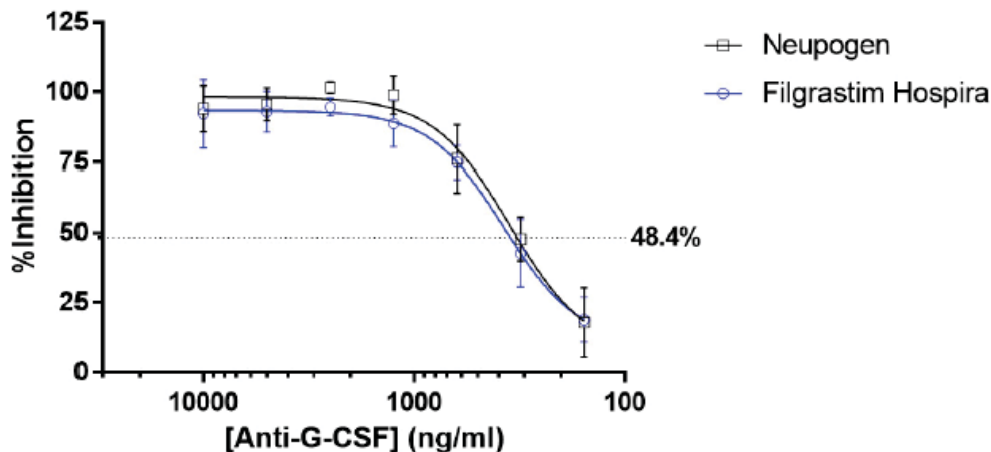
LPC1 = mean of interpolated conc. of each dilution curve at $CP + (t_{0.01df} * SD)$

Throughout the validation runs, LPC1 had values ranging from 80% inh to 94% inh.

- Re-validation

The re-validation study was designed to test whether there was any difference in sensitivity when test cells are stimulated with Hospira filgrastim or US-Neupogen. Anti-hG-CSF antibodies were prepared in a 2-fold serial dilution starting at 10 µg/mL and spanning the in-study cut point. They were tested in the presence of 0.5 ng/mL of Hospira filgrastim and US-Neupogen in a total of 6 assays by 2 analysts over at least 3 days. The dilution curves were as follows:

Figure 1. Comparative NAb Assay Sensitivity using Filgrastim Hospira and Neupogen for Cell Stimulation



The calculated 5% false positive rate for assay sensitivity were similar between the Hospira filgrastim and US-Neupogen, 448.03 ng/mL and 410.84 ng/mL respectively. This indicates that the NAb assay responds similarly to both drugs.

The re-validation also included testing additional LPCs to monitor assay performance. The additional LPCs included LPC2: 3.5 µg/mL and LPC3: 4.5 µg/mL. Throughout the validation runs, the performance of LPC2 and LPC3 were not different from the currently validated LPC1b. The range for LPC1b was 55.4% inhibition to 86.3% inhibition with a mean of 76.3% inhibition and a median of 78.5% inhibition, during the assay sensitivity runs. LPC2 failed to have a signal above the cut point in 2 of 6 sensitivity runs and had a mean of 72.7% inhibition and median of 73.1% inhibition.

Reviewer's Comment: *The comparative data demonstrates that the NAb assay responds equally to Hospira filgrastim and US-Neupogen. The assay sensitivity was increased upon re-validation; however, there was no change to the LPC1b which is based on the sensitivity. The new sensitivity data would indicate that the new LPC (99th percentile) should be ~ 500 ng/mL or roughly 8-fold lower. However, testing of LPC2 (3.5 µg/mL), demonstrated that it could not consistently produce a signal above the in-study cut point. This indicates that although the sensitivity has changed, LPC1b may still be the most appropriate control. The assay sensitivity and LPCs are acceptable.*

Drug Tolerance

Drug tolerance is evaluated by spiking normal human serum pool (NC-1) with anti-hG-CSF antibodies at concentrations equivalent to the positive controls. Varying concentration of Hospira filgrastim and US-Neupogen were then added in a geometrical dilution series starting at 500 ng/mL. The final concentration where the signal was above the cut point was the tolerance level.

The original validation had a lower drug tolerance than the re-validation study. The current drug tolerance for the LPC1b is 6.2 ng/mL for Hospira filgrastim and 18.5 ng/mL for US-Neupogen. The small drug tolerance difference is not a concern for assay validation. The values of 6.2 ng/mL and 18.5 ng/mL are both below the C_{max} levels established under the single dose and multiple dose studies (16 ng/mL and 30 ng/mL, respectively). This indicates that the NAb assay may not be able to detect low level NAb under C_{max} conditions; however, the half-life of filgrastim is 2.8-4 hours and the sampling schedule is no less than 10 days after dosing.

Reviewer's Comment: *The relatively low drug tolerance levels for the LPC is not a concern due to the sampling timing for immunogenicity testing samples. Due to low anticipated on-board drug levels in immunogenicity samples, the potential for interference from drug product is significantly reduced. Both drugs have similar drug tolerance levels indicating that the assay is suitable for single platform use. This is acceptable.*

Assay Specificity and Matrix Interference

The NAb assay was tested to determine the assay specificity for G-CSF and if there is any impact on the presence of other cytokines on cell-based assay performance. Varying levels of GM-CSF were spiked into all positive controls from the original validation. It was determined that the HPC is still positive in the presence of up to 200 pg/mL of GM-CSF and LPC1b is still positive in the presence of up to 50 pg/mL GM-CSF. If the re-validated cut point is applied to the specificity tables those values change to 300 pg/mL and 100 pg/mL, respectively.

To assure the absence of matrix interference, the sponsor took a multifaceted approach to include: 1) demonstration that known cytokines do not induce signaling via G-CSF pathway, 2) demonstrate the study samples do not induce receptor signaling by routinely testing samples in the absence of filgrastim, 3) heat treating samples to further reduce matrix interference. Data from C1121012 study samples run without filgrastim indicate that there is no matrix interference.

Reviewer's Comment: *The Assay specificity and matrix interference studies demonstrate that the reporter cell line is appropriate and is not influenced by other cytokines which may activate expression of the reporter gene construct.*

An IR was sent 12/13/2017 asking for more details related to how well the heat treatment abrogates stimulation by other cytokines and how the heat treatment impacts samples. The sponsor responded 01/02/2018, that by testing the pre-dose in-study samples in the absence of filgrastim and demonstrating that there is no matrix interference eliminates the need for specific cytokine spiking studies. The sponsor also provided data (included below) showing that heat-treatment had no negative impact on the detection of GCSF inhibition by the positive control samples.

Table 2. Effect of Heat Treatment on NAb Positive Controls

Assay Control	Replicate 1 (RLU)	Replicate 2 (RLU)	Mean RLU	Standard Deviation	%CV	%Inhibition
HPC	7577	7219	7398	253	3.4	102.4
LPC2	8266	8674	8470	289	3.4	94.2
LPC1	9845	10125	9985	198	2.0	82.7
NC	20444	22639	21542	1552	7.2	-5.4
HPC HI	6760	7271	7016	361	5.1	105.3
LPC2 HI	8208	6848	7528	961	12.8	101.4
LPC1 HI	9381	9999	9690	437	4.5	84.9
BL2	20588	21074	20831	343	1.6	0.0
BL1	7981	7439	7710	384	5.0	100.0

Source: BIO Filgrastim-000882

Abbreviations: BL = blank; HPC = high positive control; HI = heat inactivation at 56°C for 30 ± 5 minutes; LPC = low positive control; NC = negative control; RLU = relative light units

Assay Precision

The acceptance criterion for both the intra-assay and inter-assay variation is ≤30 % CV for the positive controls. The variability on the NAb assay is relatively low with calculated variance of 5.4% for intra-assay and 7.2% for inter-assay testing.

Reviewer's Comment: *The assay variations meets the recommended acceptance criteria of ≤30 % CV. This is acceptable.*

Reviewer's Comment: *The overall validation data for the NAb assay demonstrates that the cell-based NAb assay is capable of detecting neutralizing antibodies to both Hospira filgrastim and US-Neupogen. Routine testing of study samples in the absence of filgrastim and heat-treatment of study sample alleviates the concerns with matrix interference for the assay. The re-validation of the assay demonstrated an improved sensitivity of the assay; however, the LPC was not adjusted in conjunction with the improved sensitivity. Assays testing LPC2 (3.5µg/mL) indicated that the practical lower positive control limit may be the current LPC1b level of 4 µg/mL. I find the NAb assay to be valid with the current in-study fixed cut point of 48.4% inhibition.*

SPR Assay Validation

A typical surface plasmon resonance (SPR) method was designed to determine the ADA immunoglobulin isotypes in confirmed ADA positive clinical study samples. For method development and qualification, a surrogate model (lactoferrin) was used due to the lack of human anti-filgrastim antibodies. The model demonstrated the ability of the SPR assay to determine the isotype of autoantibodies.

The filgrastim SPR method involved immobilizing PF-06881893 to the sensor chip. Anti-filgrastim antibodies would bind to the ligand then antibodies to IgM, IgA, IgG1, IgG2, IgG3, IgG4 are passed over the chip to identify isotypes. For the method, normal human serum pool from 4 healthy individuals was used as the negative control and as the diluent for the positive control antibodies. A summary of the control signals and cut points is

below (Table 9). Samples were run in 10% serum. A summary of the qualitative isotype results is in Table 10.

Table 9. Summary of PF-06881893 Binding for the Positive and Negative Control Samples

Clinical Study	Run Sequence	Binding Response (RU)			
		Negative Control	PC-1 (0.3 µg/mL)	PC-2 (1 µg/mL)	Cut Point
C1121012	1	57.6	120.0	259.9	75.2
	2	45.2	102.4	212.9	53.1
	3	44.2	170.9	421.8	52.9
	4	45.9	99.1	212.8	49.4
	5	50.7	87.4	151.6	55.6
ZIN-FIL-1501	1	0.3	3.4	22.4	1.7
ZIN-FIL-1502	1	38.6	82.6	192.9	77.1
	2	52.7	119.9	188.9	105.4
Data Summary					
Mean		41.9	98.2	207.9	58.8
Standard Deviation		17.8	47.2	111.3	29.8
%CV		42.4	48.1	53.5	50.6

Table 10. Summary of Qualitative Isotyping Results for the Positive and Negative Control Samples

Clinical Study ^a	Run Sequence	Control	Isotype Response (RU)				
			IgM	IgG4	IgG3	IgG2	IgG1
C1121012	3	PC-1	6.0	10.3	-3.1	-0.8	-1.8
		PC-2	5.4	50.8	-5.4	-1.3	-2.0
		NC	5.3	-1.0	-2.5	-0.6	-1.8
	4	PC-1	25.7	7.5	-3.2	-0.8	-1.8
		PC-2	25.8	20.8	-3.9	-1.0	-1.8
		NC	24.2	3.2	-3.0	-0.7	-1.8
ZIN-FIL-1502	2	PC-1	-11.3	-2.0	-1.9	-1.0	4.7
		PC-2	-12.2	-1.9	-1.9	-1.1	4.4
		NC	-9.7	-2.0	-1.8	-1.3	0.7

^a. The ZIN-FIL-1501 sample was negative for filgrastim binding by SPR and was not tested in the qualitative isotyping portion of the assay.

For the 3 clinical studies that were analyzed, the SPR assay was capable of detecting and measuring the isotype of anti-filgrastim antibodies. A summary of the clinical trial results is below.

Clinical Study	ADA pos. tested	SPR screened pos	Isotype
ZIN-FIL-1501	1	0	Not done
ZIN-FIL-1502	15	6	All IgM and IgG1
C1121012	68	33	15 Positive: • 3 IgM and IgG4 • 1 IgM, IgG4, IgG1 • 5 IgG1 only • 1 IgG1 and IgG2 • 5 IgG3 only 18 Negative

Reviewer's Comment: The SPR method demonstrates that it can detect anti-filgrastim antibodies present in many confirmed ADA-positive samples and subsequently detect

immunoglobulin isotypes in the clinical trial study samples. The SPR method is acceptable.

Review Summary

Overall, the immunogenicity assay (ADA, NAb, and SPR) validation runs demonstrated the ability to be used in a single platform model for testing both Hospira filgrastim and US-Neupogen equally. There were minimal differences in assay sensitivity, drug tolerance, and precision between the two drugs for both the ADA assay and the NAb assay. The resulting analysis of the non-inferiority study indicated that Hospira filgrastim has similar levels of ADA incidence as the US-Neupogen and supports the approval of Hospira filgrastim as a biosimilar.



Scott
Lute

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Harold
Dickensheets

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Date: 6/06/2018 02:16:07PM
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BLA STN 761080

Product USAN Name: filgrastim-aafi
Nivestym (PF-06881893)

Manufacturer
Hospira, Inc., a Pfizer Company

Reviewer
Malini Wileman, Ph.D.
(Drug Substance, Analytical Similarity, Assay Validation)

Application Technical Lead
Howard Anderson, Ph.D.

Office of Biotechnology Products
Division of Biotechnology Review and Research II

Recommendation: Based on the Drug Substance and Analytical Similarity assessments, this BLA is recommended for approval.

OBP CMC Review Data Sheet

1. **BLA#:** 761080

2. **Review Date:** June 5, 2018

3. **Primary Review Team:**

a. **CDTL:** Donna Przepiorka

b. **Medical Officer:** Lea Cunningham

c. **Product Quality Team:** Howard Anderson (Application Technical Lead)
Malini Wileman (DS Quality, Method Validation, Analytical Similarity)
Scott Lute (DP Quality, Immunogenicity)
Maria Reyes Candau-Chacon (Quality Assessment Lead)
Maxwell Van Tassell (DS Microbiology)
Aimee Cunningham (DP Microbiology)

d. **Facilities:** Marion Michaelis

e. **Clinical Pharmacology:** Theingi Thway

f. **Statistics:** Lola Luo

g. **OBP Labeling:** Vicky Borders-Hemphill

h. **RBPM:** Kelly Ballard

4. **Major GRMP Deadlines:**

a. **Filing Meeting:** November 3, 2017

b. **Mid-cycle meeting:** March 2, 2018

c. **Wrap-up meeting:** June 7, 2018

d. **Primary review due:** June 12, 2018

e. **Secondary review due:** June 21, 2018

f. **BsUFA action date:** July 21, 2018

5. **Communications with Sponsor:**

Communication/Document:	Date:
Information Request	December 08, 2017
Information Request	January 3, 2018
Information Request	April 18, 2018
Information Request	May 10, 2018

6. **Submission Reviewed:**

Submission:	Date Received:	Review Completed (yes or no)
761080/0001	9/21/2017	Yes
IR response 761080/0011	12/22/2017	Yes
IR response 761080/0013	1/12/2018	Yes
IR response 761080/0024	5/2/2018	Yes

IR response 761080/0026	5/17/2018	Yes
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7. Drug Product Name/Code/Type:

- a. Proprietary Name: Nivestym (proposed)
- b. Trade Name: Nivestym (proposed)
- c. Non-Proprietary Name/USAN: filgrastim-aafi
- d. CAS Name: R-metHuGCSF; Recombinant methionyl human GCSF (CAS Registry number: 121181-53-1)
- e. Common Name: PF-06881893
- f. INN Name: Filgrastim
- g. Compendial Name: N/A
- h. OBP systematic name: RPROT P09919 (CSF3_HUMAN) Granulocyte colony stimulating factor (PF06881893)

8. Pharmacological Category: Granulocyte colony-stimulating factor (recombinant methionyl human G-CSF)

9. Dosage Form: Injection, Pre-filled syringe and Single Dose Vial

10. Strength/Potency:

- (i): The concentration/strength of the Drug Product: 300 mcg/0.5 mL and 480 mcg/0.8 mL in a single-dose pre-filled syringe; 300 mcg/mL and 480 mcg/1.6 mL in a single-dose vial
- (ii): Type of potency assay(s): Cell proliferation assay, in vitro proliferative response of M-NFS-60 cells

11. Route of Administration: Intravenous and Subcutaneous

12: Referenced Drug Master Files (DMF): Covered in Drug Product review

13. Inspectional Activities: A pre-license inspection of Hospira Zagreb d.o.o. (a wholly owned subsidiary of Pfizer Inc., 10291 Prigorje Brdovečko, Zagreb, Croatia; FEI: 3010630287) was conducted from February 12-20, 2018 in support of BLA 761080, under FACTS assignment number 11819901. Information about the FDA inspection team is provided below:

Reyes Candau-Chacon (Inspection Lead; DMA)
Max Van Tassell (DMA)
Marion Michaelis (DIA)
Howard Anderson (OBP)
Malini Wileman (OBP)

This was the first FDA inspection of the Hospira Zagreb facility. The inspection covered drug substance and drug product manufacturing areas, manufacturing support areas, the quality control laboratories, and the warehouse. The inspection was conducted in accordance with the applicable sections of CP 7356.002M, Inspections of Licensed Therapeutic Drug Products; CP 7346.832, Pre-Approval Inspections/Investigations; and ICH Q7. This inspection was limited to the manufacturing of PF-06881893. An FDA Form 483 was issued to the firm at the closeout meeting on February 20, 2018. The observations noted on the FDA Form 483 are summarized below:

1. The environment [REDACTED] (b) (4) is inadequate to prevent contamination of the sterile product.
2. Procedures designed to prevent microbiological contamination of drug products purporting to be sterile do not include adequate validation of the sterilization process.
3. Procedures for [REDACTED] (b) (4)
4. PF-06881893 sterile drug product [REDACTED] (b) (4)
5. SOP001476/12 [REDACTED] (b) (4) lacks adequate instructions for classification of all rejects.
6. Procedures for handling of PF-06881893 [REDACTED] (b) (4) is inadequate for microbial control.
7. [REDACTED] (b) (4) is inadequate.
8. [REDACTED] (b) (4) used in PF-06881893 manufacture is inadequate.

14. Consults Requested by OBP: N/A (OND Requested CDRH ODE and OC consults)

15. Quality by Design Elements: The following was submitted in the identification of QbD elements (check any that apply):

	Design Space
X	Design of Experiments
X	Formal Risk Assessment/Risk Management
	Multivariate Statistical Process Control
	Process Analytical Technology
	Expanded Change Protocol

16. Precedents: None

Summary of Quality Assessments

- I. Primary Reviewer Summary Recommendation
We recommend approval of this Biologics License Application (BLA). The data submitted support the conclusion that the manufacture of PF-06881893 is well controlled and leads to a product that is pure and potent. The conditions used in manufacturing have been sufficiently validated, and a consistent product has been manufactured from multiple production runs. It is recommended that PF-06881893 be approved for human use (under the conditions specified in the package insert).
- II. List of Deficiencies to be Communicated
There are no CMC-related product quality deficiencies precluding approval of this BLA.

III. List of Post-Marketing Commitments/Requirements
None.

IV. Review of Common Technical Document- Quality Module 1

A. Environmental Assessment of Claim of Categorical Exclusion

A categorical exclusion to the environmental assessment requirements is requested by Hospira Inc., a Pfizer Company. This is in compliance with the categorical exclusion criteria 21 CFR Part 25.31 (b): Action on a BLA when the estimated concentration of the substance(s) at the point of entry into the aquatic environment will be below 1 part per billion. The sponsor claims that to their knowledge there are no extraordinary circumstances as described in 21 CFR 25.15(d).

The sponsor's environmental analysis and claim of categorical exclusion are adequate, therefore exclusion may be granted.

V. Primary Container Labeling Review

The primary container labeling was reviewed separately by Vicky Borders-Hemphill

VI. Review of Common Technical Document- Quality Module 3.2

CTD Modules 3.2.S, and the documents in 3.2.R that are pertinent to the Drug Substance (DS) are reviewed in this document. Module 3.2.P and the documents in 3.2.R that are pertinent to the DP were reviewed separately by Scott Lute.

VII. Review of Immunogenicity Assays- Module 5.3.1.4

The immunogenicity assays were reviewed separately by Scott Lute

Description of Drug Substance and Drug Product

S. Drug Substance

3.2.S.1.1 Nomenclature

PF-06881893 is a recombinant human N-methionyl granulocyte colony stimulating factor manufactured in *Escherichia coli* (*E. coli*). Other names for PF-06881893 used in the submission are Filgrastim, Filgrastim DS, Filgrastim Hospira, Filgrastim Hospira DS. rmetHuG-CSF or Filgrastim is a proposed biosimilar to US-licensed Neupogen. The sponsor's proposed proprietary name for the product is NivestymTM.

3.2.S.1.2 Structure

PF-06881893 is a 175 amino acid, single-chain, non-glycosylated protein (18,799 Da). The primary structure of PF-06881893 is identical to natural G-CSF, except for an additional methionine residue at the N-terminus of PF-06881893. It has an α -helical structure, a 4 helical-bundle motif and contains an N-terminal methionine and 2 intramolecular disulfide bonds: Cys37-Cys43 and Cys65-Cys75. PF-06881893 also contains a free cysteine at position 18 (Cys18).

3.2.S.1.3 General Properties

The general properties of PF-06881893 Drug Substance (DS) are summarized below (Table 1).

Property	Description
Molecular weight	Monoisotopic: (b) (4) Da; Average: 18,799 Da
pH	(b) (4)
Protein concentration	NLT (b) (4) mg/mL
Isoelectric point	6.05
Extinction coefficient	0.86 mL mg ⁻¹ cm ⁻¹
Disulfide linkages	Cys37-Cys43, Cys65-Cys75, Cys18 is not cross-linked

Table 1: Summary of PF-06881893 general properties

Endogenous G-CSF is a growth factor produced by monocytes, fibroblasts, and endothelial cells. It regulates the maturation, proliferation, and differentiation of the precursor cells of neutrophilic granulocytes.

rmetHuG-CSF or filgrastim is used for the treatment of neutropenia in patients. It stimulates the production of white blood cells by binding and activating G-CSF receptor found on leukocytes.

3.2.S.2 Manufacture

3.2.S.2.1 Manufacturers

Manufacture and release testing of DS occurs at:

Hospira Zagreb d.o.o. (a wholly owned subsidiary of Pfizer Inc.)
Prudnička cesta 60
10291 Prigorje Brdovečko,
Croatia

FEI: 3010630287
DUNS: 500625201

External contract partners for DS manufacturing are listed in Table 2 below:

3.2.R.6 Analytical Similarity

The data and information discussed in this section was provided by Hospira (sponsor) and corresponds to Hospira analyses of the products under evaluation. The statistical results and conclusions have been corroborated by the CMC reviewer, using sponsor-provided raw data.

Formulation

The proposed biosimilar product, PF-019881893 is developed as a clear, colorless, liquid, injection, filled in a prefilled syringe (PFS) in the same dose strengths (300 mcg/0.5 ml and 480 mcg/0.8 ml), and single-dose vial (SDV; 300 mcg/1.0 ml and 480 mcg/1.6 ml) approved for US-licensed Neupogen. These DP presentations for PF-06881893 match the presentations for US-licensed Neupogen. The route of administration for PFS is subcutaneous (SC) injection, and the route of administration for SDV is SC injection or intravenous (IV) infusion. A comparison of the formulation of PF-16881893 and US licensed Neupogen is provided in Tables 76 a and 76b;

Ingredient/Dose Strength	PF-06881893			
	PFS (600 mcg/mL)		SDV (300 mcg/mL)	
	300 mcg/0.5 ml	480 mcg/0.8 ml	300 mcg/1.0 ml	480 mcg/1.6 ml
Filgrastim (mcg)	300	480	300	480
Acetate (mg)	0.295	0.472	0.59	0.94
Sorbitol (mg)	25	40	50	80
Polysorbate 80 (mg)	0.02	0.032	0.04	0.064
Sodium (mg)	0.0175	0.028	0.035	0.056
(b) (4)			q.s.	q.s.
	q.s.	q.s.	q.s.	q.s.
Water for Injection USP, q.s. (mL)	q.s. to 0.5 mL	q.s. to 0.8 mL	q.s. to 1 mL	q.s. to 1.6 mL

Table 76a: DP presentations of PF-06881893 PFS and SDV

Ingredient/Dose Strength	US-licensed Neupogen			
	PFS (600 mcg/mL)		SDV (300 mcg/mL)	
	300 mcg/0.5 ml	480 mcg/0.8 ml	300 mcg/1.0 ml	480 mcg/1.6 ml
Filgrastim (mcg)	300	480	300	480
Acetate (mg)	0.295	0.472	0.59	0.94
Sorbitol (mg)	25	40	50	80
Polysorbate 80 (mg)	0.02	0.032	0.04	0.064
Sodium (mg)	0.0175	0.028	0.035	0.056
Water for Injection USP, q.s. (mL)	0.5	0.8	1	1.6

Table 76b: DP presentations of US-licensed Neupogen PFS and SDV

Reviewer comment: The excipients and their concentrations in the PF-06881893 DP are same as those of US-licensed Neupogen (3.2.P.1).

