

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

761084Orig1s000

PRODUCT QUALITY REVIEW(S)



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

LABELS AND LABELING ASSESSMENT

Date of Assessment:	May 24, 2022
Assessor:	Vicky Borders-Hemphill, PharmD Labeling Assessor Office of Biotechnology Products (OBP)
Through:	Anshu Rastogi, PhD, Product Quality Assessor OBP/Division of Biotechnology Review and Research 1
Application:	BLA 761084
Applicant:	Kashiv BioSciences, LLC
Submission Date:	November 29, 2021
Product:	pegfilgrastim-pbbk
Dosage form(s):	injection
Strength and Container-Closure:	6 mg/0.6 mL solution in a single-dose prefilled syringe for manual use only
Purpose of assessment:	The Applicant submitted a biologics license application Agency assessment
Recommendations:	The prescribing information (submitted on May 23, 2022), Patient Labeling Information and Instructions for Use (submitted on May 6, 2022), container labels (submitted on May 24, 2022), and carton labeling (submitted on May 12, and May 24, 2022) were assessed and found to be acceptable (see Appendix C) from an OBP labeling perspective.

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

n/a = not applicable for this assessment

DISCUSSION

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

CONCLUSION

The prescribing information (submitted on May 23, 2022), Patient Labeling Information and Instructions for Use (submitted on May 6, 2022), container labels (submitted on May 24, 2022), and carton labeling (submitted on May 12, and May 24, 2022) were assessed and **found to be acceptable (see Appendix C)** from an OBP labeling perspective.

APPENDICES

Appendix A: Proposed Labeling

Prescribing Information (submitted on November 29, 2021

<\\CDSESUB1\evsprod\bla761084\0036\m1\us\114-labeling\draft-labeling\draft-label-text\draft-label-text-prescr-info.pdf>)

Patient Information Labeling (submitted on November 29, 2021

<\\CDSESUB1\evsprod\bla761084\0036\m1\us\114-labeling\draft-labeling\draft-label-text\draft-patient-information.pdf>)

Instructions for Use (submitted on November 29, 2021

<\\CDSESUB1\evsprod\bla761084\0036\m1\us\114-labeling\draft-labeling\draft-label-text\draft-ifu.pdf>)

2 Page(s) of Draft Labeling have been Withheld in Full as b4 (CCI/TS) immediately following this page

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

Container⁴ Label Evaluation

Proper Name (container label)	Acceptable
Regulations: 21 CFR 610.60(a)(1), 21 CFR 201.10(g)(2), 21 CFR 610.62(a), 21 CFR 610.62(b), 21 CFR 610.62(c), 21 CFR 610.60(c), 21 CFR 201.50(b), 21 CFR 201.10(a), 21 CFR 201.10(h)(2)(i)(1)(i)	☒ Yes ☐ No ☐ N/A
<i>Recommended labeling practices (placement of dosage form outside of parenthesis and/or below the proper name)</i>	☒ Yes ☐ No ☐ N/A

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

Comment/Recommendation: The Applicant revised "Amneal Pharmaceuticals LLC" in place of "Kashiv BioSciences, LLC". The name of the manufacturer and address is the applicant listed on FDA form 356h, "Manufactured by: Kashiv BioSciences, LLC Piscataway NJ 08854", thus revise back to the Manufacturer's name and address. The distributor information "Distributed by: Amneal Pharmaceuticals LLC" may be included but only if the manufacturer's information is also provided. *The Applicant revised as requested*

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 21 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.

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

Lot number or other lot identification (container label)	Acceptable
Regulations: 21 CFR 610.60(a)(3), 21 CFR 610.60(c), 21 CFR 201.18, 21 CFR 201.100(b)(6), 21 CFR 201.10(h)(2)(i)(1)(iii)	☒ Yes ☐ No ☐ N/A

Expiration date (container label)	Acceptable
Regulations: 21 CFR 610.60(a)(4), 21 CFR 201.17	☒ Yes ☐ No ☐ N/A
<i>Recommended labeling practices references: USP General Chapters <7> Labeling, Draft Guidance Safety Considerations for Container Labels and Carton Labeling Design to Minimize Medication Errors, April 2013 lines 178-184, which, when finalized, will represent FDA's current thinking on topic</i>	☒ Yes ☐ No ☐ N/A

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

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

Multiple-dose containers (container label)	Acceptable
Regulations: 21 CFR 610.60(a)(5), 21 CFR 201.55 <i>(recommended individual dose)</i>	☐ Yes ☐ No ☒ N/A

Statement: "Rx only" (container label)	Acceptable
Regulations: 21 CFR 610.60(a)(6), 21 CFR 201.100(b)(1)	☒ Yes ☐ No ☐ N/A

<i>Recommended labeling practices (prominence of Rx Only statement) reference: Draft Guidance Safety Considerations for Container Labels and Carton Labeling Design to Minimize Medication Errors, April 2013 line 147, which, when finalized, will represent FDA's current thinking on topic</i>	☒ Yes ☐ No ☐ N/A

Comment/Recommendation: Add 'Rx Only' to the syringe container label
The Applicant revised as requested

Medication Guide (container label)	Acceptable
Regulations: 21 CFR 610.60(a)(7), 21 CFR 208.24(d)	☐ Yes ☐ No ☒ N/A

No Package for container (container label)	Acceptable
Regulation: 21 CFR 610.60(b)	☐ Yes ☐ No ☒ N/A

No container label (container label)	Acceptable
Regulation: 21 CFR 610.60(d)	☐ Yes ☐ No ☒ N/A

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

Visual inspection	Acceptable
Regulation: 21 CFR 610.60(e)	☒ Yes ☐ No ☐ N/A

Comment/Recommendation: Confirm that sufficient area of the container remains uncovered for its full length or circumference to allow for visual inspection when the label is affixed to the container and indicate where the visual area of inspection is located
Kashiv confirms that sufficient area of the container remains uncovered at the circumference to allow for visual inspection when the label is affixed to the container

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

NDC numbers (container label)	Acceptable
Regulations: 21 CFR 201.2, 21 CFR 207.35	☒ Yes ☐ No ☐ N/A