Analytical similarity testing

Criticality of Quality Attributes and Tier Assignment: The CQAs for PF-06881893 DS and DP were assigned based on the overall severity score, calculated as shown.

Severity=impact x uncertainty, where

- Impact determined based on potential to affect activity, efficacy, safety
- Uncertainty represents the assessment of the relevance, abundance, and specificity of the information for that attribute.

The Tier assignment was guided by the Risk Priority Number (RPN), relevance of the CQA to *in vivo* mechanism of action, suitability of the output to statistical analysis, and potential redundancy between attributes.

RPN=impact x occurrence

- Impact determined based on potential to affect activity and efficacy, or if a regulatory/compendial requirement.
- Occurrence is considered high for attributes related to filgrastim structure and function, medium or low for a product-related impurity or substance that is known to be adequately controlled by the manufacturing process (Section 3.2.S.3.2).

The critical quality attributes (CQAs) for PF-06881893 are DS attributes such as process- and product-related impurities, and product-related substances that are produced during manufacture, and DP attributes such as protein concentration and particulate matters, along with product-related substances and impurities that may change during long-term storage of DP at 2-8°C. The sponsor ranked quality attributes (QA) for similarity evaluation based on their potential impact on activity, efficacy, PK/PD,

safety, and immunogenicity. Attributes with the highest level of risk and significance to similarity that are evaluable by quantitative assays were assessed using Tier 1 statistical assessments. This evaluation consists of equivalence testing. Attributes having a moderate level of risk, high risk attributes present at low levels, or attributes orthogonal to those evaluated as Tier 1 (in this instance, additional testing of biological activity), are evaluated using Tier 2 statistical assessment of quality range. The remaining attributes are assigned to the Tier 3 analysis. Where redundant methods are used for the same QA such as for determining higher order structure, DSC was assigned to Tier 2, but orthogonal methods for higher order structure determination, including Far-UV CD and SV-AUC, H-D exchange, were all assigned to Tier 3. In addition, the method or assay used to measure a QA was assessed to determine if the data produced are amenable to statistical analysis. For Tier 2 assessments, results were evaluated by using quality ranges; analytical similarity for the attribute meets Tier 2 criteria if 90% or greater of test values fall within the statistical quality range (± 3 standard deviations of estimated mean of the comparator product). QAs evaluated in Tier 3 are assessed using side-by-side graphical comparisons of the raw data or descriptive statistics. This classification based on Tier assignment and justification for this assignment is provided in reviewer-prepared Table 77.

Table 77: Analytical similarity assessment plan for PF-06881893

Quality Attribute	Tier Assignment	Assay	Method	Rationale for Tier Assignment
Functional	1	Potency	<i>In vitro</i> cell-based bioassay	Direct link to mechanism of action
DP Attributes	2	Protein Concentration	UV-Vis spectroscopy	Linked to dose accuracy.
Functional		Receptor Binding	Competitive Receptor Binding Assay (CRBA)	Measures the first step in the filgrastim <i>in vivo</i> biological response. Redundant relative to Tier 1 <i>in vitro</i> potency.
		Receptor Binding Affinity and Kinetics (Relative K_D)	Surface Plasmon Resonance (SPR)	Characterization test that measures the relative binding affinity constant. Redundant relative to Tier 1 <i>in vitro</i> potency.
Product related Substances and Impurities		Total Filgrastim Related Proteins	RP-HPLC (USP)	Filgrastim related proteins include multiple product related impurities such as oxidized filgrastim and reduced species that may have unknown clinical impact.

		Total Charge Variants	Cation Exchange Chromatography (CEX)	Filgrastim charge variants include species containing deamidation and aspartate succinimide that may have unknown clinical impact depending on the site of modification(s).
		Oxidation	RP-HPLC (USP)	Oxidation of Met122, 127, and 138 are identified as CQAs and a number of species containing Met oxidation are separated in the RP-HPLC method. The levels of Met oxidation determined by the validated RP-HPLC method are amenable to statistical analysis.
Higher Order Structure		Tertiary Structure	Differential Scanning Calorimetry (DSC)	Sensitive method that is also indicative of protein stability when samples are evaluated in a matched matrix.
Functional	3	Receptor Binding Kinetics (k _{on} , k _{off} , K _D)	SPR	Characterization test with high variability. Redundant relative to the Tier 2 Relative K _D attribute.
Primary Structure		Molecular Weight	RP-UPLC-MS	Results are expected to be identical and the assay output is not suitable for quantitative statistical analysis
		Amino Acid Sequence	Glu-C Peptide Mapping	
		Free Thiol	Ellman's Test	Characterization test with relatively high variability used as an orthogonal primary structure characterization method to confirm the

				presence of one free cysteine residue per molecule.
Higher Order Structure		Secondary Structure	Far-UV Circular Dichroism (CD)	Used to measure the relative percentages of secondary structure elements. Quantitative outputs from the software fitting have higher variability than DSC.
		Tertiary Structure	Top-down MS	Results expected to be identical between PF-06881893 and US-licensed Neupogen. Assay output is not suitable for quantitative statistical analysis.
			Intrinsic fluorescence	Wavelengths of Maximum fluorescence emission are reported in discrete whole number wavelengths and have a non-normal distribution not suitable for a formal statistical comparison.
			Hydrogen-Deuterium (H-D) exchange	The rates and distributions of deuterium exchange are indicative of secondary and tertiary structures and are compared graphically.
			SV-AUC	Characterization test with high variability
			Nuclear Magnetic Resonance (NMR)	Provide qualitative comparison of structural fingerprint
			X-ray crystallography	Provide structural finger-print of the molecule

Product related Substances and Impurities		High Molecular Weight Species	Size Exclusion Chromatography (SEC, Ph. Eur.)	Attribute present below or near the LOQ of the analytical method. Data not amenable to statistical analysis.
			SV-AUC	Characterization test with high variability. Attribute present below or near the LOQ of the method. Data not amenable to statistical analysis.
		Molecular Weight Variants	SDS-PAGE (Ph. Eur.)	Method is a limit test (2%). Data not amenable to statistical analysis.
			Capillary Electrophoresis (CE)-SDS	Attribute expected to be present below or near the LOQ, not amenable to statistical analysis.
		Oxidation (Met1, Met1227, Met138, Trp59, Trp119)	Glu-C Peptide Mapping	Characterization test with high variability. Redundant relative to the Tier 2 RP-HPLC Oxidation analysis.
		Deamidation	RP-HPLC (USP)	Attribute present below or near LOQ of the method. Redundant relative to the Tier 2 RP-HPLC Total Filgrastim Related Proteins analysis.
			CEX	Attribute present below or near LOQ of the method. Redundant relative to the Tier 2 CEX Total charge variants analysis.
		Deamidation (Gln21, Gln71, Gln19, Gln108, Gln132)	Glu-C Peptide Mapping	Characterization test with high variability. Attribute present below or near the reporting limit of analytical method.

		Aspartate Isomerization	Glu-C Peptide Mapping	Characterization test with high variability. Attribute present below or near the reporting limit of analytical method.
		Succinimide	RP-HPLC (USP)	Attribute present below or near LOQ of the method. Redundant relative to Tier 2 RP-HPLC Total Filgrastim Related Proteins analysis.
		Reduced Species	RP-HPLC (USP)	Attribute present below or near LOQ of the analytical method. Redundant relative to Tier 2 RP-HPLC Total Filgrastim Related Proteins analysis.
		Other species (N-terminal truncations, fMet1, Asp110 and Gly150 Glu substitution)	Glu-C Peptide Mapping	Characterization test with high variability. Attribute present below or near the reporting limit of the analytical method
		Other characteristics	Isoelectric Point	Capillary Isoelectric Focusing (cIEF)
Process-related Impurities	No Tier assignment	Residual Host-cell Proteins	HCP ELISA	Attribute present below or near the LOQ of the analytical method.
Residual Host-cell DNA		qPCR		
DP Attributes		pH	USP<791>	Discrete numerical analytical output not suitable for statistical analysis. FDA guidelines for biosimilars allow for
	Polysorbate 80	RP-HPLC-ELSD		

				minor differences in clinically inactive components
		Osmolality	USP<785>	Statistical analysis not required. The osmolality of parenteral drugs only needs to be controlled within a range that ensures an isotonic product.
		Appearance, Color, and Clarity	Visual, Ph. Eur. <2.2.2> and <2.2.1>	Descriptive testing results not suitable for statistical analysis.
		Visible Particles	USP<790>	
		Sub-visible Particles	Low volume HIAC, and MFI ($\geq 25 \mu\text{m}$, $\geq 10 \mu\text{m}$, $\geq 5 \mu\text{m}$, $\geq 2 \mu\text{m}$)	Characterization test with high variability

Table 77: Analytical similarity assessment plan for PF-06881893

Reviewer comments: CQAs used for analytical similarity assessment between PF-06881893 and US-licensed Neupogen are the same as those used in PF-06881893 characterization testing (3.2.S.3) as well as in PF-06881893 product control strategy (3.2.S.2 and 3.2.P.3). A number of CQAs were identified based on prior knowledge about the filgrastim protein and its therapeutic mechanism of action. These include potential amino acid modifications, critical higher order structure motifs, and receptor binding, that may assist in the evaluation of purity, safety, or efficacy of the product.

Several methods used in this exercise are also used for routine quality control and were validated per ICH Q2(R1) (reviewed in Section 3.2.S.4.3). Other methods such as those used for DS characterization, were qualified according to sponsor SOPs. ^1H NMR spectroscopy, H/D exchange, X-ray crystallography, and SV-AUC, used to study higher order structure of PF-06881893 and US-licensed Neupogen were performed at contract laboratories. MFI, used for the determination of subvisible particles was also performed at a contract laboratory.

Total charge variants by CEX chromatography, total filgrastim-related proteins by RP-HPLC, and Met122, Met127 variants of filgrastim, in PF-06881893 and US-licensed Neupogen are evaluated by Tier 2 analysis for analytical similarity evaluation. Analytical similarity of individual variants is evaluated by Tier 3 statistical evaluation.

Study Design: The testing plan as well as the lots used in each assay were prospectively defined by the sponsor. A total of 50 DP lots of US-licensed Neupogen were purchased over time and enrolled in the analytical similarity assessment within the manufacturers' assigned expiry date, and were tested for

analytical similarity using a subset of PF-06881893 characterization and release methods. The manufacturing dates for the US-licensed Neupogen were estimated as 30 months prior to expiry date. The testing plan, including the PF-06881893 and US-licensed Neupogen lots to be tested for each attribute, was prospectively defined in the analytical similarity assessment protocol (LF-332-R-504-15). Neupogen lots were selected across the shelf-life of the product and are representative of both dose strengths and both the prefilled syringe (PFS) and single dose vial (SDV) presentations. All of the three US-licensed Neupogen lots used in the comparative non-clinical (lot 1047463) and clinical (lots 1050859 and 1060852) studies are included in the analytical similarity assessment.

The PF-06881893 lots tested in the analytical similarity assessment include the engineering, clinical, and process performance qualification (PPQ) lots. A total of 26 DP lots (16 PFS and 10 SDV) manufactured from 14 DS lots were included in the similarity assessment. All lots were manufactured using the proposed commercial manufacturing process. In 12 instances, the same DS lot was used to derive DP lots with two different dose strengths. While the sponsor considered these lots as “independent” lots for analytical similarity assessment, this is not true of attributes such as potency and process- or product-related impurities that are a direct result of the manufacturing process, and are not affected by the presentation or storage. The sponsor was sent an information request by the CMC Statistics reviewer to re-evaluate the Tier 1 potency evaluation for analytical similarity (see below for review of Tier 1 assessment for potency), and by the CMC reviewer for re-evaluation of all Tier 2 attributes (see below), using independent DP Lots for the analytical similarity assessment. For QAs that may be affected by container closure or DP storage at 2-8°C, DP lots derived from the same DS batch may be considered independent (Table 78).

DS Batch Number	Date of Manufacture	Storage	DP Lot Number	Dose Strength	Date of Manufacture
1755093	Oct 2013	(b) (4)	2854103	300 mcg/0.5 mL	Oct 2013
			2855103	480 mcg/0.8 mL	
1750123	Dec 2013		2057024	480 mcg/0.8 mL	Feb 2014
			2056024	300 mcg/0.5 mL	
1805034	Mar 2014		2053054	300 mcg/0.5 mL	May 2014
			2054054	480 mcg/0.8 mL	
1804074	Jul 2014		2073094	300 mcg/0.5 mL	Sep 2014
			2074094	480 mcg/0.8 mL	
1804094	Oct 2014		2074114	300 mcg/0.5 mL	Nov 2014
			2075114	480 mcg/0.8 mL	
FSM VUS S01P/14	Dec 2014		EX6-084A	300 mcg/1.0 mL	Mar 2016
			EX6-084B	480 mcg/1.6 mL	
FSM VUS S02P/14	Dec 2014		EX6-049A	300 mcg/1.0 mL	Jan 2016
			EX6-049B	480 mcg/1.6 mL	
1451025	Feb 2015		2572075	300 mcg/0.5 mL	Jul 2015
			2573075	480 mcg/0.8 mL	
1452025	Feb 2015		2574075	300 mcg/0.5 mL	Jul 2015
			2575075	480 mcg/0.8 mL	
1455035	Mar 2015		2576075	300 mcg/0.5 mL	Jul 2015
			2577075	480 mcg/0.8 mL	
1460055	May 2015		PD6-267	300 mcg/1.0 mL	May 2016

		(b) (4)	PD6-269	300 mcg/1.0 mL	
1463055	Jun 2015		PD6-270	480 mcg/1.6 mL	Jun 2016
1453065	Jun 2015		PD6-268	300 mcg/1.0 mL	May 2016
			PD6-272	480 mcg/1.6 mL	Jun 2016
1551016	Jan 2016		PD6-271	480 mcg/1.6 mL	Jun 2016

Table 78: PF-06881893 DP lots used for similarity assessment

Reviewer comments: For attributes that depend primarily on the DS manufacturing process, it is imperative to use results from DP lots manufactured solely from single DS lots to assure independence of the data points. Such attributes include potency, process- and product-related impurities and product-related substances that are produced during manufacture of the DS. Attributes such as aggregates or deamidated species that may be impacted by DP manufacture or long term storage of the DP at 2-8°C, may be assessed by comparing available data from DP lots, that may not necessarily be derived from independent DS lots.

The statistical analysis for Tier-1 attribute (i.e. potency) was conducted by the Agency CMC statistics reviewer, Dr. Wang. I conducted the statistical analysis for Tier 2 attributes. The acceptance criterion (AC) for Tier-2 is as follows: Analytical similarity is supported for the quality attribute if the 90 % test lot values fall within the quality range of mean $\pm$ 2SD or mean $\pm$ 3SD.

Lots selected for analytical similarity assessment range in age and are representative of both dose strengths, for both presentations (PFS and SDV). The number of US-licensed Neupogen and PF-06881893 lots used for the assessment of each attribute are sufficient to allow for the determination of meaningful similarity acceptance criteria.

Biological characterization studies

The functional assays are directly relevant to the established filgrastim therapeutic mechanism of action of binding to high affinity receptors located on neutrophils and inducing cell proliferation.

Tier 1: In-vitro biological activity-bioassay

The *in vitro* bioassay measures bioactivity of PF-06881893. The cell-based assay measures PF-06881893 concentration-dependent proliferation of murine myelogenous leukemia cells (NFS-60 cell line). These cells express the G-CSF receptor and are known to be induced to proliferate upon filgrastim binding and signal transduction. Proliferation is determined by measuring ATP generated in a dose-response curve. Data from this analysis are presented as relative potency (%) of PF-06881893 and US-licensed Neupogen based on a comparison to the PF-06881893 Reference Standard (b) (4).

The sponsor's statistical analysis for this attribute was initially based on the 26 lots of PF-066881893 DP manufactured from 14 different DS lots. However, FDA considers an "independent" lot to be a single DP lot produced from a single drug substance lot. Therefore in response to an information request sent to the sponsor on Dec 13, 2017, from the CMC Statistics reviewer, requesting re-evaluation of the equivalence testing for potency using the first DP lot manufactured from each DS lot, the sponsor performed Tier 1 statistical analysis of the bioassay results using data from independent lots.

Independent lots were identified as the first DP lot manufactured from each DS lot. The updated similarity assessment includes the comparison of 14 PF-06881893 independent lots (8 PFS and 6 SDV, ranging in age from 0-21 months) to 15 US licensed- Neupogen DP lots (7 PFS and 8 SDV). All US-licensed Neupogen lots tested were included in the analysis. These lots were selected across the shelf life of the product (ranging in age from 9 months to 27 months, at the time of testing) and representative of dose strengths, and presentations. The US-licensed Neupogen lots used also include the product lots 1050859 and 1060852 used in the comparative clinical studies.

A Tier 1 equivalence test was performed by the sponsor because biological activity is a high risk attribute based on the CQA assessment. This statistical equivalence was independently corroborated by the CMC Statistics reviewer Dr. Wang. Graphical representation of the relative potency results is shown in Figure 15 below. (For details refer to the CMC statistics review).

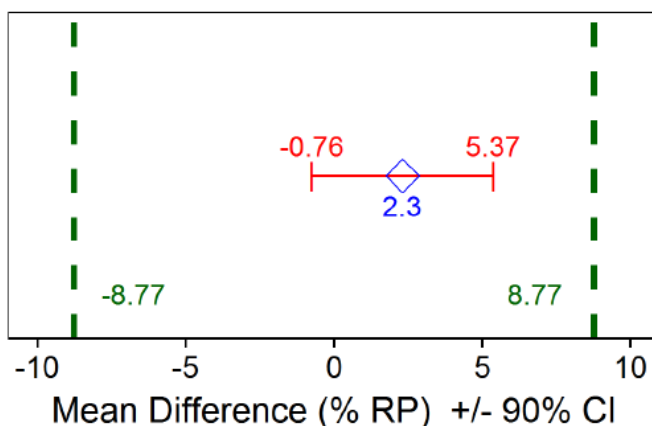


Figure 15: In vitro potency of PF-06881893 and US-licensed Neupogen

Reviewer comments: The 90% confidence interval of the mean difference, [-0.76, 5.37], is within the equivalence margins [-8.77, 8.77]. For additional details on this evaluation refer to the review by Dr. Wang. The results demonstrate that the in vitro potency for PF-06881893 is equivalent to that of US-licensed Neupogen. The US-licensed Neupogen lots used in the assessment are representative of the shelf life of the product and both dose strengths in both PFS and SDV presentations. The sponsor's justification for the use of 15 US-licensed Neupogen lots used in the assessment is adequate since the number and age of the lots used meet the requirement for using lots that represent both PFS and SDV presentations, span the age of the manufacturing history, and the number is sufficient to allow a meaningful statistical analysis.

The cell-based proliferation assay is a validated method used for routine release and stability testing of PF-06881893 DS and DP. The assay is suitable for its intended use as reviewed in 3.2.S.4.3, and for its use in the analytical similarity assessment between PF-06881893 and US-licensed Neupogen.

Tier 2: Receptor binding assay:

Binding of filgrastim to the G-CSF receptor is measured by the competitive receptor binding assay (CRBA). The assay measures the competitive binding of PF-06881893 or US-licensed Neupogen with biotin-labeled rhuG-CSF to an immobilized G-CSF receptor. Receptor binding is a high impact quality

attribute. As an orthogonal functional assay to the bioactivity testing, the CRBA characterization test is assigned to statistical Tier 2 for analysis. Sponsor provided data for 19 DP lots derived from 11 independent DS lots of PF-08881893, and 18 US-licensed Neupogen PFS lots, and 12 SDV lots ranging in age from 10-26 months. In an IR sent on May 10, 2018, the sponsor was asked to re-evaluate the quality attributes analyzed using Tier 2 (quality ranges) using the first DP lot manufactured from each DS lot. The receptor binding results are expressed as percent relative potency based on a direct comparison to the dose-response curves for the reference standard. Similarity is assessed by Tier 2 statistics based on a quality range using the multiplier $k=3$ (Mean $\pm$ 3xSD) and evaluating the proportion of PF-06881893 DP lots that fall within the quality range defined by the US-licensed Neupogen product results (82-115%; Table 79).

Test article	Number of lots	% relative potency					PF-06881893 lots within range
		Mean	S.D.	Min	Max	Quality Range	
PF-06881893	11	103.3	4.4	97	112		100%
US-licensed Neupogen	30	98.5	5.6	91	117	81.6- 115	

Table 79: Tier 2 statistical analysis summary for receptor binding by PF-06881893 and US-licensed Neupogen measured by CRBA

The receptor binding results for 100% of the PF-06881893 DP lots fall within the mean $\pm$ 3SD quality range defined by US-licensed Neupogen lots.

Reviewer comments: The results of the receptor binding assay support a demonstration that PF-06881893 is highly similar to US-licensed Neupogen.

Assay qualification: The receptor binding assay is a characterization assay (3.2.S.3.1). The sponsor has qualified the assay by assessing the performance of the method with respect to parameters and their acceptance criteria provided in assay qualification report LF-332-R-272-13 (Table 80).

Parameters	Target Value	Result
Specificity	Non-specific response <10%	No measured response
Linearity	60 - 130%	40 - 140%
Accuracy	80 - 120% Recovery in Linear Range	94 - 105% Recovery
Range	60 - 130%	40 - 140%
Precision		
Intra-Assay Repeatability	%CV < 30%	3.3 - 9.9%
Repeatability	%CV < 30%	6.7 - 9.2%
Intermediate Precision	%CV < 30%	8.1%
Robustness	Discussed in Report	See section 6.6

Table 80: Summary of CRBA assay qualification acceptance criteria and assay results

Reviewer comment: CRBA has been adequately qualified for its intended use.

Tier 2: Receptor binding affinity and

Tier 3: Receptor binding kinetics

Surface plasmon resonance (SPR) is an optical method for measuring the change in the refractive index at the surface of the immobilized receptor (G-CSF receptor Fc fusion protein) on a sensor chip when a solution containing the ligand (filgrastim) flows over the sensor. The assay measures the association and dissociation rates for measuring the kinetics of filgrastim binding to the receptor, and data are evaluated for analytical similarity between PF-06881893 and US-licensed Neupogen. Several kinetic binding parameters are determined, including on-rate k_{on} ($M^{-1}s^{-1}$) and off-rate, k_{off} (s^{-1}), respectively. The binding affinity constant, K_D , is expressed as a percentage relative to the PF-06881893 DP Reference Standard, and is measured as $K_D = k_{off}/k_{on}$.

Relative receptor binding affinity (Relative K_D) was assigned as a Tier 2 attribute for statistical analysis and analyzed by evaluating the proportion of PF-06881893 DP lots that fall within the quality range [mean $\pm$ (3 $\times$ standard deviations)] as defined by US-licensed Neupogen results and the bioassay specifications for PF-06881893 of 80-125%. In an IR sent on May 10, 2018, the sponsor was asked to re-evaluate the Tier 2 analytical quality attributes using the first DP lot manufactured from each DS lot. The Relative K_D results for 91% of the PF-06881893 DP lots fall within the quality range defined by the US-licensed Neupogen lots (Table 81).