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

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

Misleading statements (container label)	Acceptable
Regulation: 21 CFR 201.6	☐ Yes ☐ No ☒ N/A

Prominence of required label statements (container label)	Acceptable
Regulation: 21 CFR 201.15	☒ Yes ☐ No ☐ N/A

Spanish-language (Drugs) (container label)	Acceptable
Regulation: 21 CFR 201.16	☐ Yes ☐ No ☒ N/A

FD&C Yellow No. 5 and/or FD&C Yellow No. 6 (container label)	Acceptable
Regulation: 21 CFR 201.20	☐ Yes ☐ No ☒ N/A

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

Strategic National Stockpile (exceptions or alternatives to labeling requirements for human drug products) (container label)	Acceptable
Regulations: 21 CFR 610.68, 21 CFR 201.26	☐ Yes ☐ No ☒ N/A

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

Statement of Dosage (container label)	Acceptable
Regulations: 21 CFR 610.60(a)(5), 21 CFR 610.60(c), 21 CFR 201.55, 21 CFR 201.100(b)(2)	☐ Yes ☐ No ☒ N/A

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

Storage requirements (container label)	Acceptable
<i>Recommended labeling practices references: USP General Chapters <7> Labeling, USP General Chapters <659> Packaging and Storage Requirements</i>	☐ Yes ☐ No ☒ N/A

Dispensing container (container label)	Acceptable
Regulation: 21 CFR 201.100(b)(7)	☐ Yes ☐ No ☒ N/A

Package⁶ Labeling Evaluation

Proper name (package labeling)	Acceptable
Regulations: 21 CFR 610.61(a), 21 CFR 201.50(b), 21 CFR 201.10(g)(2)	☒ Yes ☐ No ☐ N/A
<i>Recommended labeling practices (placement of dosage form outside of parenthesis and/or below the proper name)</i>	☒ Yes ☐ No ☐ N/A

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

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

Comment/Recommendation: The Applicant revised "Amneal Pharmaceuticals LLC" in place of "Kashiv BioSciences, LLC". The name of the manufacturer and address is the applicant listed on FDA form 356h, "Manufactured by: Kashiv BioSciences, LLC Piscataway NJ 08854", thus revise back to the Manufacturer's name and address. The distributor information "Distributed by: Amneal Pharmaceuticals LLC" may be included but only if the manufacturer's information is also provided. *The Applicant revised as requested*

Lot number or other lot identification (package labeling)	Acceptable
Regulation: 21 CFR 610.61(c), 21 CFR 201.18	☒ Yes ☐ No ☐ N/A

Expiration date (package labeling)	Acceptable
Regulations: 21 CFR 610.61(d), 21 CFR 201.17	☒ Yes ☐ No ☐ N/A

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

Preservative (package labeling)	Acceptable
Regulation: 21 CFR 610.61(e)	☒ Yes ☐ No ☐ N/A

Number of containers (package labeling)	Acceptable
Regulation: 21 CFR 610.61(f)	☒ Yes ☐ No ☐ N/A

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

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

Handling: "Do Not Shake", "Do not Freeze" or equivalent (package labeling)	Acceptable
Regulation: 21 CFR 610.61(i)	☒ Yes ☐ No ☐ N/A

Multiple dose containers (recommended individual dose) (package labeling)	Acceptable
Regulation: 21 CFR 610.61(j)	☐ Yes ☐ No ☒ N/A

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

Known sensitizing substances (package labeling)	Acceptable
Regulations: 21 CFR 610.61(l), 21 CFR 801.437 (User labeling for devices that contain natural rubber)	☐ Yes ☐ No ☒ N/A

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

Comment/Recommendation: Revise to the compendial names in the inactive ingredient list as follows: prefilled syringe contains 6 mg pegfilgrastim-pbbk (based on protein weight) in a sterile, clear, colorless to slightly yellow, preservative-free solution (pH 4.0) containing acetic acid (0.35 mg), polysorbate 20 (0.02 mg), sodium hydroxide (0.02 mg), and sorbitol (30 mg) in Water for Injection, USP. *The Applicant revised as requested and consistent with the amounts provided in the proposed PI for acetic acid and sodium hydroxide.*

Source of the product (package labeling)	Acceptable
Regulation: 21 CFR 610.61(p)	☒ Yes ☐ No ☐ N/A

Minimum potency of product (package labeling)	Acceptable
Regulation: 21 CFR 610.61(r)	☒ Yes ☐ No ☐ N/A

Comment/Recommendation: In addition to comments provided earlier on the package (carton) label, we request that the statement "No U.S. standard of potency" be removed from the carton. Although this statement appears on the carton label of the reference product, Neulasta, our current thinking is that 21 CFR 610.61(r) is not applicable, and thus no statement is required for the Fylnetra carton label.⁷ *The Applicant revised as requested*

⁷ Weiner J. Memorandum. Applicability of 21 CFR 610.61(r). BLA 761084. Silver Spring (MD): FDA, CDER, ORP (US); 2022 May 19.

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

Divided manufacturing (package labeling)	Acceptable
Regulation: 21 CFR 610.63 (Divided manufacturing responsibility to be shown)	☐ Yes ☐ No ☒ N/A

Distributor (package labeling)	Acceptable
Regulation: 21 CFR 610.64, 21 CFR 201.1(h)(5)	☒ Yes ☐ No ☐ N/A

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

Strategic National Stockpile (exceptions or alternatives to labeling requirements for human drug products) (package labeling)	Acceptable
Regulations: 21 CFR 610.68, 21 CFR 201.26	☐ Yes ☐ No ☒ N/A

NDC numbers (package labeling)	Acceptable
Regulations: 21 CFR 201.2, 21 CFR 207.35	☒ Yes ☐ No

	☐ N/A

Preparation instructions (package labeling)	Acceptable
Regulation: 21 CFR 201.5(g) and 21 CFR 610.61(i)	☒ Yes ☐ No ☐ N/A
<i>Recommended labeling practices references: Draft Guidance Safety Considerations for Container Labels and Carton Labeling Design to Minimize Medication Errors, April 2013 (lines 426-430), which, when finalized, will represent FDA's current thinking on topic</i> <i>USP General Chapters <7> Labeling</i>	☐ Yes ☐ No ☒ N/A

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

Misleading statements (package labeling)	Acceptable
Regulation: 21 CFR 201.6	☐ Yes ☐ No ☒ N/A

Prominence of required label statements (package labeling)	Acceptable
Regulation: 21 CFR 201.15	☒ Yes ☐ No ☐ N/A

Spanish-language (Drugs) (package labeling)	Acceptable
Regulation: 21 CFR 201.16	☐ Yes ☐ No ☒ N/A

FD&C Yellow No. 5 and/or FD&C Yellow No. 6 (package labeling)	Acceptable
Regulation: 21 CFR 201.20	☐ Yes ☐ No ☒ N/A

Phenylalanine as a component of aspartame (package labeling)	Acceptable
Regulation: 21 CFR 201.21(c)	☐ Yes ☐ No ☒ N/A

Sulfites; required warning statements (package labeling)	Acceptable
Regulation: 21 CFR 201.22(b)	☐ Yes ☐ No ☒ N/A

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

Statement of Dosage (package labeling)	Acceptable
Regulations: 21 CFR 201.55, 21 CFR 201.100(b)(2)	☒ Yes ☐ No ☐ N/A

Dispensing container (package labeling)	Acceptable
Regulation: 21 CFR 201.100(b)(7)	☐ Yes ☐ No ☒ N/A

Medication Guide (package labeling)	Acceptable
Regulations: 21 CFR 610.60(a)(7), 21 CFR 208.24(d)	☐ Yes ☐ No ☒ N/A

Prescribing Information Evaluation

PRESCRIBING INFORMATION

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

Highlights of Prescribing Information	
DOSAGE AND ADMINISTRATION	Acceptable
<i>Recommended labeling practices reference: USP nomenclature for diluents and intravenous solutions</i>	☐ Yes ☐ No ☒ N/A

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

Full Prescribing Information	
2 DOSAGE AND ADMINISTRATION	Acceptable
Regulation: 21 CFR 201.57(c)(3)(iv)] <i>Confirm appropriateness of specific direction on dilution, preparation, and administration of the dosage form and storage conditions for stability of the reconstituted or diluted drug; ensure verbatim statement for parenterals: "Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit."</i>	☒ Yes ☐ No ☐ N/A

<i>Recommended labeling practices reference: USP nomenclature for diluents and intravenous solutions and storage instructions for reconstituted and diluted products; confirm the appropriateness of infusion bags, infusion sets (e.g., tubing, infusion aids, or filter membranes) incompatibilities with these components</i>	☐ Yes ☐ No ☒ N/A

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

Full Prescribing Information	
11 DESCRIPTION	Acceptable
Regulations: 21 CFR 201.57(c)(12), 21 CFR 610.61 (m), 21 CFR 610.61(o), 21 CFR 610.61 (p), 21 CFR 610.61 (q)	☒ Yes ☐ No ☐ N/A
<i>Recommended labeling practices references: USP General Chapters <1091>, USP General Chapters <7></i>	☒ Yes ☐ No ☐ N/A

Comment/Recommendation: We added the type and calculated amount of antibiotics added during manufacture (See 21 CFR 610.61(m). <i>The applicant revised as requested</i> We added the dosage form <i>The applicant revised as requested</i> Revise to the compendial name "acetic acid" <i>The Applicant revised as requested</i> Revise to the compendial name "sodium hydroxide" <i>The Applicant revised as requested</i>

Full Prescribing Information	
15 & 16 Hazardous Drug	Acceptable
Regulation: 21 CFR 201.57(c)(17)(iv) Section 15: References 1. OSHA Hazardous Drugs. OSHA. http://www.osha.gov/SLTC/hazardousdrugs/index.html Section 16: xxxx is a hazardous drug. Follow applicable special handling and disposal procedures. ¹	☐ Yes ☐ No ☒ N/A

Full Prescribing Information	
16 HOW SUPPLIED/ STORAGE AND HANDLING	Acceptable
Regulation: 21 CFR 201.57(c)(17)	☒ Yes ☐ No ☐ N/A
<i>Recommended labeling practices: to ensure placement of detailed storage conditions for reconstituted and diluted products</i>	☒ Yes ☐ No ☐ N/A

Comment/Recommendation: Added the RT storage range [68°F to 77°F (20°C to 25°C)] for syringes stored at RT for more than 72 hours *The Applicant revised as requested*

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

Patient Information Labeling Evaluation

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

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

PATIENT INFORMATION LABELING	
<u>INGREDIENTS</u>	Acceptable
<i>Recommended labeling practice: To ensure labeling of inactive ingredients are in alphabetical order (see USP General Chapters <1091>)</i>	☒ Yes ☐ No ☐ N/A

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

Instructions for Use Evaluation

INSTRUCTIONS FOR USE	
<u>TITLE (NAMES AND DOSAGE FORM)</u>	
<i>Recommended Labeling Practices references: Proprietary name in upper case letters on line 1, proper name (line 2) in lower case letters in parentheses, and dosage form followed by the route of administration (line 3) in lower case</i>	☒ Yes ☐ No ☐ N/A

<i>letters (see Draft Instructions for Use – Patient Labeling for Human Prescription Drug and Biological products and Drug-Device and Biologic-Device Combination Products – Content and Format Guidance for Industry (July 2019). For the recommended dosage form (see USP General Chapters: <1> Injections, Nomenclature and Definitions, Nomenclature form).</i>	

INSTRUCTIONS FOR USE	
STORAGE AND HANDLING	Acceptable
<i>Recommended labeling practices for IFU: Draft Instructions for Use – Patient Labeling for Human Prescription Drug and Biological products and Drug-Device and Biologic-Device Combination Products – Content and Format Guidance for Industry (July 2019). To ensure that applicable storage and handling requirements are consistent with the information provided in the PI (Reference: Section 2 (Dosage and Administration) and Section 16 (How Supplied Storage and Handling) of the PI)</i>	☒ Yes ☐ No ☐ N/A