	Number of lots	Mean relative K_D (%)	S.D. relative K_D (%)	Quality range	Lots within quality range
US-licensed Neupogen	17	103.18	9.1	75.9 to 130.5	
PF-06881893	11	106.18	11.4		10 of 11 lots, 91%

Table 81: Tier 2 assessment for relative K_D

Receptor binding affinity and kinetics parameters (k_{on} , k_{off} , and K_D) were assigned as Tier 3 attributes. Descriptive statistics were performed with the experimentally determined values of k_{on} , k_{off} , K_D for US-licensed Neupogen and PF-06881893 (Table 82).

	Number of lots	Mean	S.D.	Minimum	Maximum
k_{on} ($M^{-1}s^{-1} \times 10^6$)					
US-licensed Neupogen	17	3.84	0.49	3.0	4.6
PF-06881893	11	3.89	0.40	3.1	4.5
k_{off} ($s^{-1} \times 10^{-5}$)					
US-licensed Neupogen	17	7.33	0.95	5.8	8.3
PF-06881893	11	8.12	0.44	7.5	8.9

	K_D ($M \times 10^{-11}$)				
US-licensed Neupogen	17	1.91	0.18	1.6	2.2
PF-06881893	11	2.12	0.28	1.8	2.7

Table 82: Descriptive statistics of k_{on} , k_{off} , and K_D values

An overlay of the binding kinetic curve for 3 lots each of PF-06881893 DP and US-licensed Neupogen is shown in Figure 16.

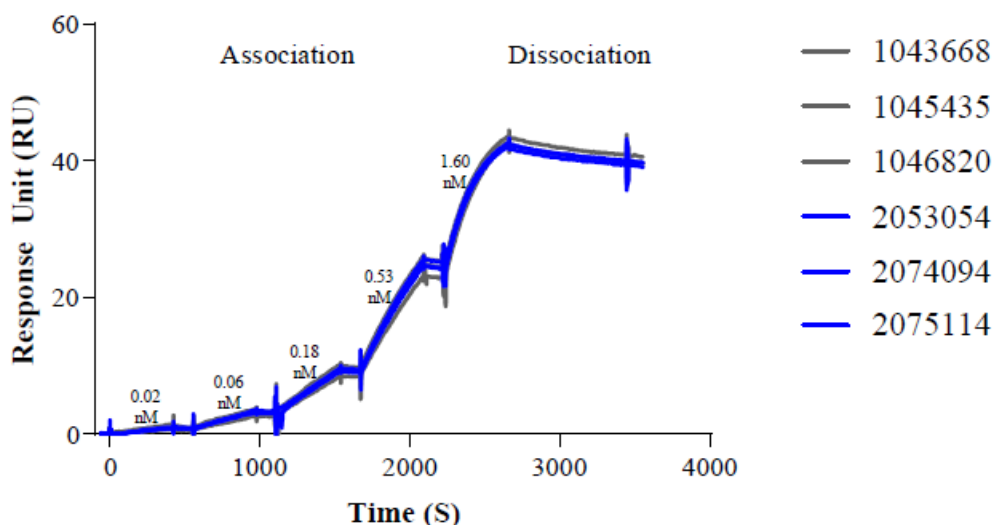


Figure 16: Overlay of receptor binding affinity and kinetics for 3 lots each of PF-06881893 and US-licensed Neupogen

Reviewer comments: The results of the relative receptor binding affinity assay for PF-06881893 DP and for US-licensed Neupogen support a determination that PF-06881893 is highly similar to US-licensed Neupogen, with 91% of the tested lots within the quality range. Lot 2576075 is the only lot with Relative K_D of 133% that is outside the quality range (upper limit 130.5). The lot met acceptance criteria for potency in the in vitro cell-based bioassay and CRBA so the slightly higher Relative K_D outside the range of US-licensed Neupogen lots, measured by this orthogonal assay, is not a cause for concern for product quality or analytical similarity. The observed mean and range of results for the 3 kinetic parameters k_{on} , k_{off} , and K_D , as well as the nearly superimposable binding kinetic curves indicate that there is no difference in the receptor binding affinity and kinetics of the filgrastim in PF-06881893 DP and US-licensed Neupogen. These results confirm that PF-06881893 and US-licensed Neupogen have similar affinities for receptor binding. These results support a determination that PF-06881893 is highly similar to US-licensed Neupogen..

Assay qualification: The SPR method is qualified as a characterization assay. A qualification report is provided in the BLA (LF-332-R-022-16 for the method protocol (b) (4)-332-04). The design for the kinetic analysis method is to run Single Cycle Kinetics using a Protein A chip, 1) to capture the recombinant human G-CSF-R Protein with the C-Fc Tag (rhGCSFR-Fc) as the ligand, and 2) to monitor the binding activity of Filgrastim injected with increasing concentration over the captured rhG-CSF-R within a single regeneration cycle. After the experimental run was complete, the results were analyzed

using the BIAcore Evaluation Software to determine the rate constants for association (k_a) and dissociation (k_d), as well as equilibrium dissociation constant (K_D).

Reviewer comment: I reviewed method qualification report LF-332-R-022-16. The method was adequately qualified for specificity, precision, robustness, and stability indicating characteristics.

Drug product attribute

Tier 2: Protein concentration

The sponsor performed analytical similarity assessment for protein concentration between PF-06881893 and US-licensed Neupogen. Protein concentration is determined by measuring absorbance using a UV-Vis spectrophotometer. The results are normalized to percent of the labeled concentration for each dose strength for statistical comparison. The initial BLA submission contained data and results for protein concentration subjected to Tier 1 equivalency testing. On Jan 3, 2018, FDA sent an IR to the sponsor requesting that protein concentration be analyzed as a Tier 2 attribute. Since the response to the IR did not use independent DP lots for the Tier 2 evaluation, in another IR sent on May 10, 2018, the sponsor was asked to re-evaluate the quality attributes evaluated by Tier 2 statistical test using the first DP lot manufactured from each DS lot. Results of the analysis are summarized in Table 83.

Test article	Number of lots	Normalized protein concentration					PF-06881893 lots within range
		Mean	S.D.	Min	Max	Quality Range	
PF-06881893	14	100.8	1.53	99.2	104.3		93%
US-licensed Neupogen	24	100.9	1.1	98.7	103.2	97.6-104.2	

Table 83: Tier 2 assessment of protein concentration for PF-06881893

In the Jan 3 IR, the sponsor was also asked to provide data demonstrating that PF-06881893 meets the statutory same strength requirement as US-licensed Neupogen. Same strength can be demonstrated with total deliverable (protein) content which is a function of protein concentration. The total deliverable content expressed as μg per container is calculated as follows:

$$\text{Total deliverable content } (\mu\text{g/container}) = \text{protein concentration } (\mu\text{g/mL}) \times \text{deliverable volume (mL)}$$

In their 12th January response to the IR the sponsor provided deliverable volume results for each of the PF-06881893 DP lots, and the US-licensed Neupogen product used in the analysis. These data are compiled and summarized in Table 84.

Test Article	Lot Number	Dose Strength	Age at Time of Test (Months)	Protein Concentration (µg/mL)	Normalized Protein Concentration (%)	Deliverable Volume (mL)	Normalized Deliverable Volume (%)	Deliverable Content (mcg per container)	Normalized Deliverable Content (%)
PF-06881893 PFS	2854103	300 mcg/0.5 mL	20	608	101.3	0.516	103.2	314	104.6
	2056024	300 mcg/0.5 mL	16	621	103.5	0.509	101.8	316	105.4
	2053054	300 mcg/0.5 mL	13	607	101.2	0.503	100.6	305	101.8
	2073094	300 mcg/0.5 mL	9	626	104.3	0.506	101.2	317	105.6
	2074114	300 mcg/0.5 mL	7	609	101.5	0.503	100.6	306	102.1
	2572075	300 mcg/0.5 mL	0	595	99.2	0.526	105.2	313	104.3
	2574075	300 mcg/0.5 mL	0	596	99.3	0.532	106.4	317	105.7
	2576075	300 mcg/0.5 mL	1	597	99.5	0.530	106	316	105.5
PF-06881893 SDV	EX6-049A	300 mcg/1.0 mL	1	300	100.0	1.089	108.9	327	108.9
	EX6-084A	300 mcg/1.0 mL	0	299	99.7	1.043	104.3	312	104
	PD6-267	300 mcg/1.0 mL	2	301	100.3	1.084	108.4	326	108.8
	PD6-268	300 mcg/1.0 mL	2	299	99.7	1.089	108.9	326	108.5
	PD6-270	480 mcg/1.6 mL	1	302	100.7	1.705	106.6	515	107.3
	PD6-271	480 mcg/1.6 mL	1	302	100.7	1.694	105.9	512	106.6
US-licensed Neupogen PFS	1038350	300 mcg/0.5 mL	30	608	101.3	0.526	105.2	320	106.6
	1038088	480 mcg/0.8 mL	30	616	102.7	0.810	101.3	499	104
	1042620	480 mcg/0.8 mL	23	606	101.0	0.822	102.8	498	103.8
	1042949	300 mcg/0.5 mL	26	608	101.3	0.521	104.2	317	105.6
	1045433	300 mcg/0.5 mL	20	607	101.2	0.523	104.6	317	105.8
	1045435	480 mcg/0.8 mL	22	619	103.2	0.822	102.8	509	106
	1047463	480 mcg/0.8 mL	17	613	102.2	0.814	101.8	499	104
	1050859	480 mcg/0.8 mL	18	611	101.8	0.833	104.1	509	106
	1051786	300 mcg/0.5 mL	13	598	99.7	0.524	104.8	313	104.6
	1056459	480 mcg/0.8 mL	9	604	100.7	0.835	104.4	504	105.1
	1057139	300 mcg/0.5 mL	9	607	101.2	0.536	107.2	325	108.5
	1060852	480 mcg/0.8 mL	15	615	102.5	NP	NP	NP	NP
US-licensed	1040808	300 mcg/1.0 mL	26	303	101.0	NP	NP	NP	NP
	1041874	480 mcg/1.6 mL	27	302	100.7	1.713	107.1	517	107.8
	1041879	480 mcg/1.6 mL	24	297	99.0	1.711	106.9	508	105.9
	1043668	300 mcg/1.0 mL	21	296	98.7	1.084	108.4	321	107

Neupogen SDV	1046820	300 mcg/1.0 mL	23	303	101.0	1.075	107.5	326	108.6
	1045436	480 mcg/1.6 mL	20	300	100.0	1.729	108.1	519	108.1
	1040884	300 mcg/1.0 mL	27	300	100.0	1.074	107.4	322	107.4
	1051319	300 mcg/1.0 mL	14	301	100.3	1.075	107.5	324	107.9
	1051323	480 mcg/1.6 mL	17	302	100.7	1.722	107.6	520	108.3
	1053036	300 mcg/1.0 mL	12	302	100.7	NP	NP	NP	NP
	1056454	300 mcg/1.0 mL	11	300	100.0	1.084	108.4	325	108.4
	1058392	480 mcg/1.6 mL	10	300	100.0	1.708	106.8	NP	NP

Table 84: Evaluation of protein content of PF-06881893 and US-licensed Neupogen NP: Not provided

The comparison of normalized deliverable protein content for the PF-06881893 DP PFS and SDV and US-licensed Neupogen PFS and SDV lots is summarized in Table 85.

Test Article	Number of Lots Tested	Normalized Deliverable Content (%)			
		Mean	Standard Deviation	Min	Max
PF-06881893 DP PFS (all)	16	103.1	1.84	100.7	105.7
PF-06881893 DP PFS PPQ	6	103.9	1.52	102.1	105.7
Neupogen PFS	11	105.4	1.39	103.8	108.5
PF-06881893 DP SDV	10	107.4	1.54	104.0	109.0
Neupogen SDV	9	107.7	0.84	105.9	108.6

Table 85: Summary of normalized protein content results for PF-06881893 and US-licensed Neupogen

The normalized deliverable content results for PF-06881893 PFS and SDV are consistent with those of US-licensed Neupogen PFS and SDV.

Reviewer comments: Protein concentration of PF-06881893 meets the acceptance criteria when subjected to the Tier 2 statistical analysis, and the data provided support a demonstration that PF-06881893 and US-licensed Neupogen are highly similar. 93% PF-06881893 lots are within the quality range established by US-licensed Neupogen. The 1 lot outside of the quality range had a normalized protein concentration of 104.3%, a value just above the upper quality range limit of 103.2% for protein concentration. However, with a normalized deliverable content of 105.6%, the lot is within the normalized deliverable content limits (Table 85). PF-06881893 is demonstrated to meet the statutory same strength requirement through an evaluation of protein concentration ($\mu\text{g/mL}$) and total deliverable (protein) content (μg per container).

Product-related substances and product-related impurities

Analytical similarity assessment is performed on PF-06881893 DP and the US licensed Neupogen to compare the product-related substances and impurities including total filgrastim related proteins, total charge variants, HMWS, molecular weight variants, oxidation, deamidation, isomerization, succinimide, reduced filgrastim, and other filgrastim related species. The methods and statistical analysis tiers are summarized in Table 86 below. Total charge variants, total filgrastim-related proteins, and Met122, Met127 variants of filgrastim, in PF-06881893 and US-licensed Neupogen are evaluated by Tier 2 analysis for analytical similarity evaluation. Analytical similarity assessment of individual filgrastim variants is evaluated by Tier 3 statistical evaluation.

Assay	Attribute	Statistical analysis tier
RP-HPLC	Total filgrastim-related proteins	2
	Oxidation (Met122, Met127)	2
	Deamidation	3
	Succinimide	3
	Reduced filgrastim	3

CEX	Total charge variants	2
	Deamidation	3
SEC	HMWS	3
SDS-PAGE	Molecular weight variants	3
CE-SDS		
Glu-C peptide mapping	Oxidation (Met1, Met 127, Met 138, Trp59, Trp119)	3
	Deamidation (Gln 21, Gln71, Gln91, Gln108, Gln132)	3
	Isomerization (Asp28)	3
	Other filgrastim-related species	3

Table 86: Summary of product-related substances and impurities analytical similarity assessment

Tier 2: Total charge variants (CEX)

The CEX method is used to analyze filgrastim charge variants in PF-06881893 and US-licensed Neupogen. Acidic variants elute prior to the filgrastim main peak and include deamidated species. Basic variants elute after the filgrastim main peak and include aspartate succinimide species. For analytical similarity assessment, the values are analyzed using Tier 2 statistics using the quality range determined using US-licensed Neupogen. The submission includes a table with data from each individual lot (Table 3.2 R.5.5-35) and a table with primary statistics (reproduced below). In the initial assessment the sponsor had evaluated all DP lots used in this experiment, including DP lots that were derived from the same DS lot. In an IR sent on May 10, 2018, the sponsor was asked to re-evaluate the quality attributes evaluated by Tier 2 statistical test using the first DP lot manufactured from each DS lot. Results of this re-analysis are presented in Table 87 and Figure 17.

Test articles	Number of lots	Peak area (%)					PF-06881893 lots within quality range
		Mean	S.D.	Minimum	Maximum	Quality Range	
PF-06881893	14	1.0	0.40	0.4	1.6		100%
US-licensed Neupogen	38	1.4	0.33	0.7	2.0	0.4-2.3	

Table 87: Summary of total charge variants results for PF-06881893 and US-licensed Neupogen (Table 3.2.R.5.5-36 in BLA)

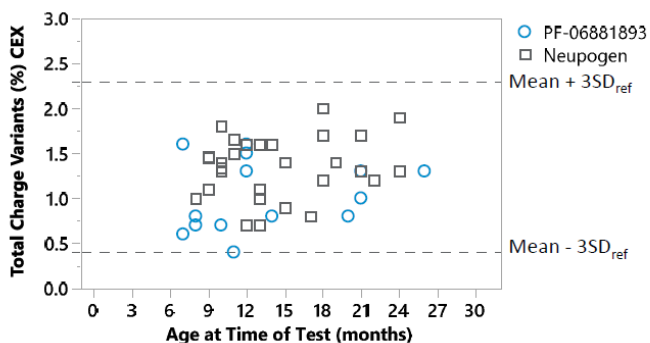


Figure 17: Tier 2 analysis of total charge variants in PF-06881893 DP and US-licensed Neupogen

Reviewer comments: As is evident from the graphical illustration, there is similar content of charge variants in PF-06881893 compared to US-licensed Neupogen with all 14 lots of the PF-06881893 product analyzed, falling within the 3xSD quality range for US-licensed Neupogen lots. The amount of charge variants appears to increase over the shelf-life of the product.

The CEX method (MPC009005) was developed in-house for quantitation of filgrastim charge variants. It has been validated as a release and stability testing method (3.2.S.4.3).

Tier 2: Total filgrastim-related proteins (RP-HPLC)

The RP-HPLC assay is conducted as validated by the sponsor for release and stability testing, and product-related substances and impurities are identified by their relative retention time, monoisotopic mass, and MS/MS fragmentation. The BLA includes a table with measurement of total filgrastim related proteins from each individual lot (3.2.R.5.5-33) and a table with summary statistics. To assess analytical similarity of the proposed biosimilar lots, the sponsor used statistical Tier 2 quality range methodology based on test results from the US-licensed Neupogen lots, as shown in Figure 18 below. Total filgrastim-related proteins were compared between 22 PF-06881893 DP lots and 27 US-licensed Neupogen lots. However, since not all 22 DP lots used in this analysis were independently derived from individual DS lots, in an IR sent on May 10, 2018, the sponsor was asked to re-evaluate the Tier 2 analytical quality attributes using the first DP lot manufactured from each DS lot. Results of this re-analysis are presented in Figure 18 and Table 88. PF-06881893 lots used at the time of testing ranged in age from 7-33 months, and US-licensed lots ranged in age from 8-30 months at the time of testing.

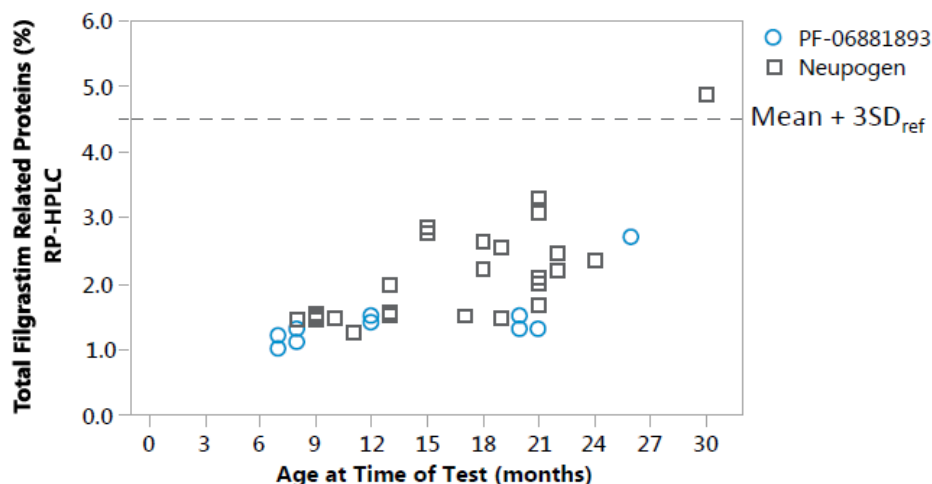


Figure 18: Tier 2 analysis of Total filgrastim-related proteins result for PF-06881893 DP and US-licensed Neupogen

The relative percentage of total filgrastim related proteins in all PF-06881893 DP lots tested was within the quality range defined by US-licensed Neupogen lots (Table 89).

Test articles	Number of lots	Peak area (%)					PF-06881893 lots within quality range
		Mean	S.D.	Minimum	Maximum	Quality Range	
PF-06881893	12	1.6	0.60	1.0	2.9		100%
US-licensed Neupogen	27	2.1	0.79	1.25	4.89	0-4.5	

Table 88: Summary of total filgrastim related protein results for PF-06881893 and US-licensed Neupogen

Results demonstrate that the level of total filgrastim related proteins for PF-06881893 DP is highly similar to that of US-licensed Neupogen.

Reviewer comments: The amount of filgrastim-related proteins appear to increase over the shelf-life of the product at recommended storage. Data support similar purity by RP-HPLC between PF-06881893 and US-licensed Neupogen because 100% of the PF-06881893 lots fell within the quality range.

The RP-HPLC method has been validated and reviewed in 3.2.S.4.3.

Tier 2: Oxidation at Met122, Met127 (RP-HPLC)

The relative percentages of Met122 oxidized filgrastim (RRT 0.81-0.85) and Met127 oxidized filgrastim (RRT 0.96-0.97) were measured by RP-HPLC. For the Met122 oxidized species, all 22 lots of PF-06881893 and 15 of 27 lots of US-licensed Neupogen tested were below the method LOQ (0.2%). Therefore the sponsor did not perform any statistical analysis. The comparison of Met127 oxidized species was subjected to Tier 2 statistical analysis and a quality range determined using US-licensed

Neupogen lots. In an IR sent on May 10, 2018, the sponsor was asked to re-evaluate the Tier 2 analytical quality attributes using the first DP lot manufactured from each DS lot. Results of this re-analysis are provided below. The LOQ and LOD for the RP-HPLC method are 0.20% and 0.05%, respectively. A value of 0.00% was assigned for <LOD result and 0.10% for <LOQ result for the purpose of graphical presentation. Results below LOQ or LOD were not included in calculating the statistical parameters mean and standard deviation (Table 89).

Test articles	Number of lots	Peak area (%)					PF-06881893 lots within quality range
		Mean	S.D.	Minimum	Maximum	Quality Range	
PF-06881893	12	0.8	0.11	0.6	1.0		92%
US-licensed Neupogen	27	0.7	0.08	0.48	0.84	0.46-0.94	

Table 89: Summary of Met127 Oxidized Filgrastim (RRT 0.96-0.98) for PF-06881893 DP and US-licensed Neupogen by RP-HPLC

Tier 3: Oxidation of Met1, Met 127, Met 138, Trp59, Trp119 (Glu-C peptide mapping)

Tabulated and graphical comparisons of data for 11 lots of PF-06881893 and 11 lots of US-licensed Neupogen are provided for percent peak area of Met 1, Met127, Met138, and Trp119, shown to be the major sites of oxidation by this assay (Table 90).

Test article	Modification	Mean (%)	S.D. (%)	Minimum(%)	Maximum(%)
PF-06881893	Met1-Ox	2.9	0.58	2.1	3.7
US-licensed Neupogen		2.9	0.34	2.4	3.7
PF-06881893	Met127-Ox	1.7	0.07	1.6	1.8
US-licensed Neupogen		1.8	0.23	1.4	2.3
PF-06881893	Met138-Ox	1.7	0.13	1.4	1.9
US-licensed Neupogen		1.7	0.22	1.4	2.2
US-licensed Neupogen	Trp119-Ox	0.6	0.11	<0.5	0.8
PF-06881893		0.6	0.15	<0.53.7	1.0

Table 90: Oxidation by Glu-C peptide mapping for PF-06881893 and US-licensed Neupogen. Results <0.5 were not included in Mean and SD calculation. Trp59-Ox and Trp59-2Ox were all ≤LOD (Revised Table 3.2.R.5.5-43 in BLA)

Reviewer comments: Oxidized variants are evaluated by two assays as summarized below (Table 91).

Oxidation site	Analysis
Met1	Glu-C peptide mapping
Met122	RP-HPLC; Assay qualification demonstrated Glu-C peptide mapping procedure induced oxidation
Met127	RP-HPLC, Glu-C peptide mapping
Met138	Glu-C peptide mapping
Trp59-1Ox	Glu-C peptide mapping, <0.5%
Trp59-2Ox	Glu-C peptide mapping, <0.5%
Trp119	Glu-C peptide mapping

Table 91: Assays for evaluating oxidized variants of PF-06881893

RP-HPLC analysis shows that 92% of PF-06881893 lots are within the quality range established by the Met127 values from the US-licensed Neupogen samples tested. Lot 2574075 was just outside the quality range at 1% Met127 levels, a value just above the upper quality range limit of 0.9%. This lot was also used in the analytical similarity assessment of potency. Met127 oxidation levels of this lot do not have an impact on potency. Therefore at 1% Met127 level, there is no cause for concern for product quality or analytical similarity for lot 2574075. The amount of Met122 oxidized filgrastim for all lots examined was below LOQ of the method.

Glu-C peptide mapping data indicate that there are no significant differences in the level of Met and Trp oxidized species in PF-06881893 versus US-licensed Neupogen. The data support a determination that PF-06881893 and US-licensed Neupogen are highly similar.

Tier 3: Deamidation (RP-HPLC)

Two RP-HPLC peaks correspond to deamidated species: (1) One peak co-elutes at RRT 1.02 to 1.03 with the partially reduced protein, and (2) the second peak elutes at RRT 1.08 (for representative chromatogram, refer to Figure 23 on page 147). The site-specific deamidated species are identified and quantified by Glu-C peptide mapping. The RP-HPLC analysis allows for the analytical similarity comparison of the relative percentages of deamidated/partially reduced filgrastim which co-elute at RRT 1.02 and 1.03, and the deamidated filgrastim that elutes at RRT 1.08. The sponsor provided data for each of the 22 PF-06881893 lots, and also for the 27 US-licensed Neupogen lots tested and analyzed in this comparisons.

For the purposes of analytical similarity, this attribute is evaluated by comparison of min-max ranges (Tier-3) by the sponsor (Table 92).

Test articles	Number of lots tested	Deamidated filgrastim	Peak area (%)			
			Mean	S.D.	Minimum	Maximum
PF-06881893	22	RRT 1.02-1.03	0.5	0.33	0.3	1.6
US-licensed Neupogen	27		0.5	0.43	<LOQ	2.02
PF-06881893	22	RRT 1.08	0.3	0.06	<LOQ	0.4

US-licensed Neupogen	27		0.4	0.10	0.20	0.67
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Table 92: Summary of deamidated filgrastim similarity assessment

Tier 3: Deamidation (CEX)

There are two CEX acidic variants that contain deamidated filgrastim. Data are provided for PF-06881893 Acidic peak 1 and 2, in comparison to the levels in US-licensed Neupogen. In general very few PF-06881893 samples show acidic peak 2 and most results are below LOQ (0.20%). Tier 3 analysis with minimum and maximum range has been provided by the sponsor, as well as the graphical representation depicted below (Figure 19).

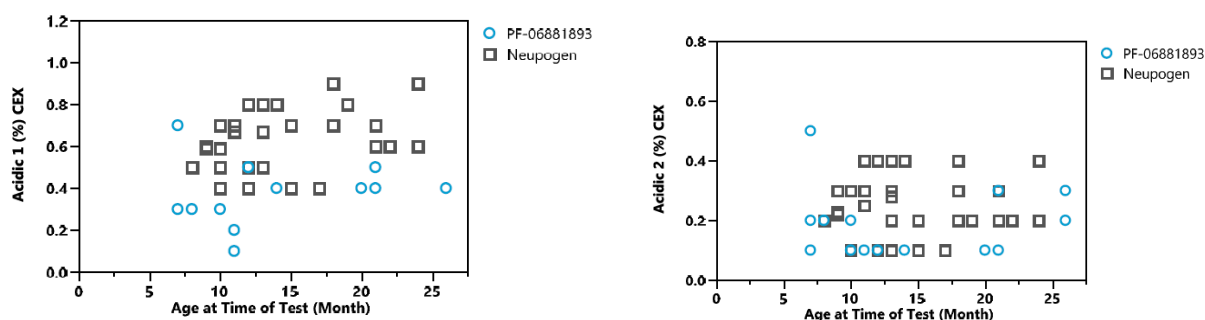


Figure 19: Comparison of deamidated filgrastim CEX results. Left: Acidic peak 1, Right: Acidic peak 2

Tier 3: Deamidation (Glu-C peptide mapping)

Site-specific deamidation of Gln12, Gln21, Gln68, Gln71, Gln91, Gln108, Gln120, Gln132, Gln159, and Gln174 was identified and evaluated by the RP-UPLC-MS Glu-C peptide mapping method. The results for Gln12, Gln68, Gln120, Gln159, and Gln174 were determined by the sponsor to be below the reporting limit of 0.5% for both PF-06881893 and US-licensed Neupogen. Of the remaining sites of deamidation, Gln71, Gln91, Gln108, Gln132, data are provided from the Glu-C peptide mapping that shows deamidation levels of these residues for all lots of PF-06881893 and some lots of US-licensed Neupogen that are <0.5%. Gln21 deamidation is determined to be the primary deamidated form in PF-06881893 by this assay and results for Gln21 deamidation levels from 11 lots of each product are provided in the submission. Tier 3 statistical analysis reporting minimum and maximum values is used for analytical similarity assessment (Table 93).

Test article	Number of lots tested	Mean (%)	S.D (%)	Minimum (%)	Maximum(%)
PF-06881893	11	0.7	0.1	<0.5	0.8
US-licensed Neupogen	11	0.8	0.21	0.5	1.1

Table 93: Summary of deamidation (peptide mapping) results. Results <0.5 were not included in Mean and SD calculation

Reviewer comments: The results from RP-HPLC, CEX, and Glu-C peptide mapping methods support that there are no significant differences in the types of deamidated species between PF-06881893 and US-licensed Neupogen. The slightly lower content of deamidated species in PF-06881893 lots is acceptable because it would favor purity of the product.

The assays used to evaluate deamidated species are validated for their intended use (3.2.S.4.3).

Tier 3: Succinimide (RP-HPLC)

Formation of succinimide intermediates at Asp residue(s) in PF-06881893 and US-licensed Neupogen was evaluated by RP-HPLC. Characterization of this variant is discussed in the impurities section (3.2.S.3.1). Filgrastim containing an aspartate succinimide is separated from the filgrastim main peak in the RP-HPLC chromatograms at RRT 0.88 to 0.89. All 22 PF-06881893 lots and 23 out of 27 US-licensed Neupogen lots tested below limit of quantitation, therefore no statistical analysis was conducted for this attribute.

Reviewer comments: RP-HPLC analysis of the amount of filgrastim succinimide support a demonstration that PF-06881893 is highly similar to US-licensed Neupogen.

Tier 3: Reduced filgrastim (RP-HPLC)

The filgrastim protein contains two intra-molecular disulfide bonds between Cys37-Cys43 and Cys65-Cys75. Partially and fully reduced species are detected by RP-HPLC. The partially reduced Cys37-Cys43 species co-elutes with a deamidated species, and the analytical similarity data are discussed in Table 93 above. The fully reduced species with both disulfide bonds reduced to free Cys residues elutes at RRT 1.04 -1.06. All except one lot of PF-06881893 demonstrated results below the assay LOQ, where US-licensed Neupogen showed low levels of the reduced filgrastim species, as shown in Table 94 below.

Test articles	Number of lots	Peak area (%)			
		Mean	S.D.	Minimum	Maximum
PF-06881893	22	NA	NA	<LOQ	0.2
US-licensed Neupogen	27	0.3	0.08	0.22	0.54

Table 94: Summary of reduced filgrastim analytical similarity results

Reviewer comments: Data demonstrates that there are no significant differences in the amount of reduced filgrastim between PF-06881893 and US-licensed Neupogen.

Tier 3: HMWS (SEC)

The Sponsor uses the SEC procedure validated for release and stability testing, to evaluate high molecular weight species (HMWS). Peaks for aggregate, oligomer 1, oligomer 2, dimer, and the main protein monomer are assigned using the RRTs defined in the validation assay. Relative percentage of each species is based on the species peak area relative to total peak area for the chromatogram, and the Sponsor reports that the method LOQ is 0.2%. The submission tabular data with percent Total HMWS

results from 26 lots of PF-06881893 (7-26 months) and 38 lots of US-licensed Neupogen (8-24 months). HMWS data by SEC were planned to be analyzed as a Tier 3 attribute, however with most results below the LOQ of the SEC method (0.20%), formal statistical analyses were not conducted (Figure 20).

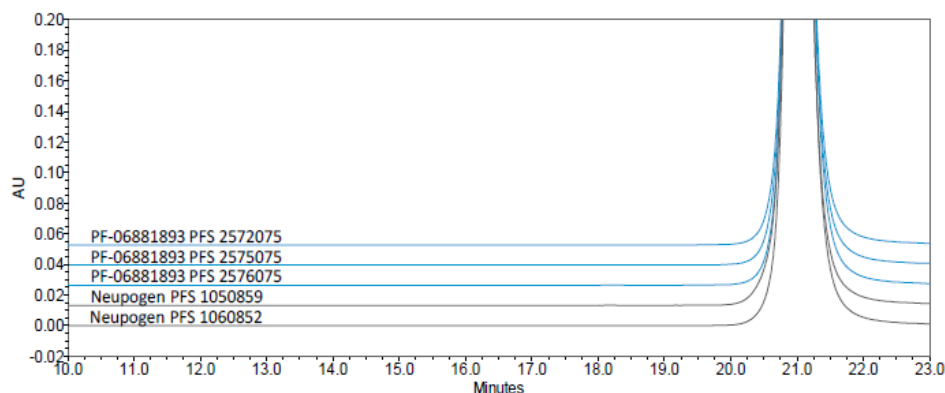


Figure 20: Overlay of representative SEC chromatograms in PFS presentation

Reviewer comments: 7/38 lots of US-licensed Neupogen and 2/26 lots of PF-06881893 have reportable HMWS ranging from 0.21%-0.54%, and 0.2%-0.5% respectively. The low levels of HMWS result are consistent with data from the orthogonal AUC-SV method used in tertiary structure determination. Data indicate that there are no significant differences in the levels of HMWS between lots of PF-06881893 and lots of US-licensed Neupogen.

In the assay validation section (3.2.S.4.3), the SEC assay has been reviewed and demonstrated to be suitable for its intended use.

Tier 3: Molecular weight variants (SDS-PAGE)

The Sponsor uses the SDS-PAGE procedure as validated by them, to evaluate molecular weight variants. The method is conducted with silver staining under reducing and non-reducing denaturing conditions. Data are evaluated using a limit test, which requires no impurity band more intense than the principal band obtained from the diluted 2% sample solution. Tabular data are provided in the submission for 16 lots of PF-06881893 and 18 lots of US-Neupogen (9 PFS + 9 SDV), with a result described as “complies” provided for all lots indicating that the limit test acceptance criteria are met. Representative gels are also provided. The analysis was assigned as a Tier 3 attribute, but since the method is a limit test with a qualitative output, no formal statistical analysis is performed.

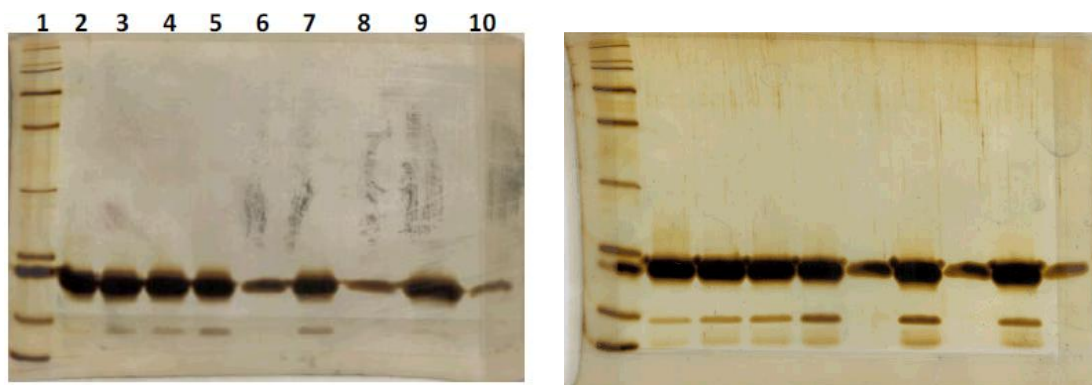


Figure 21: Representative SDS-PAGE gel images L: reduced, R: non-reduced. 1: MW ladder, 2: Neupogen 1064709, 3: RS (b) (4) 4: RS, 5: SDV PD6-269 (100%), 6: SDV PD6-269 (2%), 7: PFS 2575075 (100%), 8: PFS2575075 (2%), 9: Neupogen SDV 1056454 (100%), 10: Neupogen 1056454 (2%); (Figure 3.2.R.5.5-65 in BLA)

Reviewer comments: The results of the analysis showed no significant differences in band patterns between PF-06881893 and US-licensed Neupogen lots with no visible additional bands. The low molecular weight species (LMWS) of approximately 14k Da is a method induced artifact due to boiling the sample for 5 minutes.

In the assay validation section (3.2.S.4.3), the SDS-PAGE assay has been reviewed and demonstrated to be suitable for its intended use.

Tier 3: Molecular weight variants (CE-SDS)

This assay is used as an orthogonal assay for evaluating molecular weight variants beyond the visual comparisons allowed by SDS-PAGE. Molecular weight variants are separated under reducing or non-reducing conditions. There were no detectable Molecular Weight Variants in any of the 12 lots of PF-06881893 DP (4-15 months age at time of testing) and 10 lots of US-licensed Neupogen (13-30 months age at the time of testing) tested by reducing and non-reducing CE-SDS. Molecular weight variants by CE SDS was assigned as a Tier 3 attribute but no statistical analysis was performed since all results were below the method LOD of 0.1%.

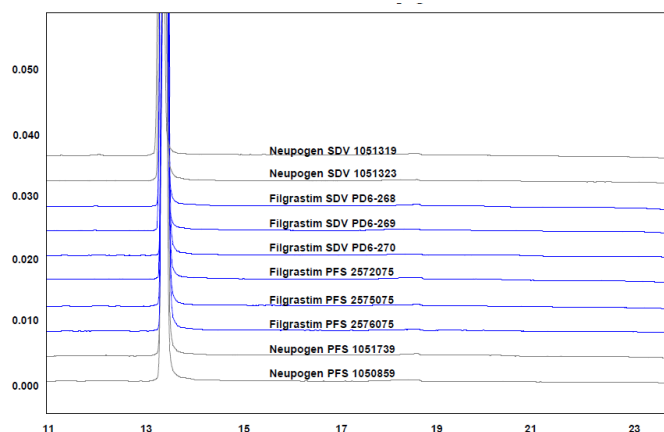


Figure 22: Example of reducing CE-SDS electropherogram of PF-06881893 and US-licensed Neupogen (Figure 3.2.R.5.5-66 in BLA

Reviewer comments: *There are no significant differences in the peak profile between PF-06881893 lots showed and US-licensed Neupogen lots, no visible additional peaks are detected.*

Assay qualification: Reducing and non-reducing CE-SDS assays have been qualified. Qualification reports for both assays, LF-332-R-140-17, and LE-332-R-141-17, are provided in the BLA.

Reviewer comment: *The assays are qualified for assay specificity, precision, linearity and range, LOD and LOQ, and assay robustness. Both assays are suitable for their intended use.*

Tier3: Isomerization (Glu-C peptide mapping)

Isomerization of Asp28 was identified and quantified by Glu-C peptide mapping. Results from 11 lots of each product were provided in the submission, and were below the reporting limit of the method <0.5%.

Reviewer comments: *The results show no significant differences in levels of isoAsp28 in the lots of PF-06881893 (<0.5%) and of US-licensed Neupogen evaluated by this assay.*

Tier 3: Other filgrastim related species (Glu-C peptide mapping)

N-terminal truncations (Des-Met1, Des-Met1-Thr2, and Des-Met1-Gly5), formylation (fMet1), and amino acid substitutions (Asp110 to Glu and Gly150 to Glu) were identified at low levels in in-process DS samples, but not detected in PF-06881893 DP. 11 lots of PF-06881893 (4-32 months) and 11 lots of US-licensed Neupogen (13-30 months age at the time of testing) were evaluated for other filgrastim-related species. Levels of Des-Met1-Thr2, and Des-Met1-Gly5, fMet1, and amino acid substitutions (Asp110 to Glu and Gly150 to Glu) were all <LOQ. All PF-06881893 lots showed < LOQ Des-Met1 levels as well, and US-licensed Neupogen lots gave levels <0.5-0.7%

Reviewer comments: *Levels of these other filgrastim related species in PF-06881893 DP are not significantly different from those of US-licensed Neupogen. Statistical analysis was not performed for these other filgrastim related species since results of all PF-06881893 DP lots and most of the US-licensed Neupogen lots were below the reporting limit (0.5%) of the method.*

Host cell protein ELISA (no Tier assigned)

The Sponsor measures *E. coli* HCP using a quantitative sandwich ELISA with pooled polyclonal anti-HCP antibodies generated from several sheep immunized with cell lysate supernatant of a null fermentation run. The anti-HCP antibody pool was characterized to show reactivity to broad range *E. coli* proteins, but not reactive to recombinant human G-CSF. Table below provides results from testing 3 lots of PF-06881893 and 5 lots of US-licensed Neupogen for HCP. The results for PF-06881893 DP are below the method limit of quantification (LOQ) of 0.5 ng/mL. The HCP results for US-licensed Neupogen from 0.51 to 3.97 ng/mL. The HCP concentration in units of ng/mg is also provided for US-licensed Neupogen lots for which the protein content was measured (Table 95).

Test article	Lot number	Age	Host cell protein content
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			ng/mL	ng/mg
PF-06881893	2075114	11	<LOQ	
	2575075	3	<LOQ	
	2576075	3	<LOQ	
US-licensed Neupogen PFS	1040887	30	3.97	N/A
	1042620	27	2.88	4.75
	1042949	30	2.65	4.36
US-licensed Neupogen SDV	1040808	30	0.51	1.68
	1041874	29	0.69	2.28

Table 95: HCP levels in PF-06881893 and US-licensed Neupogen

Host cell DNA qPCR (no Tier assigned)

Host cell DNA concentration is measured using quantitative PCR with primers and a probe specific to the DNA Polymerase I gene of *E. coli* BL21. Table 96 below provides results.

Test article	Lot number	Age	Host cell DNA (ng/mL)
PF-06881893	2075114	11	≤LOQ
	2575075	3	≤LOQ
	2576075	3	≤LOQ
US-licensed Neupogen PFS	1040887	30	≤LOQ
	1042620	27	≤LOQ
	1051786	17	≤LOQ
US-licensed Neupogen SDV	1040808	30	≤LOQ
	1041874	29	≤LOQ

Table 96: Host cell DNA analysis of PF-06881893 and US-licensed Neupogen

Reviewer comments: Process impurities are not expected, or required, to match between proposed biosimilar and reference products. In both assays, the measured amount of host cell impurities in PF-06881893 was low and levels were not significantly different from those of US-licensed Neupogen. Data presented suggest a low risk for HCP or Host Cell DNA impurities to negatively impact PF-06881893 product quality.

Validation of assay to measure host cell protein and DNA is reviewed in 3.2.S.4.3

Structural analysis: Primary Structure

The analysis of the primary structure includes verification that the primary sequence of PF-06881893 is identical to US-licensed Neupogen, as well as confirmation of the presence of expected cysteine residues and intramolecular disulfide bonds by Intact Mass and Free Thiol analysis.

Tier 3: Glu-C peptide mapping

Amino acid sequence is evaluated by Glu-C peptide mapping coupled with reversed phase-ultra high performance liquid chromatography-mass spectrometry (RP-UPLC-MS). Proteolytic digestion is conducted under reducing conditions that do not allow for confirmation of disulfide linkages. The sponsor evaluated resulting peptides to confirm that the mass of each peptide is consistent with the theoretical sequences of filgrastim. The method yields 8 principal fragments (Table 28 in Section 3.2.S.3.1), plus multiple low frequency fragments due to semi-specific cleavage. The Glu-C peptide map provides 100% amino acid sequence coverage. Comparative TICs demonstrating the RP-UPLC separation of peptides after Glu-C digestion have been provided. 11 PF-06881893 lots (8 PFS, 3 SDV) and 11 US-licensed Neupogen lots (6 PFS and 5 SDV) have been used in the assessment. The fragmentation pattern is unique to the specific amino acid sequence and based on the amino acid sequence, relative retention time (RRT), and monoisotopic mass of the peptides generated by Glu-C digestion, data confirm that the peptide sequence exactly matches the theoretical sequence. Comparative TIC showing the RP-UPLC separation of peptides generated by Glu-C digestion of PF-06881893 and US-licensed Neupogen lots are shown in Figure 23.

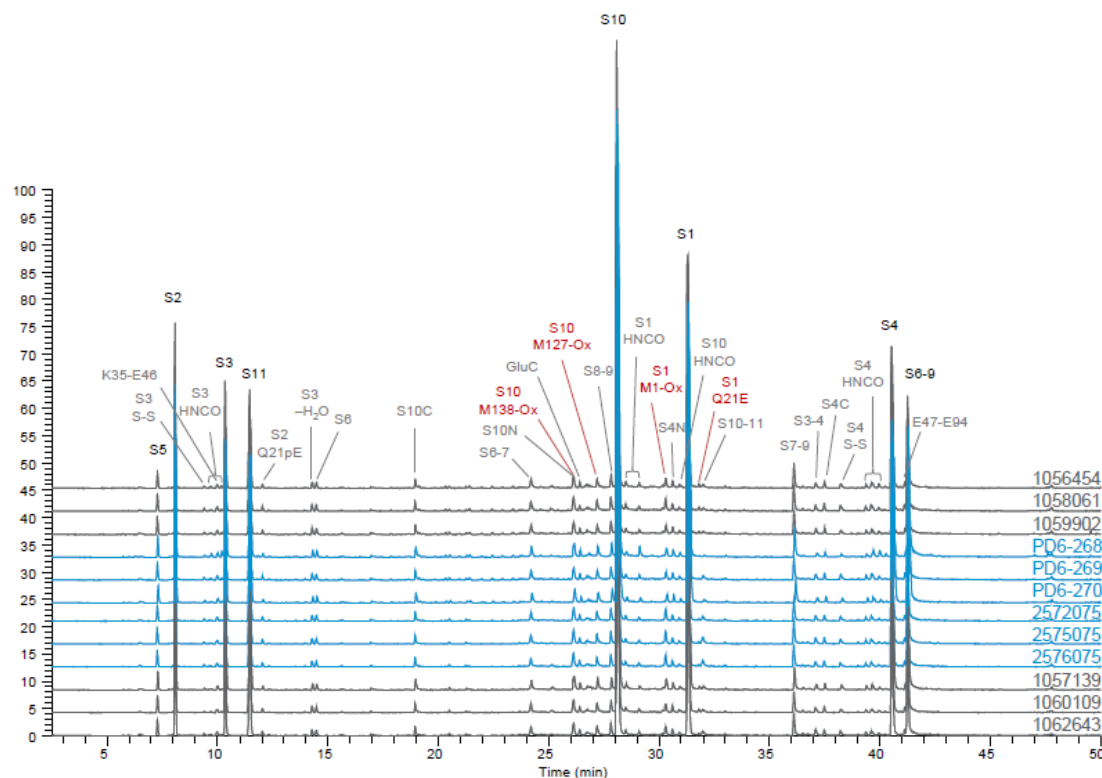


Figure 23: Overlay of Glu-C peptide map TICs of PF-06881893 (blue) and US-licensed Neupogen (gray)

Reviewer comments: Based on the qualitative comparisons of peak intensities and retention times among the US-licensed Neupogen and PF-06881893 lots, there are no clear differences. Overlays of chromatograms show no significant differences in the profiles.

The Glu-C peptide mapping method coupled with RP-HPLC has been validated for its intended use (3.2.S.4.3).