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

INSTRUCTIONS FOR USE	
MANUFACTURER INFORMATION	Acceptable
21 CFR 201.1, 19 CFR 134.11	☒ Yes ☐ No ☐ N/A
<i>Draft Instructions for Use – Patient Labeling for Human Prescription Drug and Biological products and Drug-Device and Biologic-Device Combination Products – Content and Format Guidance for Industry (July 2019). 21 CFR 610.61 (add the US license number for consistency with the carton labeling), 21 CFR 610.64 (Name and address of distributor may appear and use a qualifying phrase for consistency with the carton labeling, when applicable)</i>	☒ Yes ☐ No ☐ N/A

APPENDIX C. Acceptable Labels and Labeling

Prescribing Information (submitted on May 23, 2022

<\\CDSESUB1\evsprod\bla761084\0049\m1\us\114-labeling\draft-labeling\draft-label-text\draft-label-text-prescr-info.pdf>)

Instructions for Use (submitted on May 6, 2022

<\\CDSESUB1\evsprod\bla761084\0046\m1\us\114-labeling\draft-labeling\draft-label-text\draft-ifu.pdf>)

Patient Information (submitted on May 6, 2022

<\\CDSESUB1\evsprod\bla761084\0046\m1\us\114-labeling\draft-labeling\draft-label-text\draft-patient-information.pdf>)

(b) (4)



1 Page(s) of Draft Labeling have been Withheld in Full as b4 (CCI/TS) immediately following this page



Vicky
Borders-Hemphill

Digitally signed by Vicky Borders-Hemphill
Date: 5/24/2022 03:21:58PM
GUID: 50814c700007a3d59329f660d8ddf02



Rachel
Novak

Digitally signed by Rachel Novak
Date: 5/24/2022 03:29:54PM
GUID: 52e163f70002ba9e745b4e39384c76d7

Recommendation: Approval

BLA/NDA Number: 761084
Assessment Number: Second round
Assessment Date: May 10, 2022

Drug Name/Dosage Form	Fylnetra (pegfilgrastim-pbbk) injection
Strength/Potency	6 mg/0.6 mL (10 mg/mL)
Route of Administration	Subcutaneous
Rx/OTC dispensed	Rx
Indication	All indications for US-licensed Neulasta
Applicant/Sponsor	Kashiv BioSciences, LLC
US agent, if applicable	N/A

The BLA was initially submitted on August 11, 2020. A Complete Response (CR) letter for product quality, microbiology, and device deficiencies was issued on August 11, 2021 (see Appendix 1). The CR letter also noted that inspection of the Kashiv BioSciences facility, intermediate product (IP) and drug substance (DS) manufacturing site, which is required for approval of the BLA, could not take place due to travel restrictions caused by the COVID-19 pandemic. A BPD Type 1 meeting (teleconference) took place on October 7, 2021 with Kashiv and members of the FDA review team to discuss Kashiv’s approach to address several of the deficiencies and additional comments listed in the CR letter (refer to meeting minutes uploaded into DARRTS, November 4, 2021). On November 29, 2021, Kashiv submitted responses to address the deficiencies and additional comments identified in the CR letter. A pre-license inspection of the Kashiv BioSciences facility was conducted between January 10, 2022 and January 14, 2022.

Product Overview:

Fylnetra (pegfilgrastim-pbbk) is a proposed biosimilar to US-licensed Neulasta (pegfilgrastim). Pegfilgrastim is a pegylated form of filgrastim (recombinant human methionyl granulocyte colony stimulating factor, G-CSF) with a 20 kDa polyethylene glycol (PEG) molecule covalently bound to the α -amino group of the N-terminal methionine residue. Pegfilgrastim retains the same biological activity as filgrastim upon binding to G-CSF receptor on the cell surface. Binding of the G-CSF portion of pegfilgrastim to the G-CSF receptor on precursor neutrophilic granulocytes initiates signal transduction that leads to the survival, proliferation, and differentiation of neutrophil progenitor cells into mature neutrophils. Pegylation increases the size of G-CSF, reducing renal clearance, and therefore, extending the median serum half-life.

Pegfilgrastim-pbbk is produced in genetically engineered *E. coli* cells (b) (4) [redacted] Pegfilgrastim-pbbk drug product (DP), Fylnetra, is manufactured to have the same strength, dosage form, and route of administration as US-licensed Neulasta. The formulation and presentation are the same between these products as well. Fylnetra is a sterile, preservative-free, clear, colorless to pale yellow liquid that consists of 6 mg of pegfilgrastim-pbbk in 0.6 mL (10 mg/mL) and is supplied in single-use pre-filled syringes for subcutaneous administration.

Quality Assessment Team:

Discipline	Assessor	Branch/Division
Drug Substance	Anshu Rastogi	OBP/DBRRI
Drug Product	Anshu Rastogi	OBP/DBRRI
Comparative Analytical Assessment	Anshu Rastogi	OBP/DBRRI
Immunogenicity Assays	Anshu Rastogi	OBP/DBRRI
OBP Team Lead	Brian Janelsins	OBP/DBRRI
Labeling	Vicky Borders-Hemphill	OBP
Facility	Michael Shanks (DS), Yarery Smith (DP)	OPMA/DBM/BMB2
Microbiology	Michael Shanks (DS), Yarery Smith (DP)	OPMA/DBM/BMB2
OPMA Team Lead	Candace Gomez-Broughton	OPMA/DBM/BMB2
Application Team Lead	Brian Janelsins	OBP/DBRRI
Tertiary Reviewer	Rachel Novak	OBP/DBRRI
RBPM	Andrew Shiber	OPRO

Multidisciplinary Assessment Team:

Discipline	Assessor	Office/Division
RPM	Courtney Hamilton	OND/ORO/DROCHEN
Cross-disciplinary Team Lead	Tanya Wroblewski	OND/OCHEN/DNH
Medical Officer	Hyon-Zu Lee, Tanya Wroblewski (TL)	OND/OCHEN/DNH
Pharmacology/Toxicology	Fred Alavi, Natalie Simpson (TL)	OND/OCHEN/DPTCHEN
Clinical Pharmacology	Xiongmeng Xu, Sudarshan Hariharan (TL)	OTS/OCP/DCEP
Statistics	Lola Luo, Yeh-Fong Chen (TL)	OTS/OB/DBIX
OTBB	Thomas Herndon, Sarah Schrieber, Cristina Ausin, Stacey Ricci	OND/OTBB