Tier 3: Intact molecular mass (RP-UPLC-MS)

Intact mass similarity is evaluated by RP-UPLC-MS with electrospray ionization (Q-TOF). Raw mass spectra for 2 lots each of PF-06881893, and US-licensed Neupogen are provided, including charge distributions ([M+10H]¹⁰ to [M+24H]²⁴⁺) and deconvoluted spectra. The deconvoluted spectra exhibited a signal peak at 18,786.7 Da for all 4 lots. Additionally, the intact mass results from 7 lots of PF-06881893 and 8 lots of US-licensed Neupogen are provided in the document and summarized in Table 97.

Test article	Number of lots	Age at time of test	Intact mass
PF-06881893	4 PFS, 3 SDV	4-23 months	18, 786.7 Da
US-licensed Neupogen	4 PFS, 4 SDV	16-30 months	18, 786.7 Da

Table 97: Similarity assessment of intact mass results for PF-06881893 and US-licensed Neupogen

The experimentally determined molecular weights for the intact filgrastim in PF-06881893 and the US-licensed Neupogen were compared with each other and with the theoretical molecular weight for filgrastim (18,786.7 Da). The molecular weights of the intact filgrastim protein in PF-06881893 and US-licensed Neupogen were identical.

Reviewer comments: These data support that there are no significant differences in molecular masses between PF-06881893 and US-licensed Neupogen. The MS data are consistent with 2 disulfide bonds and 1 free cysteine.

Assay qualification: The RP-UPLC TOF-MS method ((b) (4)-332-07) was qualified for PF-06881893 characterization. An assay qualification report has been submitted demonstrating assay specificity, accuracy, precision, robustness, and system suitability.

Reviewer comment: The qualification data demonstrate that the method is specific, precise, accurate, and robust.

Tier 3: Free thiol

When properly folded, filgrastim contains one free thiol and two disulfide bonds. The free thiol is detected using DTNB (5,5-dithio-bis-2-nitrobenzoic acid) reagent, which reacts with free thiol to release a 2-nitro-5-thiobenzoic acid chromophore. TNB is detected by UV at 412 nm. Absorbance at 412 nm (A₄₁₂) is directly proportional to the concentration of reacted thiol. The experimentally determined free thiol content was compared between PF-06881893 (4 PFS, 3 SDV) and US-licensed Neupogen (3 PFS, 3 SDV). The range of free thiol in PF-06881893 is 1.03-1.07 and that for US-licensed Neupogen is 1.02-1.08.

Reviewer comment: The mean value of the free thiol content in both products, 1.05, is the same and is consistent with the presence of 1 free thiol..

Assay qualification: The Ellman's assay is a characterization assay (method (b) (4)-332-05-R00). A qualification report for the use of this assay in the determination of free thiol content in PF-06881893 is provided (LF-332-R-194-16).

Reviewer comment: The assay is qualified by evaluation of system suitability, assay repeatability, intermediate precision, robustness, linearity, and LOD. It is demonstrated to be suitable for its intended use.

Structural analysis: Higher order structure

Tier 2: Tertiary Structure-Differential Scanning Calorimetry

Protein melting temperatures (T_m) determined by DSC are sensitive to changes in structure. By subjecting samples to temperature scans from 30°C to 90°C at a rate of 1°C/min, the T_m values for 16 lots PF-06881893 PFS and SDV and 18 lots of US-Neupogen (12 PFS and 6 SDV) were initially compared using a Tier 2 statistical approach. An IR was sent on May 10, 2018, and the sponsor was asked to re-evaluate the quality attributes evaluated by Tier 2 statistical test using the first DP lot manufactured from each DS lot. In their IR response received on May 17, 2018, the sponsor provided a re-analysis of the T_m values using 11 PF-06881893 lots and all the US-licensed Neupogen lots tested in this assay. These results are summarized in Table 98.

Test Article	Number of Lots Tested	Mean (°C)	Standard Deviation (°C)	Range (°C)	Quality Range (°C)	PF-06881893 Lots within Quality Range (%)
PF-06881893	11	68.9	0.25	68.6 – 69.3	68.2 – 70.0	100
US-licensed Neupogen	18	69.1	0.30	68.4 – 69.6		

Table 98: Tier 2 analysis of T_m results determined by DSC

The measured T_m results for all of the PF-06881893 lots were within the quality range defined based on US-licensed Neupogen's mean and standard deviation.

Reviewer comments: The DSC data support that there are no significant differences in higher-order structure between PF-06881893 and US-licensed Neupogen because values met Tier 2 acceptance criteria. The average apparent T_m for PF-06881893 (69.0°C) is very close in value to the average apparent T_m range observed for US-licensed Neupogen lots, 69.1 °C. The mean $\pm$ 3SD quality range is based on data from US-licensed Neupogen lots, and all PF-06881893 lots are within the pre-defined quality range.

Tier 3: Secondary Structure-Far-UV CD

Protein secondary structure is determined by Far-UV CD spectroscopy to estimate the distribution of α -helix, β -sheet, β -turn, and random coil conformations. 16 PF-06881893 and 23 US-licensed Neupogen lots were analyzed by this method. Representative CD spectrum overlay of 3 PF-06881893 PFS lots with 3 US-licensed Neupogen PFS lots, and also 3 PF-06881893 SDV lots with 3 US-licensed

Neupogen SDV lots are provided. These data show superimposable traces with minima near 208 and 222 nm. The intensity of the SDV samples is less than the PFS samples due to difference in protein concentrations (0.3 mg/mL SDV vs. 0.6 mg/mL PFS) though the overall spectral pattern is consistent for all samples. Quantitation data are provided for the α -helix, β -sheet, β -turn, and random coil conformations of 16 PF-06881893 samples and 26 US-licensed Neupogen samples (Table 99).

Test Article	Parameter	Mean (%)	Standard Deviation (%)	Range (%)
PF-06881893	α -helix	74.8	1.20	72.9 - 77.7
	Total β -structures	14.4	0.66	12.9 - 15.4
	Random Coil	12.0	0.78	10.1 - 13.2
US-licensed Neupogen	α -helix	74.4	0.90	71.4 - 75.4
	Total β -structures	14.7	0.50	14.1 - 16.2
	Random Coil	12.4	0.55	11.8 - 14.2

Table 99: Comparison of secondary structures calculated from Far-UV CD spectra for PF-06881893 and US-licensed Neupogen

Reviewer comment: No differences in far-UV spectra were identified. The secondary structure comparison by Far-UV CD confirms the predominantly alpha-helix conformations of PF-06881893 samples as compared to US-licensed Neupogen with comparable α -helix, β -sheet, β -turn, and random coil content. This was determined by both graphical overlays using 3 independent lots of PF-06881893 PFS and SDV samples each, and tabular summaries of the quantitative results.

Assay qualification: Method qualification report (LF-332-R-087-15) provides the results of the method qualification for the determination of secondary structure by far-UV circular dichroism of PF-06881893. The method was qualified using DP Lot 2057024 and DS Lot 1755093.

Reviewer comment: The assay is repeatable between analysts and test dates, specific for properly folded filgrastim protein, and robust with varying buffer pH, sorbitol, and polysorbate 80 concentrations. The method is sensitive to protein concentrations used in the assay.

Tier 3: Tertiary Structure-Top-down MS analysis of disulfide bond linkages

Top-down MS analyzes intact proteins without proteolytic digestion and preserves the native disulfide bonds from method induced disulfide scrambling. The disulfide linkages for filgrastim in PF-06881893 and US-licensed Neupogen were confirmed using a top-down LC-MS method based on the Intact Mass (RP UPLC-MS) method.

Assay qualification: Intact molecular mass (RP-UPLC-MS) assay data were analyzed using ProSight Lite, to assign disulfide bonds.

Tier 3: Tertiary Structure-Intrinsic Fluorescence

Tertiary structures were compared between PF-06881893 and US-licensed Neupogen using Intrinsic Fluorescence emission spectra. 16 PF-06881893 lots and 23 US-licensed Neupogen lots were analyzed.

Figure 24 provides a representative graphical overlay comparing 3 lots each of PF-06881893 PFS and SDV samples, with similar number of US-licensed Neupogen samples. For analytical similarity assessment the wavelength of maximum fluorescence emission (λ_{max}) was evaluated and compared using 16 PF-06881893 PFS and SDV lots, and 23 US-licensed Neupogen lots.

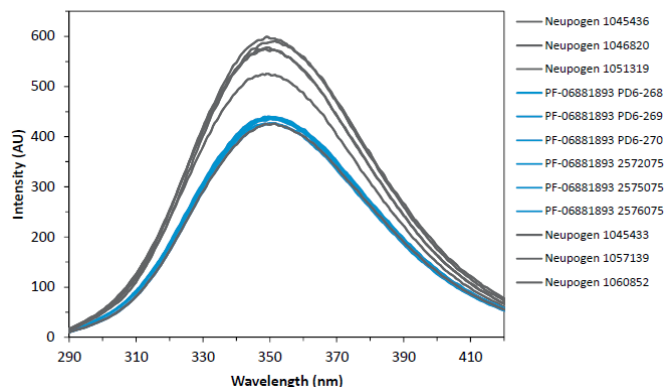


Figure 24: Intrinsic fluorescence spectra of PF-06881893 and US-licensed Neupogen (Figure 3.2.R.5.5-12 in BLA)

The fluorescence intensity of the US-licensed Neupogen varies due to being tested at different times, though the emission maxima for all samples is within 348-352 nm., (mean emission maximum 350). The sponsor states that the lower fluorescence intensity for PF-06881893 samples may be due to photo-bleaching, small differences in matrix components, or protein concentration, though the emission maxima is in the 349-352 nm range (mean emission maximum 351) for the 16 PF-06881893 samples analyzed for similarity assessment.

Reviewer comment: The λ_{max} values are not significantly different between PF-06881893 and US-licensed Neupogen based on the graphical overlays of samples, and the experimental determination of λ_{max} .

Assay qualification: This is a characterization method that is validated with respect to selectivity, range, intermediate precision and sample stability (MVR000985-RPT-56537).

Reviewer comment: Assay acceptance criteria are met and the method is demonstrated to be validated for its intended use.

Tier 3: Secondary and Tertiary Structure-Hydrogen Deuterium Exchange

The Sponsor evaluated tertiary structure similarity by comparing rates of proton exchange for PF-06881893 and US-licensed Neupogen. Proton exchange rates are sensitive to solvent accessibility, and solvent-proximal sequences exchange protons at higher rates compared to sequences located in protected regions of the protein. Proteins are incubated in deuterium oxide (heavy water) for different time intervals (0 sec., 30 sec., 2 min., 10 min., 1 hour, and 4 hours) in triplicate, digested on-column with pepsin, and the resulting peptides are separated by RP-UPLC for MS analysis. The rates of deuterium incorporation are calculated using the average mass difference between the deuterated peptide at a given timepoint relative to the t=0 control. The Sponsor compared HDX rates between 3 independently-derived lots of PF-06881893 and 4 lots of US-licensed Neupogen in a pairwise fashion

using differential heat maps. PF-06881893 lot 2075114 was tested twice, at age 4 months and again at age 12 months (Table 100). Results were compared using graphical overlays of deuterium build up curves and differential heat maps.

PF-06881893/age	US-licensed Neupogen, age	Result
2075114, 4 months	1050859, 19 months	Difference of deuterium incorporation rate for all peptides is less than 10% between samples
2075114, 12 months	1036971, 28 months	
2057024, 13 months	1051786, 10 months	
PD6-268, 5 months	1051319, 30 months	

Table 100: PF-06881893 and US-licensed Neupogen lots analyzed by Hydrogen-Deuterium exchange

Reviewer conclusions: The results demonstrate that PF-06881893 and US-licensed Neupogen have very similar rates of HDX across the entire protein sequence. This similarity indicates that the proteins are folded into similar secondary and tertiary structure.

Tier 3: Tertiary Structure-Sedimentation Velocity-Analytical Ultracentrifugation (SV-AUC)

SV-AUC measures the sedimentation rate of a protein molecule based on mass and shape of monomeric and multimeric species. This method reliably resolves and detects monomeric filgrastim and high molecular weight species (HMWS) greater than dimer. For analytical similarity evaluation, mean sedimentation coefficients (S) for the monomer, percent monomer, and percent HMWS, were compared using Tier 3 statistical analysis.

Data from 16 lots of PF-06881893, and 32 lots of US-licensed Neupogen, are provided in the submission and summarized in Table 101 below.

Test Article	Parameter	Mean	Standard Deviation	Range
PF-06881893	Sedimentation Coefficient (S)	1.70	0.020	1.67-1.74
	Monomer %	99.7	0.35	99.1- 100.0
	HMWS (%)	0.3	0.36	0.0-0.9
US-licensed Neupogen	Sedimentation Coefficient (S)	1.72	0.028	1.68 - 1.77
	Monomer %	99.6	0.44	98.2 – 100.0
	HMWS (%)	0.4	0.45	0.0 - 1.8

Table 101: SV-AUC analysis pf PF-06881893 and US-licensed Neupogen

Two filgrastim species were identified in the SV-AUC sedimentation coefficient distributions for the PF-06881893 and the US-licensed Neupogen samples: monomeric filgrastim and HMWS greater than

dimer. The S value, percent monomer, and percent HMWS determined from the SV-AUC analysis are consistent for PF-06881893 and US-licensed Neupogen.

Reviewer comments: The SV-AUC data (sedimentation coefficient and apparent size and shape) show no significant differences between PF-06881893 and US-licensed Neupogen and support that there are no significant differences in tertiary structure.

This method has been validated as a release and stability testing assay (3.2.S.4.3).

Tier 3: Tertiary Structure-Nuclear Magnetic Resonance (NMR)

^1H NMR spectroscopy is useful for the determination of higher order structure of a protein at high resolution. The conformation of a protein has a unique NMR signature enabling analysis of structural similarity. Samples were concentrated and subjected to buffer exchange into filgrastim placebo to ensure identical matrices for different test articles. The Sponsor then conducted qualitative comparisons of the ^1H spectra and provided overlay spectra for 2 lots of PF-06881893 and 2 lots of US-licensed Neupogen.

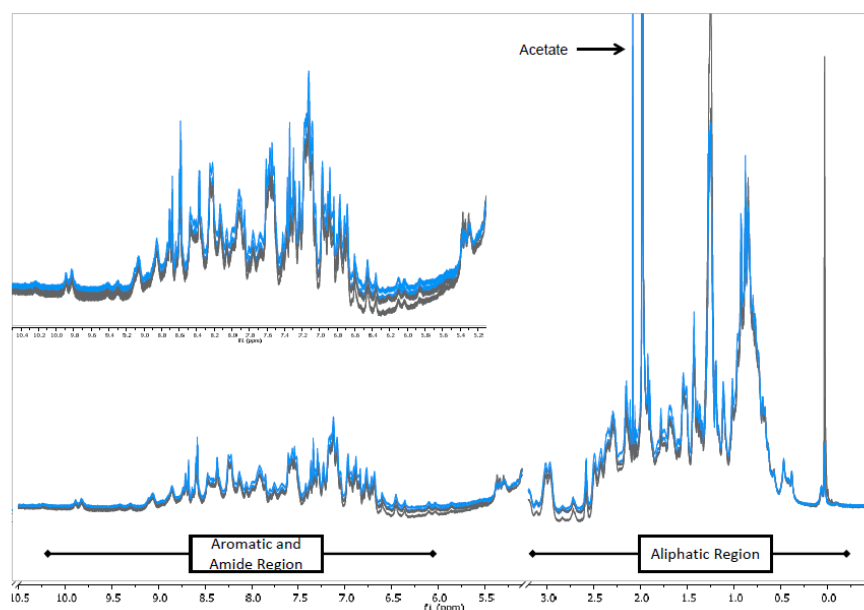


Figure 25: Overlay of ^1H spectrum of PF-06881893 and US-licensed Neupogen

In addition $2\text{D } ^1\text{H}-^{15}\text{N}$ NMR spectroscopy was conducted for overlay comparisons between two different lots of the same product; PF-06881893 samples (PD6-268 and 2075114), and US-licensed Neupogen samples (1041874 and 1050859). Comparisons are also provided between pairs of PF-06881893 and US-licensed Neupogen samples, collected on different days using a single instrument. The signals in a $^1\text{H}-^{15}\text{N}$ HSQC spectrum correspond to N-H pairs (primarily amide groups). This technique provides better resolution than ^1H NMR and may be considered a structural fingerprint of the 3D structure of the protein.

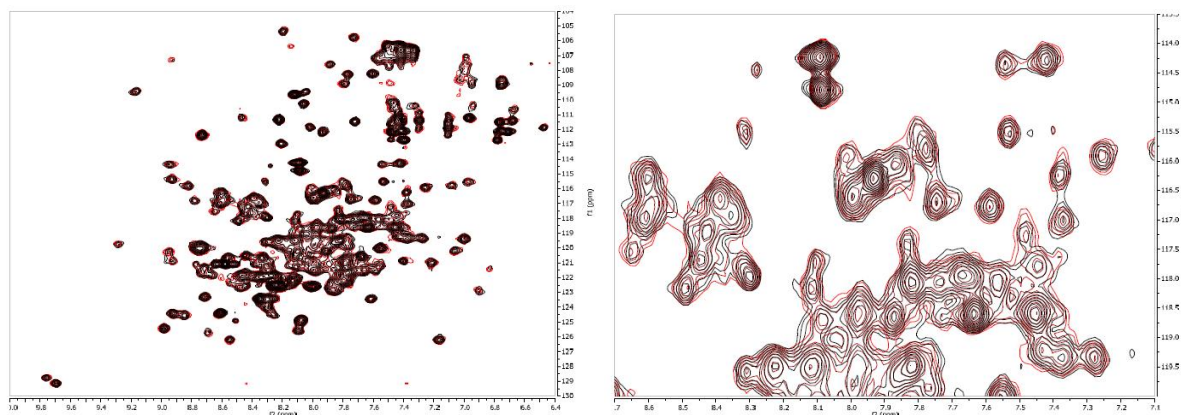


Figure 26: Overlay of ^1H - ^{15}N HSQC spectrum

Reviewer conclusions: *There are no significant differences between PF-06881893 and US-licensed Neupogen in terms of folding and higher order structure as measured by NMR and 2D ^1H - ^{15}N NMR. The major cross-peaks completely overlay and there are no chemical shift differences observed between the peaks in the PF-06881893 and US-licensed Neupogen spectra.*

Tier 3: Tertiary Structure-X-ray Crystallography

X-ray crystallography is a high-resolution technique that provides detailed information on the primary, secondary, and tertiary structure of a protein. PF-06881893 (lot 2075114) and US-licensed Neupogen (lot 1050859) samples were processed to remove the buffer matrix by CEX and buffer exchanged in 10 mM sodium acetate. Crystals were grown and their structure analyzed.

According to the sponsor, the superposition of the PF-06881893 and US-licensed Neupogen structures resulted in only minor visual differences which may be attributed to crystal packing. Both PF-06881893 and US-licensed Neupogen diffracted up to a resolution of 1.7 Å and each molecule displayed an antiparallel helix bundle with a left-handed twist.

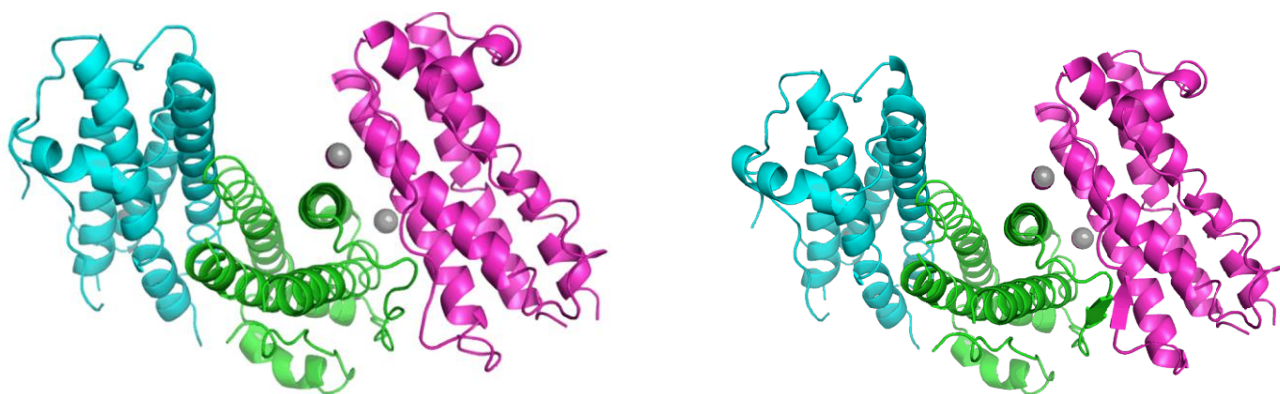


Figure 27: Ribbon diagram of OF-06881893 and US-licensed Neupogen at 1.7 Å

Reviewer comment: *The X-ray crystallography data indicate that the higher order structure of the filgrastim in PF-06881893 and US-licensed Neupogen is nearly identical and confirms the amino acid sequence data completed by LC-MS.*

Drug Product Attributes

The Sponsor evaluated PF-06881893 and US-licensed Neupogen lots for pH, Osmolality, Polysorbate 80, and Appearance/Color/Clarity. These attributes are considered “non-comparative” by the Sponsor because FDA guidance allows for minor differences in clinically inactive components, as long as they are controlled within a range. Therefore, results are not evaluated statistically for analytical similarity. The submission includes tabular results for 18 lots of PF-06881893 and 5 lots of US-licensed Neupogen (3 PFS + 2 SDV). These results are summarized in Table 102.

Attribute	Method	PF-06881893	US-licensed Neupogen
pH	USP <791>	4.0-4.1	4.0
Osmolality	USP <785>	274-301	302-306
Polysorbate 80 (%)	RP-HPLC	0.003-0.004	0.003-0.004
Appearance, color, clarity	Ph.Eur.<2.2.2>, Ph.Eur.<2.2.1>	Clear and colorless solution	Clear and colorless solution

Table 102: Summary of analytical similarity evaluation of DP attributes of PF-06881893 and US-licensed Neupogen

Reviewer conclusions: Results show no differences in the pH, osmolality, Polysorbate 80 content, and appearance of PF-06881893 and US-licensed Neupogen. Compendial methods were used for the evaluation of pH, osmolality, appearance, color, and clarity. A validated RP-HPLC method was used to assay Polysorbate 80 levels (3.2.S.4.3).

Visible Particles (no Tier assigned)

Visible particles are tested according to USP<790>. The submission includes tabular data for 18 lots of PF-06881893 (8 PFS and 10 SDV) and 5 lots of US-licensed Neupogen (3 PFS + 2 SDV). The result for all lots of both products was “Practically free of visible particles.”

Subvisible Particles (no Tier assigned)

Subvisible particles were evaluated for similarity using orthogonal techniques, Low Volume HIAC and MicroFlow Imaging (MFI) methods. Both assays were conducted according to USP<787> and particles were measured and reported separately for ranges $\geq 2 \mu\text{m}$, $\geq 5 \mu\text{m}$, $\geq 10 \mu\text{m}$, and $\geq 25 \mu\text{m}$. Figures below provide the particles per mL measured for each lot by Low Volume HIAC.

Low volume HIAC: The mean, standard deviation, and range of the Low Volume HIAC subvisible particle concentrations for PF-06881893 and US-licensed Neupogen are summarized in Table 103:

Test Articles	Particle Size	Number of Lots Tested	Particles per mL			
			Mean	SD	Minimum	Maximum
PF-06881893 PFS	$\geq 2 \mu\text{m}$	16	4765	2952.3	546	11623
Neupogen PFS		16	7196	4287.5	1895	15431
PF-06881893 SDV		6	67	17.1	47	91
Neupogen SDV		14	724	308.7	187	1301
PF-06881893 PFS	$\geq 5 \mu\text{m}$	16	915	481.2	48	1767
Neupogen PFS		16	1476	1030.3	178	3969
PF-06881893 SDV		6	14	10.1	6	30
Neupogen SDV		14	67	24.1	27	104
PF-06881893 PFS	$\geq 10 \mu\text{m}$	16	169	100.2	17	344
Neupogen PFS		16	301	243.6	50	959
PF-06881893 SDV		6	8	6.9	2	19
Neupogen SDV		14	6	5.2	0	17
PF-06881893 PFS	$\geq 25 \mu\text{m}$	16	4	3.9	1	16
Neupogen PFS		16	11	10.7	1	39
PF-06881893 SDV		6	2	0.8	1	3
Neupogen SDV		14	1	1.6	0	6

Table 103: Summary of low volume HIAC subvisible particle results, by size range, for PF-06881893 and US-licensed Neupogen (Table 3.2.R.5.5-72 in BLA)

Graphical results are also provided, plotted as a function of product age at the time of testing for PF-06881893 DP and US-licensed Neupogen. The PF-06881893 DP and US-licensed Neupogen product have overlapping ranges in the plots of number of subvisible particles versus age at time of testing

There is no noticeable difference between lots of different dose strength. There is however a difference between PFS and SDV presentations of PF-06881893 and US-licensed Neupogen. The higher particle count in PFS is explained by the presence of (b) (4) in the PFS container closure system (reviewed in 3.2.P.8).

MFI: The particle concentrations (particle/mL) were reported separately for the $\geq 2 \mu\text{m}$, $\geq 5 \mu\text{m}$, $\geq 10 \mu\text{m}$, and $\geq 25 \mu\text{m}$ particle size groups. The mean, standard deviation, and range of the MFI subvisible particle concentrations for PF-06881893 DP and US-licensed Neupogen are summarized in Table 104:

Test Articles	Particle Size	Number of Lots Tested	Particles per mL			
			Mean	SD	Minimum	Maximum
PF-06881893 PFS	$\geq 2 \mu\text{m}$	16	60224	29656.5	23378	123104
Neupogen PFS		15	73180	42866.1	15826	178934
PF-06881893 SDV		6	3081	860.6	1782	4298
Neupogen SDV		15	9882	5537.3	5432	27793
PF-06881893 PFS	$\geq 5 \mu\text{m}$	16	3625	1977.1	902	6704
Neupogen PFS		15	6253	5144.4	1434	22269
PF-06881893 SDV		6	109	60.3	37	207
Neupogen SDV		15	267	172.0	109	790
PF-06881893 PFS	$\geq 10 \mu\text{m}$	16	141	78.8	33	275
Neupogen PFS		15	387	405.9	111	1608
PF-06881893 SDV		6	15	9.6	2	27
Neupogen SDV		15	10	8.8	2	36
PF-06881893 PFS	$\geq 25 \mu\text{m}$	16	7	3.1	0	13
Neupogen PFS		15	11	10.8	0	33
PF-06881893 SDV		6	3	4.1	0	10
Neupogen SDV		15	2	3.1	0	10

Table 104: Summary of MFI subvisible particles by size range for PF-06881893 and US-licensed Neupogen (Table 3.2.R.5.5-74 in BLA)

As with the HIAC results, the particle concentrations for PF-06881893 and US-licensed Neupogen are higher in PFS lots compared to SDV lots. Particle size data plotted as a function of time show no correlation between subvisible particle concentration and age of lots at the time of testing.

Reviewer comments: *The subvisible particle concentrations of PF-06881893 PFS and SDV lots are not significantly different from those of US-licensed Neupogen PFS and SDV lots, respectively, for all 4 size groups $\geq 2 \mu\text{m}$, $\geq 5 \mu\text{m}$, $\geq 10 \mu\text{m}$, and $\geq 25 \mu\text{m}$. There is no correlation between the subvisible particle concentration and the age of the product lots at the time of testing for either the PF-06881893 DP or US-licensed Neupogen.*

Other characteristics

Isoelectric Point (pI)

Isoelectric point (pI) was measured by imaged capillary isoelectric focusing (icIEF) to confirm that the pI of filgrastim in PF-06881893 is similar to that in US-licensed Neupogen. pI is a non-CQA and is analyzed as a Tier 3 attribute by tabular and graphical comparisons. All measured pI values (6 lots of PF-06881893 and 5 lots of US-licensed Neupogen) were 6.05 for PF-06881893 DP and US-licensed Neupogen lots tested (Figure 28).

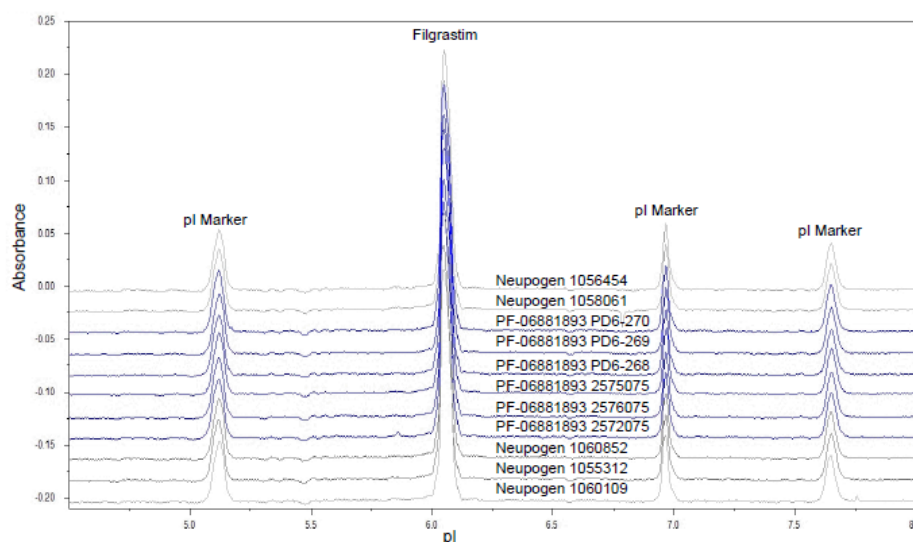


Figure 28: Electropherogram overlay of icIEF results for PF-06881893 and US-licensed Neupogen

Reviewer conclusions: *There are no significant differences in pI value between PF-06881893 and no age-related differences are observed. The pI value is dependent on the primary structure of the filgrastim protein and can be influenced by the higher order structure. The results of the pI analysis support a determination of highly similar between PF-06881893 and US-licensed Neupogen.*

Assay qualification: *The icIEF assay has been qualified for its intended use by validation of assay specificity, precision, linearity, and range (LF-332-R-027-17).*

Comparative forced degradation studies

When DP is exposed to stress conditions, degradant profile and rates of degradation can be an indicator of degradation pathways. Therefore two comparative forced degradation studies were performed on PF-06881893 DP and US-licensed Neupogen PFS and SDV. Three lots each of PF-06881893 PFS and US-licensed Neupogen lots were exposed side by side to each stress condition (Table 105 to Table 108).

	PF-06881893 PFS			US-licensed Neupogen PFS			
DP Lot number	2074094	2855103	2075114	1051786	1057139	1056459	1042621
Age (months)	11	22	9	15	8	8	25
0.01% H ₂ O ₂ (100 ppm), RT	0, 2, 8, 24, 32 hours			√		√	√
Agitation: 200 rpm, RT	0, 1, 2, 4, 7 days			√		√	√
Heat: 50°C	0, 1, 2, 4, 7 days				√	√	√
Light: ICH Q1B Option 2	0X, 0.2X, 0.5X, 1X, and 1.5X				√	√	√
Low pH: pH 2.0	0, 1, 2, 4, 7 days			√		√	√
High pH: pH 10.0	0, 1, 2, 4, 7 days			√		√	√
Freeze at -20°C or ≤ -65°C, Thaw at RT	0, 5 cycles				√	√	√

Table 105: √: US-licensed Neupogen lots analyzed by forced degradation side-by-side with the three PF-06881893 lots at all time points. 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. RT: room temperature

Attributes	Analytical Methods	Agitation	Peroxide	Heat	Light	High pH	Low pH	F/T
Filgrastim Related Proteins	RP-HPLC	X	X	X	X	X	X	X
High Molecular Weight Species	SEC	X	X	X	X	X	X	X
Charge Variants	CEX	X	X	X	X	X	X	X
Molecular Weight Variants	Non-Reducing CE-SDS	X	X	X	X	X	X	X
	Reducing CE-SDS	X	X	X	X	X	X	X
Free Thiol	Ellman's Test	X	X	X	X	X	X	X
Molecular Weight	Intact MS	X	X	X	X	X	X	X
Chemical Degradation	LC-MS Peptide Mapping	X	X	X	X	X	X	X
Secondary Structure	Far-UV CD	-	-	-	-	-	-	X
Tertiary Structure	DSC	-	-	-	-	-	-	X
In Vitro Potency	In Vitro Cell-Based Bioassay	X	X	X	X	X	X	X
Receptor Binding	Competitive Receptor Binding Assay	X	X	X	X	X	X	X
Receptor Binding Affinity and Kinetics	SPR	X	X	X	X	X	X	X

Table 106: Analytical testing plan for PFS study

	PF-06881893 SDV			US-licensed Neupogen SDV		
DP Lot number	PD6-267	PD6-269	PD6-271	1051319	1056455	1058392
Age (months)	3	3	2	28	21	17

0.01% H ₂ O ₂ (100 ppm), RT	0, 2, 8, 24, 32 hours	√	√	√
Agitation: 200 rpm, RT	0, 1, 2, 4, 7 days	√	√	√
Light: ICH Q1B Option 2	0X, 0.2X, 0.5X, 1X, and 1.5X	√	√	√
High pH: pH 10.0	0, 1, 2, 4, 7 days	√	√	√

Table 107: √: US-licensed Neupogen SDV lots analyzed by forced degradation side-by-side with the three PF-06881893 lots at all time points. 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. RT: room temperature

Attributes	Analytical Methods	Agitation	Peroxide	Light	High pH
Filgrastim Related Proteins	RP-HPLC	X	X	X	X
High Molecular Weight Species	SEC	X	-	X	X
Charge Variants	CEX	-	-	X	X
Molecular Weight Variants	Non-Reducing CE-SDS	X	-	X	X
Free Thiol	Ellman's Test	-	X	X	X
Molecular Weight	Intact MS	X	X	X	X
Chemical Degradation	LC-MS Peptide Mapping	-	X	X	-
In Vitro Potency	In Vitro Cell-Based Bioassay	X	X	X	X
Receptor Binding	Competitive Receptor Binding Assay	X	X	X	X

Table 108: X: Measurement performed at all or select time points, -: Not analyzed

Raw data are provided in the BLA for every attribute measured at every stress condition and time point at which data are collected. The results of the studies are summarized:

Agitation: Exposure of PF-06881893 and US-licensed Neupogen to agitation stress resulted in no significant differences in the rates of aggregation and increase in filgrastim related proteins. One of the 3 US-licensed Neupogen PFS lots also showed a decrease in potency. Representative data plots to compare rates of degradation for each of the 2 forced degradation studies, by RP-HPLC and SEC are provided (Figure 29).

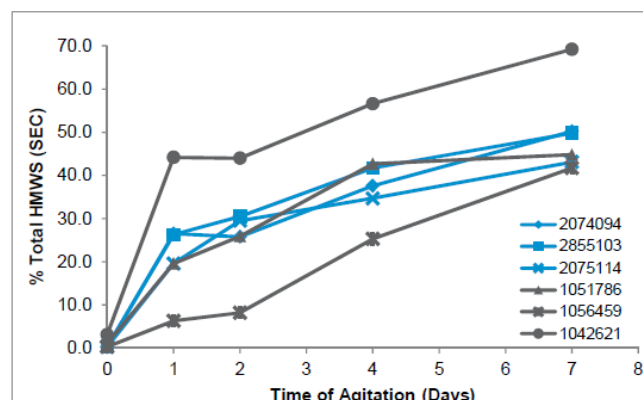
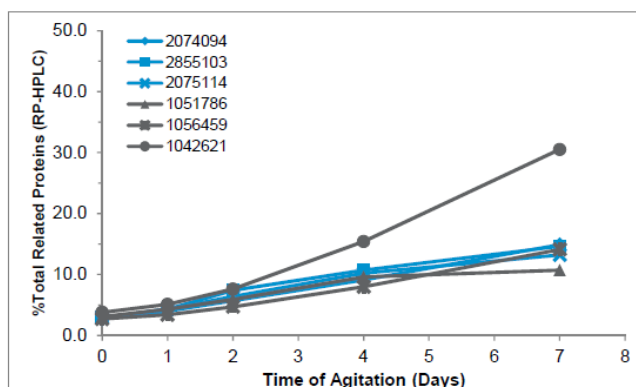


Figure 29: Left: Total filgrastim-related proteins by RP-HPLC in agitation stressed PFS product. Right: Total HMWS by SEC in agitation stressed samples in PFS

Peroxide: Addition of hydrogen peroxide to PF-06881893 and US-licensed Neupogen showed no differences in the rates of oxidation and increase of filgrastim related proteins. RP-HPLC analyzed levels of oxidized filgrastim (Met127), and levels of filgrastim-related proteins after peroxide treatment are provided (Figure 30).

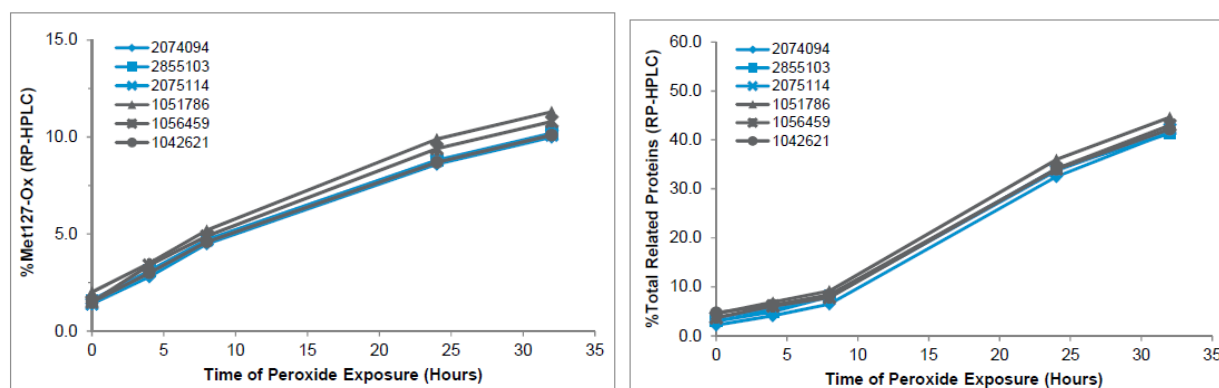


Figure 30: Left: Met127-Oxidized filgrastim levels in PF-06881893 and US-licensed Neupogen PFS after peroxide stress. Right: Total filgrastim-related proteins by RP-HPLC in peroxide stressed PFS samples

Heat: Both PF-06881893 and US-licensed Neupogen show no differences in the rate of increase in aggregation, with minor increases in filgrastim related proteins, measured by SEC.

Light: Exposure of PF-06881893 and US-licensed Neupogen to combined UV and white light resulted in no differences in the rates of aggregation, increase in filgrastim related proteins, and decrease of potency. Minor increases of oxidation, fragmentation, and increase of charge variants showed also no differences in levels in both products (Figure 31).

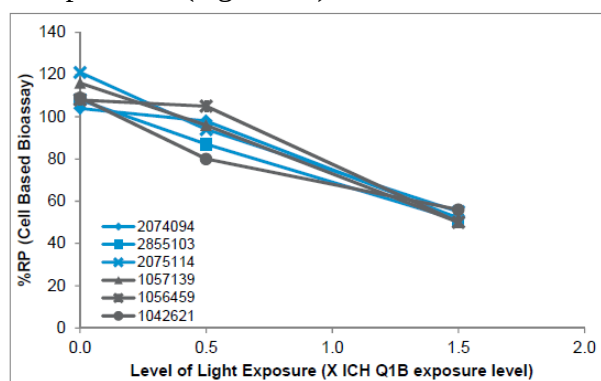


Figure 31: Relative potency by *in vitro* cell-based bioassay for light stressed PF-06881893 and US-licensed Neupogen PFS

pH: Low pH did not cause degradation of PFS samples. SDV samples were not subjected to low pH. Exposure of PF-06881893 and US-licensed Neupogen PFS and SDV to high pH (pH 10) stress did not

show differences in rates of aggregation and increase in filgrastim related proteins, with minor increases in fragmentation and charge variants.

Freeze/thaw: PF-06881893 and US-licensed Neupogen PFS lots were frozen at either -20°C (in syringe) or ≤ -65°C (in polypropylene tube) and thawed at ambient temperature for 5 cycles. In addition to common physicochemical and functional assays, biophysical tools including DSC and Far-UV CD were employed to compare the products. Results from the analyses indicate that both PF-06881893 and US-licensed Neupogen are stable upon freeze/thaw stress.

Reviewer conclusions: Two sets of side-by-side forced degradation studies were performed to compare the degradation profiles of PF-06881893 and US-licensed Neupogen in PFS and SDV presentations. Under the stress conditions applied (agitation, peroxide, heat, light, low pH, high pH, and freeze/thaw), PF-06881893 and US-licensed Neupogen lots of the same presentation, PFS or SDV, showed the same major and minor degradation pathways, with no significant differences in degradation rates, and degradant profiles.

Comparative stability

Analytical similarity was assessed between PF-06881893 and US-licensed Neupogen by comparing long-term, accelerated, stressed stability results of the two products. Stability studies for both PF-06881893 and US-licensed Neupogen DP lots were conducted at the long-term storage condition of 2 to 8°C (5°C), an accelerated condition of 25 ± 2°C/60 ± 5% Relative Humidity (25°C/60% RH), and a stressed condition of 40 ± 2°C/75 ± 5% RH (40°C/75% RH). The quantitative stability indicating attributes used in this comparison include protein concentration (by UV-Vis), potency (by *in vitro* cell-based bioassay), and purity (by SEC, CEX, and RP-HPLC).

The following lots of PF-06881893 and US-licensed Neupogen were used in this study (Tables 109 and 110):

Presentation	Lot Number	Dose Strength	Drug Substance Lot Number	DP Manufacturing Date	Stability Initiation Date	Available Data
PFS	2855103	480 mcg/0.8 mL	1755093	Oct 2013	Dec 2013	Up to 36 months at 5°C, 6 months at accelerated, 6 weeks at stressed
	2854103	300 mcg/0.5 mL				
	2057024	480 mcg/0.8 mL	1750123	Feb 2014	Apr 2014	Up to 30 months at 5°C, 6 months at accelerated, 6 weeks at stressed
	2056024	300 mcg/0.5 mL				
	2054054	480 mcg/0.8 mL	1805034	May 2014	Jul 2014	
	2053054	300 mcg/0.5 mL				
	2074094	480 mcg/0.8 mL	1804074	Sep 2014	Nov 2014	Up to 24 months at 5°C, 6 months at accelerated, 6 weeks at stressed
	2073094	300 mcg/0.5 mL				
	2075114	480 mcg/0.8 mL	1804094	Nov 2014	Jan 2015	
	2074114	300 mcg/0.5 mL				
	2572075	300 mcg/0.5 mL	1451025	Jul 2015	Jul 2015	Up to 36 months at 5°C, 6 months at accelerated, 6 weeks at stressed
	2573075	480 mcg/0.8 mL				
	2574075	300 mcg/0.5 mL				

	2575075	480 mcg/0.8 mL	1452025	Jul 2015	Jul 2015	
	2576075	300 mcg/0.5 mL	1455035	Jul 2015	Jul 2015	
	2577075	480 mcg/0.8 mL				
SDV	EX6-049A	300 mcg/1.0 mL	FSM VUS S02P/14	Jan 2016	Feb 2016	Up to 9 months at 5°C, 6 months at accelerated, 6 weeks at stressed
	EX6-049B	480 mcg/1.6 mL		Jan 2016	Feb 2016	
	EX6-084A	300 mcg/1.0 mL	FSM VUS S01P/14	Mar 2016	Apr 2016	
	EX6-084B	480 mcg/1.6 mL		Mar 2016	Apr 2016	
	PD6-267	300 mcg/1.0 mL	1460055	May 2016	Jul 2016	Up to 6 months at 5°C, 6 months at accelerated, 6 weeks at stressed
	PD6-268	300 mcg/1.0 mL	1453065	May 2016	Jul 2016	
	PD6-269	300 mcg/1.0 mL	1460055	May 2016	Jul 2016	
	PD6-270	480 mcg/1.6 mL	1463055	Jun 2016	Jul 2016	
	PD6-271	480 mcg/1.6 mL	1551016	Jun 2016	Jul 2016	
	PD6-272	480 mcg/1.6 mL	1453065	Jun 2016	Jul 2016	

Table 109: PF-06881893 lots used in comparative stability studies

Presentation	Lot Number	Dose Strength	Expiry Date	Estimated Date of Manufacture	Stability Conditions and Time Points	
					Conditions	Time Points
PFS	1047463	480 mcg/0.8 mL	Jul-16	Jan-14	5°C	13, 18, 22, 26, 30 months
	1051786	300 mcg/0.5 mL	Nov-16	May-14	5°C 25°C/60% RH 40°C/75% RH	8, 14, 18, 24, 30 months 0, 1, 2, 3, 6 months 0, 2, 4, 6 weeks
	1056459	480 mcg/0.8 mL	Jun-17	Dec-14	5°C 25°C/60% RH 40°C/75% RH	9, 12, 18, 24 months 0, 1, 2, 3, 6 months 0, 2, 4, 6 weeks
	1057139	300 mcg/0.5 mL	Jun-17	Dec-14	5°C 25°C/60% RH 40°C/75% RH	9, 12, 18, 24 months 0, 1, 2, 3, 6 months 0, 2, 4, 6 weeks
SDV	1045436	480 mcg/1.6 mL	Jun-16	Dec-13	5°C	13, 18, 22, 26, 30
	1051319	300 mcg/1.0 mL	Oct-16	Apr-14	5°C 25°C/60% RH 40°C/75% RH	9, 15, 18, 24, 30 months 0, 1, 2, 3, 6 months 0, 2, 4, 6 weeks
	1053036	300 mcg/1.0 mL	Feb-17	Aug-14	25°C/60% RH 40°C/75% RH	0, 1, 2, 3, 6 months 0, 2, 4, 6 weeks
	1056454	300 mcg/1.0 mL	Apr-17	Oct-15	5°C	11, 14, 18, 24

	1058392	480 mcg/1.6 mL	Sep-17	Mar-15	5°C 25°C/60% RH 40°C/75% RH	10, 12, 15, 18 months 0, 1, 2, 3, 6 months 0, 2, 4, 6 weeks
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Table 110: US-licensed Neupogen lots used in comparative stability study

The BLA contains raw data of comparative stability results and its graphical analysis. The graphical data are represented in the form of linear regression models of the scatter plot of each stability indicating attribute as a function of time for each product lot. This analysis was performed for all lots evaluated at the long-term, accelerated, and stressed stability conditions. In the graphical displays below, the shaded areas represent the two-sided 95% confidence regions of the average slope expected for each product. solid lines represent the current effective stability specifications and dashed lines represent the proposed commercial DP stability specifications as presented. The comparability of the stability profiles of PF-06881893 DP and US-licensed Neupogen is based on the visual trending and average slope assessment.

Protein concentration: There are no significant differences in the stability profile of protein concentration at the long-term, stability-indicating conditions, between PF-06881893 DP and US-licensed Neupogen (Figure 32).