1. Names:

- a. Proprietary Name: Fylnetra
- c. Non-Proprietary Name/USAN: pegfilgrastim-pbbk
- d. CAS Name: 208265-92-3
- e. Common Name: TPI-120
- f. INN Name: Pegfilgrastim
- h. OBP systematic name: CONJ: RPROT P09919 (CSF3_HUMAN); PEG [TPI120]

Submissions Assessed:

Submission(s) Assessed	Document Date
761084/Seq. 0036 (Resubmission)	November 29, 2021
761084/Seq. 0037 (Product Quality Amendment)	January 14, 2022
761084/Seq. 0039 (Product Quality Amendment)	February 14, 2022
761084/Seq. 0042 (Audit Report)	March 15, 2022
761084/Seq. 0043 (Response to OBP information request)	April 13, 2022

Quality Assessment Data Sheet:

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

DMF #	DMF Type	DMF Holder	Item referenced	Code ¹	Status ²	Date Assessment Completed	Comments
(b) (4)	3	(b) (4)	(b) (4)	3	Adequate	N/A	
	5			3	Adequate	N/A	
	3			3	Adequate	N/A	
	3			3	Adequate	N/A	
	2			3	Adequate	N/A	

1. Action codes for DMF Table: 1- DMF Assessed; Other codes indicate why the DMF was not assessed, as follows: 2- Assessed previously and no revision since last assessment; 3- Sufficient information in application; 4- Authority to reference not granted; 5- DMF not available; 6- Other (explain under “comments”).

2. Action codes for Status column: Adequate, Adequate with Information Request, Deficient, or N/A (There is not enough data in the application; therefore, the DMF did not need to be assessed).

- B. Other documents: IND, Referenced Listed Drug (RLD), or sister application:
Not applicable.

3. Consults:

Discipline/Topic	Date Requested	Status	Recommendation	Assessor
CDRH (pre-filled syringe assembly and needle guard component)	November 29, 2021	Completed	Approve with no PMCs (review uploaded in ICCR platform on April 20, 2022)	Porsche Bennett, Alan Stevens (TL)

4. Environmental Assessment of Claim of Categorical Exclusion:

Kashiv requested categorical exclusion from the environmental assessment requirement under 21 CFR 25.31(a), (c), and (d). These claims of categorical exclusions are acceptable for BLA 761084.

Executive Summary:

I. Recommendations:

A. Recommendation and Conclusion on Approvability:

The Office of Biotechnology Products (OBP), OPQ, CDER recommends approval of STN 761084 for Fylnetra, manufactured by Kashiv BioSciences. Sufficient analytical data are provided in the application to support that Fylnetra is highly similar to US-licensed Neulasta, notwithstanding minor differences in clinically inactive components, and that the strength, dosage form, and route of administration of Fylnetra are the same as that of US-licensed Neulasta. In addition, the data submitted in this application are adequate to support the conclusion that the manufacture of Fylnetra is well-controlled and leads to a product that is pure and potent. It is recommended that this product be approved for human use under conditions specified in the package insert.

B. Approval Action Letter Language:

- Manufacturing location:
 - Drug Substance:
 - Kashiv BioSciences, LLC in Chicago, IL
 - Drug Product:
 - (b) (4)

- Fill size and dosage form:
 - 6 mg/0.6 mL single-dose pre-filled syringe, injection

- Dating period:
 - Drug Product: 24 months from the date of manufacture when stored at $5 \pm 3^{\circ}\text{C}$. The date of manufacture shall be defined as the date of final sterile filtration of the formulated drug product.
 - Drug Substance: (b) (4) months from the date of manufacture when stored at (b) (4) $^{\circ}\text{C}$.
 - (b) (4)

- Exempt for lot release:
 - Yes, Fylnetra is a specified product exempted according to 21 CFR 601.2a.

C. Benefit/Risk Considerations:

Fylnetra is a proposed biosimilar to US-licensed Neulasta and was developed to have the same dosage form, route of administration, and strength as for US-licensed Neulasta. The formulation and presentation are also identical between the products. Kashiv BioSciences requested Fylnetra be approved for all indications approved for US-licensed Neulasta. The comparative analytical assessment provided in the original BLA submission, and subsequent amendments during the first

review cycle, supports the conclusion that Fylnetra is highly similar to US-licensed Neulasta. No new comparative analytical assessment data were submitted in the BLA resubmission.

Deficiencies and additional comments related to manufacturing and controls described in the CR letter have been adequately addressed in the BLA resubmission. Overall, the OPQ review of manufacturing and controls in the BLA resubmission and initial BLA submission have confirmed that the process and methods used for the IP, DS, and DP manufacturing and testing are sufficient to assure a consistent and safe product. Individual assessments for each discipline are located in separate documents in Panorama.

A pre-license inspection of the Kashiv BioSciences facility, IP and DS manufacturing site, performed during the resubmission review cycle was recommended for approval. A pre-license inspection was not conducted during the first review cycle due to travel restrictions caused by the COVID-19 pandemic.

As discussed below in Section 1.D., three PMCs will be issued related to manufacturing and controls. The PMCs can be addressed post-market rather than pre-market because the risk is minimal (e.g., verification of data under similar conditions, manufacturing process analysis, and long-term data needed).

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

1. Perform a real-time drug product (DP) shipping study from the DP manufacturing site to Kashiv BioSciences and to the distribution center to confirm validation of the commercial TPI-120 DP shipping conditions, such as described in protocol PTL-2374 (Section 3.2.P.3.5). The final study report should include assessments for the impact of real-time shipping on (i) product quality (comparison of product pre- and post-shipment), (ii) temperature for the duration of transport, and (iii) physical damage to the DP containers.
2. Perform a study to evaluate the impact of the removal of kanamycin from the (b) (4) manufacturing process. If the data support removal of kanamycin, a plan for the removal of kanamycin from the manufacturing process is expected to be provided. The plan should include an evaluation of consistency of the fermentation process and comparability of the (b) (4) manufactured with and without kanamycin. The results should be reported per 21 CFR 601.12.
3. Reassess the (b) (4) acceptance criteria for testing of drug substance (DS) and drug product (DP) at release and on stability when 10 commercial DS and DP batches have been manufactured and tested. The final study report should include sufficient information and data to support the acceptance criteria, and all relevant protocols (e.g., qualification and requalification protocols of reference standards) as well as DS and DP specifications should be updated as needed.