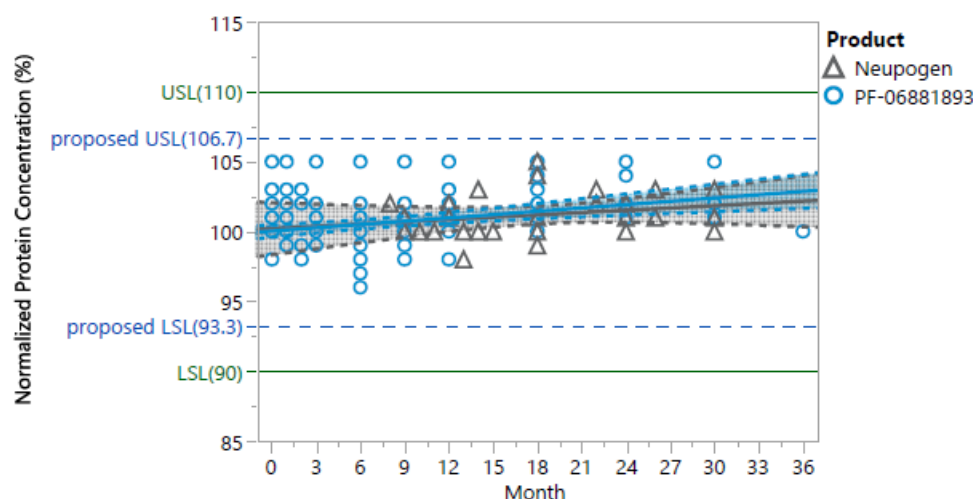


Figure 32: Comparison of normalized protein concentration at the long-term stability condition for PF-06881893 and US-licensed Neupogen (Figure 3.2.R.5.5.8-2 in BLA)

No differences in qualitative stability profiles for normalized protein concentration (percent labeled concentration) are observed for the PF-06881893 DP and US-licensed Neupogen lots at the 25°C/60% RH accelerated stability testing condition (Figure 33).

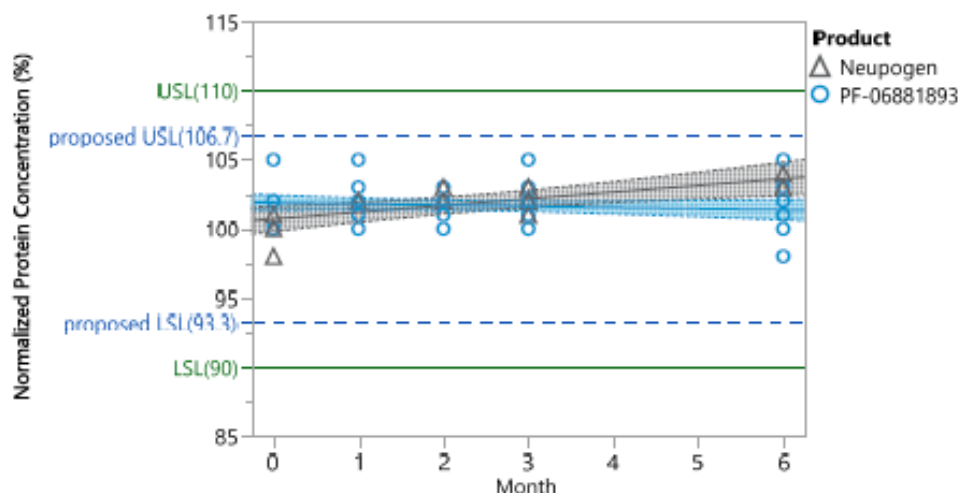


Figure 33: Comparison of normalized protein concentration for accelerated stability condition for PF-06881893 and US-licensed Neupogen (Figure 3.2.R.5.5.8-4 in BLA)

PF-06881893 DP and US-licensed Neupogen lots showed a slight increase in protein concentration over 6 weeks storage at the stressed condition (Figure 34). However, scatter plots of the 40°C/75% RH stressed stability data for individual lots visually show that there are no differences in stability profiles.

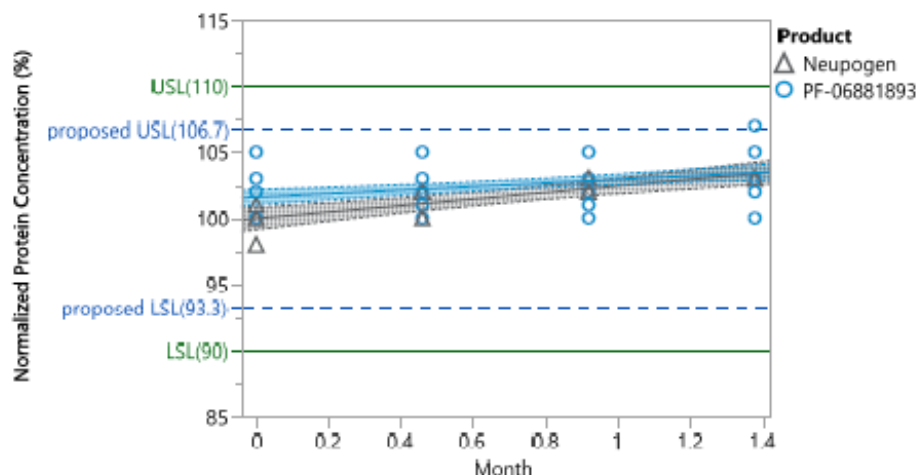


Figure 34: Comparison of normalized protein concentration at the stressed stability condition for PF-06881893 and US-licensed Neupogen (Figure 3.2.R.5.5.8-6 in BLA)

Reviewer comment: There are no differences in stability profiles of protein concentration of PF-06881893 DP and US-licensed Neupogen under long-term, accelerated, and stressed stability conditions. There is no noticeable difference between the PFS and SDV presentations or different dose strengths.

Potency: The percent relative potency evaluated by the in vitro cell-based bioassay does not substantially change over long-term stability conditions and there is no noticeable difference between the different presentations and dose strengths (Figure 35).

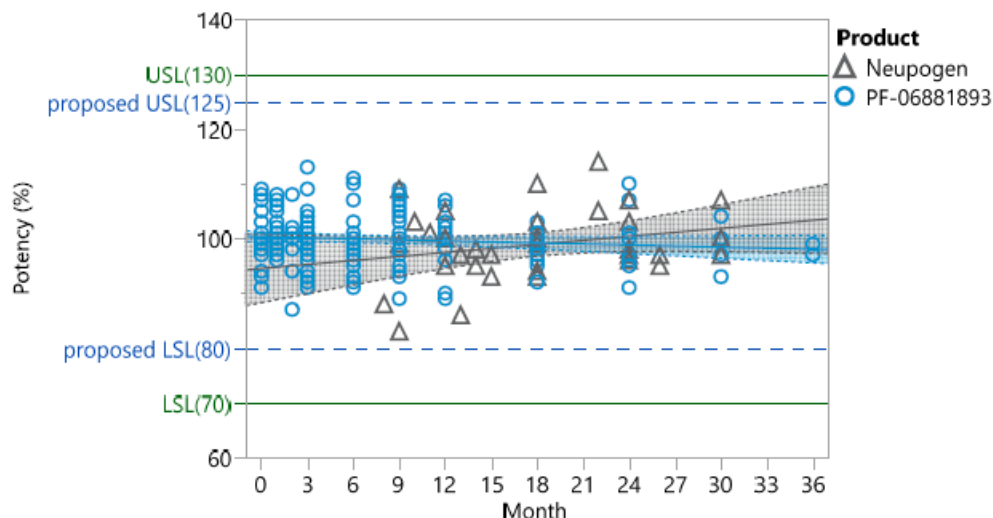


Figure 35: Comparison of *in vitro* cell based bioassay results for long term-stability at 5°C for PF-06881893 and US-licensed Neupogen (Figure 3.2.R.5.5.8-8 in BLA)

The % potency does not substantially change over time also at the 25°C/60% RH accelerated, and 40°C/75% RH stressed stability conditions for both PF-06881893 DP and US-licensed Neupogen. (Figure 36).

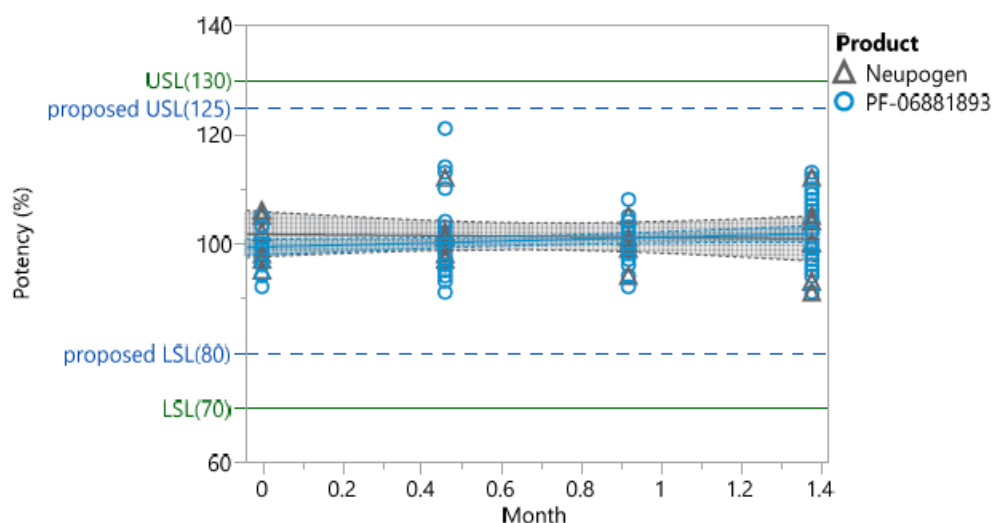


Figure 36: Comparison of *in vitro* cell based bioassay results for stressed stability at 40°C/75% RH for PF-06881893 and US-licensed Neupogen (Figure 3.2.R.5.5.8-12 in BLA)

High molecular weight species: The total HMWS long-term stability data are presented in scatter plots as a function of time in months with fitted regression lines for each PF-06881893 DP lot and each US-licensed Neupogen lot. At the long-term storage condition, the majority of the data points are $\leq$ LOQ (0.2 %) and visually, total HMWS does not appear to increase over long-term stability. There is no noticeable difference between different presentations and dose strengths.

At the accelerated storage condition, visual trending data indicate that US-licensed Neupogen HMWS levels appear to increase at a slightly faster rate than PF-06881893 DP (Figure 37). However it is to be noted that the US-licensed Neupogen lots are at least 8-13 months past their estimated manufacturing date, compared to the PF-06881893 DP lots that are placed on stability testing within 0-2 months after manufacture. The HMWS amounts for both PF-06881893 and US-licensed Neupogen lots are within their proposed and current effective stability specification, respectively.

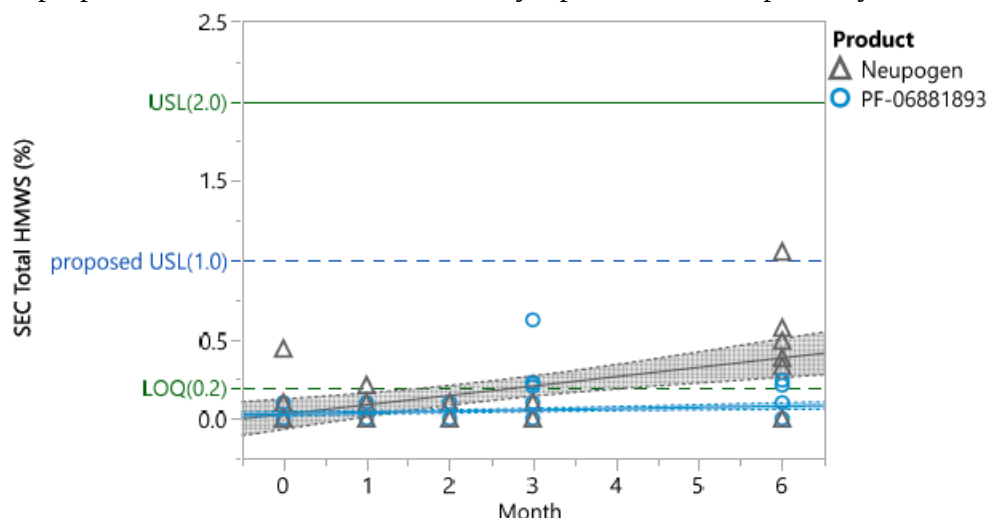


Figure 37: Comparison of SEC % total HMWS for accelerated stability at 25°C/60% RH for PF-06881893 and US-licensed Neupogen lots (Figure 3.2.R.5.5.8-16 in BLA)

At the stressed 40°C/75% RH condition, for both products, PFS lots on average appear to have a faster rate of increase in percent total HMWS than the SDV lots. The stability profiles of total HMWS, however, are visually similar between PF-06881893 DP and US-licensed Neupogen lots of the same presentation.

Reviewer comment: . There is no appreciable change in HMWS over time for either product under long-term and accelerated stability conditions. Increases in HMWS were observed under stressed stability conditions for both products. There are no differences in the HMWS stability profiles of PF-06881893 DP and US-licensed Neupogen under long-term, accelerated, and stressed stability conditions

Charge variants: Graphical analysis of long term stability data of total charge variants, is presented for PF-06881893 and US-licensed Neupogen. Visually, total charge variants do not exhibit a substantial change over time for either PF-06881893 DP or the US-licensed Neupogen product (Figure 38).

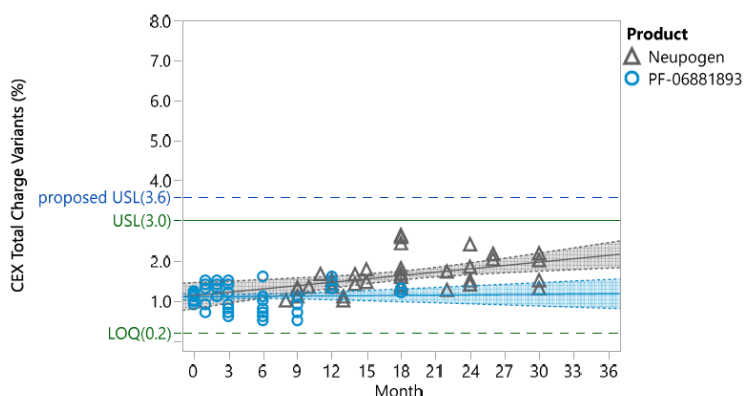


Figure 38: Comparison of CEX % total charge variants for long term stability at 5°C for PF-06881893 and US-licensed Neupogen (Figure 3.2.R.5.5.8-24 in BLA)

For the accelerated storage conditions, at the initial time point for US-licensed Neupogen, charge variant levels are higher than that of PF-06881893 lots. This may be due to difference in age of the products when they were placed on stability. However, no differences were noted in qualitative stability trends for both PF-06881893 and US-licensed Neupogen lots with an increase in total charge variants over time (Figure 39).

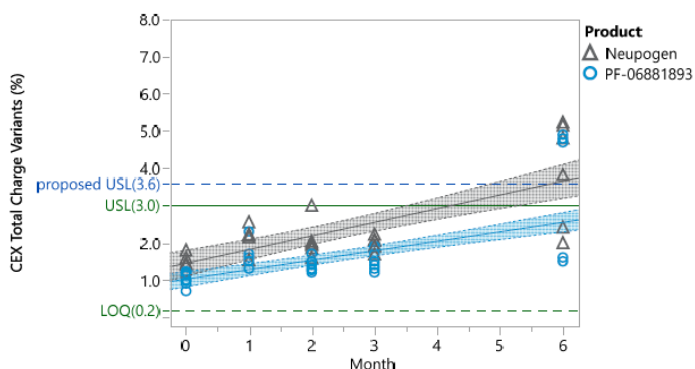


Figure 39: Comparison of CEX % total charge variants at accelerated stability at 25°C/60% RH (Figure 3.2.R.5.5.8-26 in BLA)

Stability data at the stressed condition also visually show no differences in profiles for PF-06881893 and US-licensed Neupogen.

Reviewer comments: There are no differences in the overall rate of increase in total charge variants between PF-06881893 and US-licensed Neupogen, at the accelerated and stressed stability conditions..

Filgrastim-related proteins: Graphical data obtained from RP-HPLC analysis are provided for visual comparison of total filgrastim-related proteins over the stability testing time points, at long-term, stressed, and accelerated conditions. The total filgrastim related proteins appear to increase over long-term stability except for PF-06881893 SDV lots. However this may be due to the fact that there is limited stability data for PF-06881893 SDV lots (up to (b) (4)) vs. up to 24 to 36 months for PFS presentations (Figure 40).

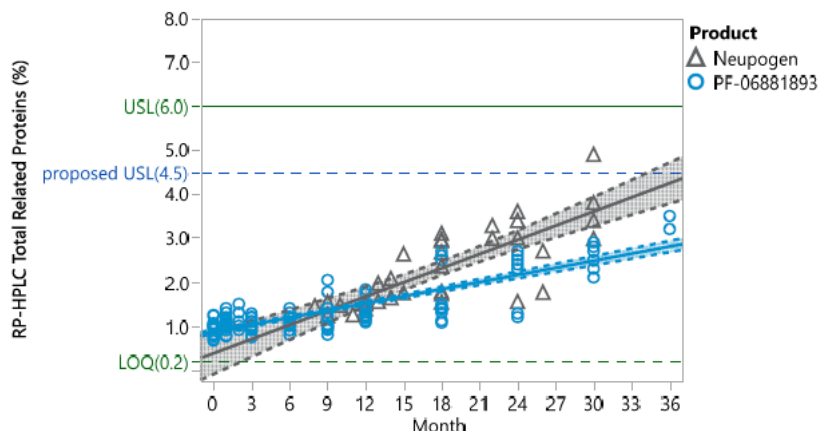


Figure 40: Comparison of RP-HPLC % total filgrastim related proteins at long term stability 5°C, for both PF-06881893 and US-licensed Neupogen (Figure 3.2.R.5.5.8-42 in BLA)

At accelerated and stressed stability conditions, no differences are observed in the qualitative stability profiles between PF-06881893 DP and US-licensed Neupogen.

Reviewer comment: The total filgrastim related proteins in PF-06881893 and US-licensed Neupogen show no differences in the rates of increase under long-term, accelerated, and stressed stability conditions.

Reviewer comment for comparative stability: Comparative stability analysis was conducted for PF-06881893 and US-licensed Neupogen at the long-term (5°C), accelerated (25°C/60% RH), and stressed (40°C/75% RH) conditions. The results for protein concentration, potency, and product-related impurities demonstrate that there are no differences in the rates and routes of degradation of PF-06881893 DP and US-licensed Neupogen.

Reviewer comments for analytical similarity section: The sponsor has provided a comprehensive and robust analysis of the structural and functional characterization of PF-06881893, as compared to US-licensed Neupogen using orthogonal techniques. These methods include assays used in the routine release and stability testing of PF-06881893, as well as characterization assays for PF-06881893 allowing a comparative analytical assessment between PF-06881893 and US-licensed Neupogen. 50 lots of Neupogen were procured over time and range in age. Individual filgrastim attributes were assigned to statistical analysis tiers where relevant. The Tier 1 attribute potency was evaluated using an equivalence testing approach by the CMC statistics reviewer, Dr. Wang. Tier 2 attributes were evaluated relative to a quality range that was defined based on the range observed for US-licensed Neupogen.

Attributes with descriptive testing results, at or below the method LOQ were not assigned to any statistical tier. Although not part of the analytical similarity assessment, residual HCP and DNA were evaluated for PF-06881893 and the results showed no differences with those for US-licensed Neupogen.

Comparative stability at the long-term storage, accelerated, and stressed conditions, and forced degradation was analyzed as recommended in FDA's Guidance for Industry: Quality Considerations in

Demonstrating Biosimilarity of a Therapeutic Protein Product to a Reference Product (Apr2015). Both these sets of analyses demonstrate that there are no differences in the rates and routes of degradation for PF-06881893 DP and US-licensed Neupogen.

The totality of the analytical evidence provided by the Applicant supports a demonstration that PF-06881893 is highly similar to US-licensed Neupogen.



Malini
Wileman

Digitally signed by Malini Wileman

Date: 6/06/2018 01:06:14PM

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Howard
Anderson

Digitally signed by Howard Anderson

Date: 6/06/2018 02:07:59PM

GUID: 508da6da00026589f1aa05fbbf3c0e87

BLA STN 761080

Product: PF-06881893, Nivestym™

Hospira, Inc., a Pfizer Company

Review of Drug Product CMC – Product Quality

Scott Lute MSc., Primary Reviewer
Howard Anderson, PhD., Team Leader

Office of Biotechnology Products
Division of Biotechnology Review and Research II

Recommendation: From the Drug Product Quality Perspective This BLA is Recommended for Approval.

OBP CMC Review Data Sheet

1. BLA#: STN 761080

2. Review Date: April 27, 2018

3. Primary Review Team:

a. Medical Officer: Lea Cunningham
b. Product Quality Team: Malini Wileman (DS Quality Reviewer)
Scott Lute (DP and Immunogenicity Reviewer)
Howard Anderson (Application Technical Lead)
c. Facilities: Marion Michaelis
d. Clinical Pharmacology: Theingi Thway
e. Statistics: Lola Luo
Tianhua Wang
f. OBP Labeling: Vicky Borders-Hemphill
g. RBPM: Kelly Ballard (OPQ), Wanda Nguyen (OHOP)

4. Major GRMP Deadlines:

a. Filing Meeting: November 3, 2017
b. Mid-cycle meeting: February 15, 2018
c. Wrap-up meeting: June 7, 2018
d. GRMP Primary review due: June 12, 2018
e. GRMP Secondary review due: June 21, 2018
f. BsUFA action date: July 21, 2018

5. Communications with Sponsor and OND:

Communication/Document:	Date:
Information Request	December 13, 2017
Information Request	March 5, 2018
Information Request	April 18, 2018
Information Request	June 6, 2018

6. Submission Reviewed:

Submission:	Date Received:	Review Completed (yes or no)
Response to Information Request	January 3, 2018	yes
Response to Information Request	March 19, 2018	yes
Response to Information Request	May 2, 2018	yes
Response to Information Request		Covered in ATL Summary

7. Drug Product Name/Code/Type:

a. Proprietary Name: PF-06881893
b. Trade Name: Nivestym™

c. Non-Proprietary Name/USAN filgrastim-aafi (pending)
d. CAS Name: 121181-53-1
e. Common Name: filgrastim
f. INN Name: Filgrastim
g. Compendial Name: N/A
h. OBP systematic name: RPROT P09919 (CSF3_HUMAN) Granulocyte colony
stimulating factor (PF06881893)
i. Other names.

8. Pharmacological Category: Recombinant methionyl human granulocyte-colony
stimulating factor

9. Dosage Form: Injection, Pre-filled Syringe and Single Dose Vial

10. Strength/Potency:
(i): Pre-filled Syringe: 300 mcg/0.5 mL and 480 mcg/0.8 mL
Single Dose Vial: 300 mcg/1.0 mL and 480 mcg/1.6 mL
(ii): Type of potency assay: in Vitro, proliferative response of M-NFS-60 cells

11. Route of Administration: Intravenous and subcutaneous

12. Referenced Drug Master Files (DMF):

DMF#	DMF Holder	Item Referenced	Letter of Cross-Reference	Comments (status)
(b) (4)			Yes	DMF is current and will comply with statements made within
			Yes	DMF is current and will comply with statements made within
			Yes	DMF is current and will comply with statements made within
			Yes	DMF is current and will comply with statements made within

(b) (4)	Yes	DMF is current and will comply with statements made within
	Yes	DMF is current and will comply with statements made within
	Yes	DMF is current and will comply with statements made within
	Yes	DMF is current and will comply with statements made within

13. Inspectional Activities: OBP did not participate in Drug Product Review
Inspection October 27, 2017. Hospira Kansas was VAI.
Inspection February 9, 2018 Hospira Zagreb was VAI.