II. Comparative Analytical Assessment

I. Analytical Assessment Overview and Conclusions

Kashiv BioSciences provided results to support a demonstration that TPI-120 is highly similar to US-licensed Neulasta, notwithstanding minor differences in clinically inactive components. The comparative analytical assessment was consistent with the FDA Guidance for Industry, Development of Therapeutic Protein Biosimilar: Comparative Analytical Assessment and Other Quality-Related Considerations, May 2019. As part of the assessment, an extensive analytical characterization of TPI-120 and US-licensed Neulasta was performed, which included comparisons of primary structure, pegylation characteristics, higher order structure, purity and product-related variants, size-variant characterization, bioactivity (potency and receptor binding), and protein concentration. Attributes were selected based on the established quality target product profile for US-licensed Neulasta and the defined critical quality attributes (CQAs) for TPI-120. Orthogonal methods were implemented and were designed to be capable of detecting analytical differences between the products if present. The methods used in the assessment appear suitable for their intended purposes and will be further assessed on inspection during the next review recycle.

The attributes were ranked by criticality and assessed accordingly by quantitative and qualitative approaches. Attributes of very high risk (i.e., protein concentration and potency by cell-based bioassay) were evaluated using a combination of quality ranges and equivalence testing, while attributes of high risk were evaluated using quality ranges. Attributes of low risk or those that are not amendable to quantitative analysis were assessed using qualitative and/or graphic evaluations. In some cases, the Agency determined its own quality ranges for low risk attributes that are amendable to quantitative analysis when sufficient data were provided.

A total of 13 lots of US-licensed Neulasta and 12 lots of TPI-120 drug product were originally used in the assessment. All lots for each product were used in the assessment of very high and high risk quality attributes, while only a subset of lots were used in the analysis of low risk quality attributes. The assessment included analysis of independent TPI-120 drug product lots generated from clinical and commercial (i.e., PPQ lots) processes. The selected US-licensed Neulasta lots span the shelf-life of the product (24 months) and TPI-120 lots ranged from <1 – 17 months. Kashiv BioSciences updated the assessment for certain evaluations with additional lots of TPI-120 (1) and US-licensed Neulasta (5) to support the original dataset. For evaluations that consisted of reporting results relative to a reference standard, two reference standards (a US-licensed Neulasta lot and an in-house primary reference standard lot) were used, and data were provided to adequately qualify and bridge the reference standards.

In addition, the comparative analytical assessment included comparative stability studies, which evaluated the stability profile (i.e., degradation pathways and rates) of TPI-120 and US-licensed Neulasta under accelerated, stressed, and forced degradation conditions by orthogonal stability-indicating methods. The quality attributes assessed, and storage conditions selected were supported by development studies and risk assessments.

In conclusion, Kashiv BioSciences performed an adequate comparative analytical assessment that evaluated a comprehensive array of quality attributes relevant to both products by appropriate methods, used an appropriate data analysis plan, and selected appropriate lots for the assessment. The results from

this assessment support a demonstration that TPI-120 is highly similar to US-licensed Neulasta, notwithstanding minor differences in clinically inactive components.

II. Results of the Comparative Analytical Assessment

The results from the comparative analytical assessment that support a demonstration that TPI-120 is highly similar to US-licensed Neulasta are summarized in Table 1 below. While not illustrated in the table below, the stability profile of TPI-120 was demonstrated to be similar to that of US-licensed Neulasta.

Table 1. Quality Attributes Analyzed in the Comparative Analytical Assessment

Physico-chemical/Functional Characteristics	Quality Attribute Assessed	Test Method		Supports a Demonstration of Highly Similar
<i>Primary Structure</i>	<i>Amino acid sequence</i>	<i>Peptide mapping LC-UV</i>		<i>Yes</i>
	<i>Intact Mass</i>	<i>LC-MS, electrospray ionization</i>		<i>Yes</i>
	<i>Disulfide mapping</i>	<i>LC-MS/MS</i>		<i>Yes</i>
	<i>Free Thiol</i>	<i>Thiol fluorometric detector</i>		<i>Yes</i>
<i>Pegylation Characteristics</i>	<i>Pegylation site</i>	<i>MALDI-TOF, LC-MS/MS</i>		<i>Yes</i>
	<i>Polydispersity</i>	<i>MALDI-TOF MS</i>		<i>Yes</i>
	<i>Free PEG</i>	<i>CAD</i>		<i>Yes</i>
		<i>SDS-PAGE (Titrisol staining)</i>		<i>Yes</i>
<i>Higher Order Structure</i>	<i>Secondary structure</i>	<i>Far-UV CD</i>		<i>Yes</i>
		<i>FTIR spectroscopy</i>	<i>α-helix content</i>	<i>Yes</i>
			<i>β-sheet content</i>	<i>Yes</i>
	<i>Tertiary structure</i>	<i>Intrinsic fluorescence</i>		<i>Yes</i>
		<i>Near-UV CD</i>		<i>Yes</i>
		<i>DSC</i>		<i>Yes</i>
		<i>NMR</i>	<i>Yes</i>	
<i>Purity and product-related variants</i>	<i>Size variants</i>	<i>SE-HPLC</i>	<i>Monomer</i>	<i>Yes</i>
			<i>Total size variants</i>	<i>Yes</i>
			<i>Di-pegylated variants</i>	<i>Yes</i>
			<i>Aggregates</i>	<i>Yes</i>
			<i>Free rhG-CSF</i>	<i>Yes</i>
	<i>Charge variants</i>	<i>CEX-HPLC</i>	<i>Main species</i>	<i>Yes</i>
			<i>Acidic variants</i>	<i>Yes</i>
			<i>Basic variants</i>	<i>Yes</i>
	<i>Hydrophobic variants</i>	<i>RP-HPLC</i>	<i>Main species</i>	<i>Yes</i>
			<i>Total variants</i>	<i>Yes</i>
			<i>Post-main variants</i>	<i>Yes</i>
			<i>Pre-main variants</i>	<i>Yes</i>
			<i>Oxidized variants</i>	<i>Yes</i>
			<i>Deamidated variants</i>	<i>Yes</i>