14. Consults Requested by OBP:
None. Clinical has requested consults from CDRH ODE and OC

15. Quality by Design Elements:
The following was submitted in the identification of QbD elements (check any that apply):

	Design Space
X	Design of Experiments
X	Formal Risk Assessment/Risk Management
	Multivariate Statistical Process Control
	Process Analytical Technology
	Expanded Change Protocol

16. Precedents: N/A

17. Administrative:

Summary of Quality Assessments

I. Primary Reviewer Summary Recommendation

I recommend approval of this 351(k) Biologics License Application. The BLA contains adequate information to support that the drug product manufacturing process is controlled and validated and produces a consistent stable drug product. The drug substance (DS) and analytical

similarity assessment are provided in the drug substance review. The anti-drug antibody method validation is also covered in a separate review.

18 months of real time stability results are provided for the PF-06881893 Drug Product stored in single dose vials (SDV) for the 6 validation lots in the BLA to support a proposed expiry of (b) (4) 18 months of real time stability results are provided for the PF-06881893 Drug Product stored in the prefill syringe (PFS) for 6 validation lots and 24 months of data from clinical lots. The BLA proposes a 30-month expiry. An IR was sent on June 6, 2018 requesting updated stability results to support a 30-month expiry as well as updated to any changes to expiry dates that may have occurred with the new stability results. The length of expiry will be assigned after a review of the updated stability results and covered in the Application Team Lead (ATL) memo.

II. List of Deficiencies to be Communicated

There are no CMC-Product Quality deficiencies precluding approval of this BLA

III. List of Post-Marketing Commitments/Requirements

There are no Post-Marketing Commitments.

IV. Review of Common Technical Document- Quality Module 1

A. Environmental Assessment of Claim of Categorical Exclusion

The Claim of Categorical Exclusion was reviewed separately by Malini Wileman as part of the Drug Substance product quality review and exclusion from the requirement is granted.

V. Primary Container Labeling Review

The primary container labeling was reviewed by Vicky Borders-Hemphill

VI. Review of Common Technical Document- Quality Module 3.2

CTD modules in 3.2.P and partial modules in 3.2.A and 3.2.R are reviewed in this document. Modules 3.2.S, 3.2.A, and 3.2.R were reviewed separately by Malini Wileman

VII. Review of Immunogenicity Assays- Module 5.3.1.4

The immunogenicity assays are covered in a separate review by Scott Lute and Harold Dickensheets.

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P: Drug Product

3.2.P.1 Description and Composition of the Drug Product

PF-06881893 drug product (DP) is a sterile, colorless aqueous solution intended for intravenous or subcutaneous injection. DP is manufactured in two presentations: pre-filled syringes (PFS) and single dose vials (SDV). The DP in each presentation contains the active ingredients and excipients shown in the following table with no preservatives:

Table 3.2.P.1-1. Qualitative Composition of PF-06881893 DP

Name of Component	Reference to Standard	Function
PF-06881893 DS ^a	In-house specification	Active ingredient (b) (4)
Sorbitol	NF	
Sodium ^b	[USP, In-house] ¹ , [NF] ²	
Acetate ^c	[USP, In-house] ¹ , [USP] ³	
Polysorbate 80	NF, In-house	
(b) (4)	NF	
Water for injection	USP	

a. DS, drug substance

b. (b) (4)

c.

Pre-Filled Syringe

The PF-06881893 DP PFS presentation is supplied in a 1 mL long USP (b) (4) glass syringe with a 27 gauge ½ inch needle, sealed with (b) (4) stopper and rigid needle shield. The PFS presentation has two nominal fill volumes of 0.5 mL (300 µg dose strength) and 0.8 mL (480 µg dose strength). There is an overfill of (b) (4) mL for the 300 µg/0.5 mL PFS presentation and (b) (4) mL overfill for the 480 µg/0.8 mL presentation (b) (4)

The quantitative composition for the PFS presentation is shown in the table below:

Table 3.2.P.1-2 Quantitative Composition of PF-06881893 DP PFS

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

a. drug substance

b. (b) (4)

c.

(b) (4)

Single Dose Vial

The PF-06881893 DP PFS presentation is supplied in a 2 mL clear USP (b) (4) glass vial with a (b) (4) stopper and crimped with a 13 mm flip-off aluminum seal. The SDV presentation has two nominal fill volumes of 1.0 mL (300 µg dose strength) and 1.6 mL (480 µg dose strength). There is an overfill of (b) (4) mL for the 1.0 mL SDV and (b) (4) mL for the 1.6 mL SDV (b) (4)

The quantitative composition for the PFS presentation is shown in the table below:

Table 3.2.P.1-2. Quantitative Composition of PF-06881893 DP SDV

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

Reviewer's Comments: The PF-06881893 DP was developed to match the presentations and dose strengths of the originator drug product, US-Licensed Neupogen®. The two presentations utilize the same formulation protein concentration (600 µg/mL PFS, 300 µg/mL SDV) as the originator. .

3.2.P.2 Pharmaceutical Development

(b) (4)

3.2.P.8 Stability

For the PFS the BLA proposes a 30-month expiry and this is supported by 18 months of data from 6 validation lots and 24 months of data from clinical lots. An IR was sent June 6, 2018 requesting updated stability data to support the 30-month expiry. This does not affect approvability and the expiry will be covered in the ATL summary.

For the SDV the BLA proposes a (b) (4) expiry and this is supported by 18 months of stability data from 6 validation lots.

3.2.P.8.1, 3.2.P.8.3 Stability Summary, Stability Data, and Conclusion

Pre-Filled Syringes:

Stability data was generated for a total of 16 lots of PF-06881893 DP PFS in both dose strengths (300 µg/0.5 mL and 480 µg/0.8 mL). All DP stability lots (engineering, clinical, non-clinical, and PPQ lots) were manufactured using the intended commercial scale DP manufacturing process and were considered primary stability lots. The stability conditions evaluated include long-term (5°C ± 3°C), accelerated (25°C/60%RH), and stressed (40°C/75%RH) stability conditions, as well as several additional stability conditions:

- In-use at 25°C/60%RH post long-term storage at 5°C
- 3 cycles of temperature cycling at 25°C/5°C
- 3 cycles of temperature cycling at -20°C/5°C
- Photostability under ICH Q1B Option 2 light exposure
- Photostability under manufacturing light exposure

A subset of the release tests used for PF-06881893 DP and Container Closure Integrity are used for stability testing. The PFS stability samples are stored horizontally in 1 mL (b) (4) glass staked needle syringes with a rigid needle shield and as (b) (4) plunger stopper, the proposed commercial primary container closure system. Long-term storage testing is performed for 36 months, accelerated for 6 months, and stressed for 6 weeks. The table below summarizes the stability tests performed on the different lots of DP PFS for long-term, accelerated, and stressed storage conditions.

Table. Summary of Stability Tests Performed on DP PFS Lots

Stability Tests	Long-Term 5°C			Stressed 25°C/60%RH		Accelerated 40°C/75%RH
	Eng Lots 1	Eng Lots 2, Clinical Lots	PPQ Lots	Eng Lots 1	PPQ Lots, Eng Lots 2, Clinical Lots	All lots
Appearance, Color and Clarity	X	X	X	X	X	X
pH	X	X	X	X	X	X
Visible Particles	X	X	X	X	X	X
Particulate Matter	X	X	X	X	X	X
Protein Concentration (UV-Vis)	X	X	X	X	X	X
Potency (In Vitro Cell-Based)	X	X	X	X	X	X
SEC	X	X	X	X	X	X
SDS-PAGE	X	X	X	X	X	X
CEX	X	X	X	X	X	X
RP-HPLC	X	X	X	X	X	X
Sterility	X	X	X	-	X	-
Bacterial Endotoxins	X	X	X	-	X	-
Polysorbate 80	X	X	X	X	X	X
Container Closure Integrity	-	-	X	-	X	-
Syringe Function	-	X	X	-	X	-

Eng Lots 1: 2854103, 2855103, 2056024, 2057024, 2053054, 2054054

Eng Lots 2: 2073094, 2074094

Clinical Lots: 2074114, 2075114

PPQ Lots: 2572075, 2573075, 2574075, 2575075, 2576075, 2577075

Long Term Storage

Sixteen lots of PF-06881893 DP PFS are included in the long-term stability study. At the time of this submission, 2 PFS DP lots have completed the program and all others are in progress. All of the available data meet the proposed commercial PF-06881893 DP specifications. Statistical analysis demonstrates a significant increase in Total Filgrastim Related Proteins by RP-HPLC, but all data points were within the specifications for all time points completed. There was no substantial change over time for any other quality attribute. The avail long-term stability data supports the proposed shelf-life of 30 months when stored at 5°C.

Accelerate Storage

Sixteen lots of PF-06881893 DP PFS are included in the 6-month accelerated stability study and all have completed the study. All data from the accelerated study meet the proposed commercial DP specifications except Total Filgrastim Related Proteins and Total Charge Variants. Both were above the commercial DP specifications at the 6-month time point, but were within specification limits up to 3 months for all lots. PF-06881893 DP PFS is stable for up to 3 months when stored at 25°C/60%RH.

Stressed Storage

Sixteen lots of PF-06881893 DP PFS are included in the 6-week stressed stability study and all lots have competed the program. Statistical analysis of the data indicates that there are significant increases in Total HMWS, Total Filgrastim Related Proteins, and Total Charge Variants. The latest time point when these tests were within specification was the 2 week time point, suggesting that PF-06881893 DP PFS is stable for up to 2 weeks when stored at 40°C/75%RH.

Reviewer's Comments: The sponsor has ongoing long term stability studies on 14 lots of PF-06881893 DP PFS for 36 months. Currently there is data from 6 DP lots derived from 3 DS lots at 30 months or more of recommended storage conditions. This data supports the current proposed shelf life of 30 months at 5°C for PFS presentations. The accelerated and stressed storage conditions predict that Total HMWS, Total Filgrastim Related Proteins, and Total Charge Variants will increase over time and that the product remains stable when stored at 25°C/60%RH for up to 3 months. The proposed shelf-life and stability program is acceptable and the sponsor will provide updated stability data in annual reports.

In-Use Stability (25°C):

In-use stability studies are designed as a hybrid storage protocol where samples stored at long term storage conditions for different time intervals are transferred to the accelerated 25°C/60%RH storage condition. Testing is performed after storage at the accelerated condition for 15, 21, and 30 days. 6 PFS lots were tested according to the protocol in Table 3.2.P.8.1-8.

Table 3.2.P.8.1-8. In-Use Stability (25°C) Study Protocol

Long-term 5°C Time Point	6 Months ^a	18 Months ^b	30 Months
Test	25°C/60% RH In-Use Study Time Point (Days)		
Appearance, Color and Clarity	0, 15, 21, 30	0, 15, 21, 30	0, 15, 21, 30
pH	0, 15, 21, 30	0, 15, 21, 30	0, 15, 21, 30
Visible Particles	0, 15, 21, 30	0, 15, 21, 30	0, 15, 21, 30
Particulate Matter (≥ 2 µm, ≥ 5 µm, ≥ 10 µm, ≥ 25 µm)	0, 15, 21, 30	0, 15, 21, 30	0, 15, 21, 30
Protein Concentration (UV-Vis)	0, 15, 21, 30	0, 15, 21, 30	0, 15, 21, 30
Potency (In Vitro Cell-Based)	0, 15, 21, 30	0, 15, 21, 30	0, 15, 21, 30
SEC	0, 15, 21, 30	0, 15, 21, 30	0, 15, 21, 30
SDS-PAGE	0, 15, 21, 30	0, 15, 21, 30	0, 15, 21, 30
CEX	0, 15, 21, 30	0, 15, 21, 30	0, 15, 21, 30
RP-HPLC	0, 15, 21, 30	0, 15, 21, 30	0, 15, 21, 30
Sterility	0, 15, 21, 30	0, 15, 21, 30	0, 15, 21, 30
Bacterial Endotoxins	0, 15, 21, 30	0, 15, 21, 30	0, 15, 21, 30
Polysorbate 80	0, 15, 21, 30	0, 15, 21, 30	0, 15, 21, 30

UV-Vis, Ultraviolet-Visible Spectroscopy; SEC, Size Exclusion Chromatography; SDS-PAGE, Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis, CEX, Cation Exchange Chromatography; RP-HPLC, Reversed Phase-High Performance Liquid Chromatography

a. Time point only includes lots 2573075, 2575075, and 2576075

b. Time point only includes lots 2074114, 2573075, 2575075, and 2576075

All 6 lots have completed the 30-day in-use stability study and all data meet the proposed commercial DP specifications for all quality attributes with no substantial change over time for any attribute. The data demonstrate that DP PFS is stable following short term 25°C storage at either the beginning, middle, or end of the proposed DP shelf-life.

Reviewer's Comments: 6 lots of in-use stability data demonstrated no impact on the quality attributes tested as part of the shelf-life specifications. This supports an allowable in-use period of up to 30 days at 25°C.

Temperature Cycling:

Temperature cycling studies investigate both -20°C/5°C (freeze/thaw) cycling and 25°C/5°C (room temperature/refrigerated) cycling. For each cycling study, 6 lots of DP PFS were stored at recommended

conditions for 12 months, 24 months, and 30 months prior to the temperature cycling. Samples were then subjected to 3 temperature cycles that consisted of:

- -20°C/5°C: each cycle is 2 days stored at -20°C followed by 2 days at 5°C
- 25°C/5°C: each cycle is 2 days stored at 25°C followed by 2 days at 5°C

After 3 temperature cycles at the 12-month, 24-month, and 30-month time point, the samples were tested along with the identical un-cycled time points. The data from both temperature cycling studies demonstrate that there is no impact on DP quality attributes from excursions to freezing temperatures or room temperatures through the proposed shelf-life at recommended conditions.

Reviewer's Comments: *The temperature cycling studies demonstrate that PF-06881893 DP PFS is stable for short term temperature excursion above and below the recommended storage condition as no impact was seen on quality attributes.*

Photostability Studies:

Photostability studies were designed to evaluate light exposure during storage and transport according to ICH Q1B (option2), and to evaluate the effect of light exposure during manufacturing.

For ICH Q1B testing, 2 lots of PF-06881893 DP PFS were either stored wrapped in aluminum foil (dark control), light exposed in primary packaging, or light exposed in packaging cartons (syringe stored in cardboard cartons). The test samples were exposed to 1.2 million lux hours of white light and 200 watt hours/m² of near UV light in a light cabinet set to 25°C/60%RH. The samples were tested according to the proposed DP shelf-life specifications. The results demonstrate that unprotected DP in PFS are susceptible to photodegradation. The following quality attributes were affected in unprotected, light exposed DP PFS compared to the dark control.

- Increase in charge variants (1.4% vs. 11.9%)
- Increase in HMWS (LOD vs. 4.7%)
- Increase in Total Filgrastim Related Proteins (3.0% vs. 34%, oxidized species specifically)
- Reduction in PS80 concentration (0.004% vs. 0.002%)

A comparison of light exposed syringe to the dark control and the corresponding samples stored in packaging cartons demonstrated that the secondary packaging is effective at protecting the PF-06881893 DP PFS from photodegradation.

The second photostability study investigated the effect of light exposure during the routine manufacturing process at the Hospira Zagreb site. 1 PFS lot was tested by exposing samples to up to (b) (4) lux hours and an integrated UV energy of up to (b) (4) watt hours/m². These values represent the average routine exposure to light in the Hospira Zagreb facility and was determined by a formal light mapping study. The results demonstrated that routine light exposure during manufacturing has no impact on PF-06881893 DP PFS product quality.

Reviewer's Comments: *The photostability data demonstrate that PF-06881893 DP PFS is susceptible to photostress during long term storage but not under routine manufacturing conditions. The studies also demonstrate that the proposed secondary packaging for DP PFS is effective at protecting the product from photodegradation. The sponsor will include handling instructions to protect the DP from light on the labeling. This is acceptable.*

Single Dose Vials

Stability studies for PF-06881893 DP SDV included 10 lots (4 engineering and 6 PPQ) manufactured between January 2016 and July 2016 at the Hospira Kansas facility. The DP lots include 300 µg/1.0 mL and 480 µg/1.6 mL dosage strengths and all were manufactured with the intended commercial scale DP manufacturing process and derived from DS lots that were stored at $\leq -65^{\circ}\text{C}$. The stability conditions evaluated include long-term ($5^{\circ}\text{C} \pm 3^{\circ}\text{C}$), accelerated ($25^{\circ}\text{C}/60\%\text{RH}$), and stressed ($40^{\circ}\text{C}/75\%\text{RH}$) stability conditions, as well as several additional stability conditions:

- Frozen at -20°C
- Photostability under ICH Q1B Option 2 light exposure
- Photostability under manufacturing light exposure

A subset of the release tests used for PF-06881893 DP and Container Closure Integrity are used for stability testing. The SDV stability samples are stored upright and/or inverted in 2 mL (b) (4) glass vials with (b) (4) closure (stopper), and an aluminum seal with a tamper-evident Flip-Off cap, the proposed commercial primary container closure system. Long-term storage testing is performed for 36 months, accelerated for 6 months, and stressed for 6 weeks. The table below summarizes the stability tests performed on the different lots of DP SDV for long-term, accelerated, and stressed storage conditions.

Long Term Storage

Ten lots of PF-06881893 DP SDV are included in the long-term stability study. At the time of this submission, the 4 engineering lots have up to 9 months of stability data and the 6 PPQ lots have up to 6 months of stability data. The 4 SDV engineering lots were stored in the inverted orientation only for up to 6 months. The 36-month long term stability study is currently in-progress for all stability lots. All the currently available data meet the proposed commercial PF-06881893 DP specifications including sterility and endotoxin testing. However, analysis of the protein concentration plots indicates a downtrend (b) (4)

see Figure 3.2.P.8.3-11 below.

Figure 3.2.P.8.3-11. Plot of Protein Concentration Stability Data for the Long-Term 5°C Storage Condition



The sponsor contributes the negative slopes to the limited number of stability time points collected at the time of statistical analysis, indicating that the trend would change with future updates to the stability data. Despite this downward trend, all available Protein Concentration stability data meet the proposed commercial DP SDV specifications for up to (b) (4) (b) (4)

Reviewer's Comments: The currently available DP SDV stability data meet the proposed DP shelf-life specifications for recommended storage up to (b) (4) for all attributes tested. The sponsor will provide stability data updates in annual reports. However, the downward trend in protein concentration is a concern that could limit the shelf-life of DP SDV. An IR will be sent to the sponsor April 18, 2018 to submit updated stability data to determine if protein concentration is a real stability concern or an artifact of limited data points.

IR Response 5/2/2018: The sponsor provided stability data through 18 months at the long term storage condition for all 10 PF-06881893 DP SDV lots. The updated data (Figure 3 below) confirm that the downward was an artifact of limited sample data points and all lots demonstrate protein concentration values above the lower specification limit for up to 18 months, (b) (4) PF-06881893 DP SDV is considered stable for the (b) (4) expiry date.

Figure 3. Plot of Protein Concentration Stability Data for the Long-Term 5°C Storage Condition



Accelerate Storage

Ten lots of PF-06881893 DP SDV are included in the 6-month accelerated stability study and all have completed the study. The 4 SDV engineering lots were stored in the inverted orientation only for the entire 6 months. All data from the accelerated study meet the proposed commercial DP specifications at 6 months, except Total Filgrastim Related Proteins. 5 of the DP lots were above the Total Filgrastim Related Protein upper specification limit at the 6-month time point, but were within specification limits at the 3-month time all lots. The same downward trend in Protein concentration was seen with 3 lots;

however, all lots were still above the lower protein concentration specification limit throughout the 6-month study. PF-06881893 DP SDV is stable for up to 3 months when stored at 25°C/60%RH.

Stressed Storage

Ten lots of PF-06881893 DP SDV are included in the 6-week stressed stability study and all lots have completed the program. The 4 SDV engineering lots were stored in the inverted orientation only for the entire 6 weeks. Statistical analysis of the data indicates that there are significant increases in HMWS, Total Filgrastim Related Proteins, and Total Charge Variants. The latest time point when these tests were within specification was the 2-week time point, suggesting that PF-06881893 DP SDV is stable for up to 2 weeks when stored at 40°C/75%RH.

Reviewer's Comments: *The accelerated and stressed storage conditions had similar results as the DP PFS presentation. PF-06881893 DP is susceptible to an increase in Total Filgrastim Related Proteins, HMWS, and Charge Variants when stored at higher temperature for extended periods of time with a direct correlation between time and temperature. The accelerated stability data, provides evidence that DP SDV will be stable at room temperature during use. These studies are acceptable.*

Frozen Stability (-20°C):

Four lots of DP SDV were included in the frozen stability study. The 36-month study is currently in progress and updates will be provided in annual reports. Currently, all four lots demonstrate stability for up to 12 months when stored at -20°C.

Photostability Studies:

Photostability studies were performed as described in the PFS photostability section above. For ICH Q1B testing, unprotected PF-06881893 SDV exhibited an increase of 2% in Total Filgrastim related Proteins relative to the protected dark control, but still below the upper specification limit. No other attribute evaluated had an appreciable change. Samples stored in secondary packaging carton demonstrated no change in attributes indicating that the secondary packaging can protect the DP SDV from photostress.

Manufacturing photostability was studied by exposing DP SDV samples to up to (b) (4) lux hours and an integrated UV energy of up to (b) (4) watt hours/m². All stability data meet the DP specifications indicating that routine exposure to light during manufacturing is not a concern.

Reviewer's Comments: *The photostability studies demonstrated that PF-06881893 DP SDV is not affected by light exposure as greatly as the PFS presentation. All data meet the DP specification for the SDV photostability studies; however, handling instructions to protect from light will be included in the labeling.*

3.2.P.8.2 Post-Approval Stability Commitment

The sponsor commits to have at least 1 commercial scale lot of PF-06881893 DP in each PFS and SDV presentation placed into the stability program at the long-term storage condition of 2 to 8°C each year that DP is manufactured. The PF-06881893 DP PFS stability samples will be placed in the horizontal position and the SDV stability samples in the inverted position. PFS and SDV lots will be enrolled in the commercial stability program with the 480 mcg/0.8 mL PFS and 300 mcg/1.0 mL SDV alternating

annually with the 300 mcg/0.5 mL PFS and 480 mcg/1.6 mL SDV presentations. The proposed stability protocol is presented in [Table 3.2.P.8.2-1](#).

Table 3.2.P.8.2-1. Post-Approval Commercial Stability Protocol for Long-Term Storage of PF 06881893 Drug Product at 2 to 8°C

Test	Test Intervals (Months)
Appearance, Color and Clarity	0, 6, 12, 18, 24, 36
pH	0, 6, 12, 18, 24, 36
Visible Particles	0, 12, 24, 36
Particulate Matter	0, 12, 24, 36
Protein Concentration (UV-Vis)	0, 6, 12, 18, 24, 36
Potency (In Vitro Cell-Based)	0, 6, 12, 18, 24, 36
SEC	0, 6, 12, 18, 24, 36
SDS-PAGE	0, 6, 12, 18, 24, 36
CEX	0, 6, 12, 18, 24, 36
RP-HPLC	0, 6, 12, 18, 24, 36
Sterility	0, 36
Bacterial Endotoxins	0, 12, 24, 36
Container Closure Integrity	12, 24, 36
Syringe Function (Break Loose and Extrusion Force) ^a	0, 12, 24, 36

UV, Ultraviolet; Vis, Visible; SEC, Size Exclusion Chromatography; SDS-PAGE, Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis; CEX, Cation Exchange Chromatography; RP, Reversed Phase; HPLC, High Performance Liquid Chromatography

a. Syringe Function is tested for prefilled syringe presentation only

Currently available stability data for PFS and SDV samples support the initial proposed shelf-life of 30 months for PFS and (b) (4) for SDV stored at 2 to 8°C. As additional long-term stability data become available, the shelf-life may be extended if the data meets the specifications. Stability updates will be submitted in the annual reporting system.

Reviewer's Comments: The sponsor commits to submitting updated stability data in annual reports and extending the shelf-life of PF-06881893 DP PFS and/or SDV if the data supports such a change. The PMC is acceptable.



Scott
Lute

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Howard
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