Physico-chemical/Functional Characteristics	Quality Attribute Assessed	Test Method	Supports a Demonstration of Highly Similar	
	<i>Truncated variants</i>	<i>CE-SDS (non-reduced)</i>	<i>Yes</i>	
	<i>Aggregate characterization</i>	<i>AUC</i>	<i>Yes</i>	
		<i>DLS</i>	<i>Yes</i>	
<i>Bioactivity</i>	<i>Cell proliferation</i>	<i>In vitro cell-based bioassay</i>	<i>Yes</i>	
	<i>Receptor binding</i>	<i>ELISA (relative potency)</i>	<i>Yes</i>	
		<i>SPR (binding affinity and kinetics)</i>	<i>KD (M)</i>	<i>Yes</i>
			<i>Ka (1/Ms)</i>	<i>Yes</i>
<i>Kd (1/s)</i>	<i>Yes</i>			
<i>Drug Product Attributes</i>	<i>Protein concentration</i>	<i>UV Absorbance Spectroscopy</i>	<i>Yes</i>	

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

Not applicable.

IV. Assessment of Analytical Study Results

Several quality attributes were noted as not meeting the pre-determined similarity acceptance criteria or showed variable results in qualitative assessments; however, adequate justification has been provided that the differences observed do not preclude a demonstration that TPI-120 is highly similar to US-licensed Neulasta.

Size variants by SE-HPLC

TPI-120 levels of monomer were slightly higher than the quality range of US-licensed Neulasta, while TPI-120 levels of di-pegylated variants, free rhG-CSF, and total size variants were slightly lower than the quality ranges of US-licensed Neulasta. With respect to aggregates, most TPI-120 lots were within the US-licensed Neulasta quality range (10 of 13), while 3 lots showed levels slightly higher than the quality range (no more than 0.12% over the upper limit). Considering that the levels are very low for di-pegylated variants (no more than 1.38% in US-licensed Neulasta and 0.74% in TPI-120), aggregates (no more than 0.19% in US-licensed Neulasta and 0.36% in TPI-120), free rhG-CSF (no more than 0.14% in US-licensed Neulasta and 0.06% in TPI-120), and total size variants (no more than 1.80% in US-licensed Neulasta and 1.03% in TPI-120), and the differences in mean values between both products are minor for di-pegylated variants (0.7%), aggregates (0.07%), free rhG-CSF (0.06%), total size variants (0.79%), and monomer (0.79%), these differences are not expected to impact potency or have a clinical impact. For example, the comparative analytical assessment results show similar bioactivity between TPI-120 and US-licensed Neulasta. No new size variant species were identified in TPI-120, and orthogonal assessments (AUC and DLS) demonstrate that TPI-120 contains the same high molecular weight types with similar characteristics and levels as those detected in US-licensed Neulasta. In conclusion, there is no residual uncertainty regarding the evaluation for purity and size-variants as performed by SE-HPLC. The small differences in size variants do not preclude a demonstration of highly similar.

Charge variants by CEX-HPLC

TPI-120 levels for main species were slightly higher than the quality range (97.49% - 99.24%) for 5 out of the 13 evaluated lots, but these lots did not exceed the upper limit beyond 0.08%. TPI-120 levels of

acidic variants, which consist of mainly di-pegylated, acetylated, and deamidated variants, were slightly lower than the US-licensed Neulasta quality range for all 13 TPI-120 lots. The levels of acidic variants are low in TPI-120 (not higher than 0.2%) and US-licensed Neulasta (0.41% to 0.73%), and the difference is minor. These differences are not expected to impact potency or have a clinical impact. For example, the comparative analytical assessment results show similar bioactivity between TPI-120 and US-licensed Neulasta. In conclusion, there is no residual uncertainty regarding the evaluation for purity and charge variants as performed by CEX-HPLC. The small differences in charge variants do not preclude a demonstration that TPI-120 is highly similar to US-licensed Neulasta.

Hydrophobic variants by RP-HPLC

TPI-120 levels of main peak were within the quality range (94.77% - 97.58%) with the exception of 2 lots that were slightly higher than the quality range by no more than 0.14%. TPI-120 levels for pre-main variants, which consist of oxidized and sequence variants, resulted in 4 lots within the quality range (1.32% - 1.97%), 6 lots below the quality range, and 1 lot above the quality range. The 1 lot above the quality range for pre-main variants was only 0.32% higher than the maximum value reported for US-licensed Neulasta and only 0.19% higher than the quality range upper limit. The mean levels of pre-main variants are low (1.35% in TPI-120 and 1.65% in US-licensed Neulasta) and the difference between the product is minimal (0.30%). In addition, TPI-120 levels for the main RP-HPLC variant (oxidized Met-127), which is included in the calculation of pre-main variants, were below the quality range for all 11 evaluated lots. All other oxidized variants in TPI-120 and US-licensed Neulasta were below or near LOQ and showed no apparent difference between the products. The mean levels of oxidized Met-127 variant are low (0.97% in TPI-120 and 1.61% in US-licensed Neulasta) and the difference between the product is minimal (0.64%). These differences are not expected to impact potency or have a clinical impact. For example, the comparative analytical assessment results show similar bioactivity between TPI-120 and US-licensed Neulasta.

Characterization of hydrophobic variants (pre- and post-main peaks) revealed that sequence variants (non-terminal, single amino acid misincorporations) are present in TPI-120 and US-licensed Neulasta at very low levels (Table 2, see below). Only one sequence variant (F114 → I/L114) was detected in TPI-120 that was not detected in US-licensed Neulasta by RP-HPLC. While the level of that variant is unclear compared to the levels of the other variants in the peak, the overall peak amount is very low. Furthermore, while there appears to be a general trend of higher percentages of sequence variants in TPI-120, these differences and the overall difference (i.e., 0.38%) reported between the two products is minimal. Given the size (1 amino acid change), location of the misincorporation (not identified as critical for binding), and amount present (no more than 0.54%), the differences in sequence variants do not preclude a demonstration that TPI-120 is highly similar to US-licensed Neulasta.

Table 2. Sequence Variants by RP-HPLC

Peak Number	Sequence Variant	TPI-120 (min-max)	US-licensed Neulasta (min-max)
RP-HPLC P2a	W119 → R119	ND – <LOQ	ND – 0.11%
RP-HPLC P2b	T103 → S103	<LOQ – 0.18%	ND – <LOQ
RP-HPLC P3c	E123 → D123	ND – 0.12%	ND – <LOQ
RP-HPLC P8	D113 → E113 D110 → E110	ND – 0.54%	0.25 – 0.44%

	F114 → I/L114 (only in TPI-120)		
RP-HPLC P10ab	D105 → E105	ND – 0.38%	ND – 0.29%

*LOD = 0.04%, LOQ = 0.10%

In conclusion, there is no residual uncertainty regarding the evaluation of purity and hydrophobic variants (e.g., oxidized, deamidated, acetylated, truncated, and sequence) as performed by RP-HPLC. The small differences in hydrophobic and sequence variants do not preclude a demonstration that TPI-120 is highly similar to US-licensed Neulasta. The resubmission included changes to the control strategy to adequately control for sequence variants (CR Comment #5) to ensure that TPI-120 will remain highly similar to US-licensed Neulasta.

Receptor binding by SPR

The data generated from the receptor binding by SPR assessment included values for KD (equilibrium dissociation constant), which is an indication of binding affinity, and Kd (dissociation constant) and Ka (association constant), which are indications of binding kinetics, from 3 TPI-120 lots and 3 US-licensed Neulasta lots. While no quality ranges were used by Kashiv BioSciences in their evaluation, likely because of inherent method variability and the limited dataset, the Agency generated quality ranges (mean ± 3SD) to assess similarity with respect to receptor binding affinity and kinetics. All 3 TPI-120 lots reported values for KD and Kd within the quality ranges; however, 1 TPI-120 lot reported a value for Ka that was slightly higher than the quality range upper limit. Additional data was provided by Kashiv BioSciences to show higher variability of Ka values for the US-licensed Neulasta lots (CV of 7.7%) compared to TPI-120 lots (CV of 3.5%). Qualification data for the method was also provided to show that there is higher variability for Ka values with respect to Kd values (8.9% vs. 3.8%, respectively). Changes in receptor binding affinity and kinetics could be an indication of structural changes that may impact receptor binding; however, data generated from the higher order structure assessments demonstrated that TPI-120 is similar to US-licensed Neulasta. Receptor binding by ELISA and the cell-based bioassay assessments, which report relative potency, also showed similar results for TPI-120 and US-licensed Neulasta. The difference in Ka values from the receptor binding by SPR assessment is likely the result of method variability. Therefore, the observed difference in Ka values does not preclude a demonstration that TPI-120 is highly similar to US-licensed Neulasta.

A. Same Strength(s)

TPI-120 has the same dosage form and route of administration as US-licensed Neulasta. Kashiv BioSciences is seeking licensure of 6 mg/0.6 mL TPI-120 in a pre-filled syringe. US-licensed Neulasta is licensed in this strength (6 mg/0.6 mL) in a pre-filled syringe.¹ Kashiv BioSciences is seeking approval of TPI-120 for the same strength as US-licensed Neulasta. Comparative protein concentration (10 mg/mL) was assessed as part of the comparative analytical assessment. The deliverable volume (0.6 mL) and fill weight data were assessed [REDACTED] (b) (4). The proposed presentation of TPI-120 has the same total content of drug substance in units of mass in a container and the same concentration of drug substance in units of mass per unit volume as US-licensed Neulasta (6 mg/0.6 mL). The strength of TPI-120 pre-filled syringe is the same as that of US-licensed Neulasta. However, insufficient information was provided to ensure the consistency of the drug product filling process in terms of protein concentration. While deliverable volume (0.6 mL) and fill weight data were assessed [REDACTED] (b) (4) [REDACTED] Kashiv BioSciences did not provide sufficient information during the first review cycle to demonstrate that all syringes that are filled will be of consistent protein concentration

¹ U.S. Prescribing Information, U.S.-Licensed Neulasta. Accessed 7/7/21 from <https://www.accessdata.fda.gov/scripts/cder/daf/>

based on the sampling strategy employed during process validation studies. However, this was adequately addressed in the resubmission (CR Comment #4) to provide sufficient assurance that all syringes filled will be of consistent protein concentration.

III. Summary of Quality Assessments:

A. CQA Identification, Risk and Lifecycle Knowledge Management

Table 3. Active Pharmaceutical Ingredient CQA Identification, Risk and Lifecycle Knowledge Management

CQA (type)	Risk	Origin	Control Strategy	Other
Potency (in vitro cell-based bioassay)	Efficacy	Intrinsic to molecule	(b) (4)	Resubmission included sufficient data to support changes to the validated potency assays (CR Comment #8).
Receptor binding by ELISA	Efficacy	Intrinsic to molecule		N/A
Primary Sequence (Identity)	Efficacy	Intrinsic to molecule		N/A
Higher order structure	Efficacy	Intrinsic to molecule		N/A
Free PEG	Safety	Manufacturing Process/Storage conditions		N/A
Low molecular weight variants (Free rhG-CSF and other truncated species)	Efficacy and safety	Manufacturing process and storage conditions		N/A
High molecular weight variants (e.g., dimer, aggregates, and dipegylated species (only for DS and DP))	Efficacy, PK, and safety	Manufacturing process and storage conditions		N/A
Charge variants (e.g., acidic and basic variants)	Efficacy, PK, and safety	Manufacturing process and storage conditions		Resubmission included sufficient information and data to support the

				suitability of the method for its intended use to test DS and DP (CR Comment #9).
Hydrophobic variants (e.g., oxidized, deamidated, acetylated, and sequence variants)	Efficacy and safety	Manufacturing process and storage conditions	(b) (4)	N/A

B. Drug Substance pegfilgrastim-pbbk Quality Summary

The following table presents CQAs derived from the DS manufacturing process and general DS attributes, as well as control strategies in place.

Table 4. Drug Substance CQA Process Risk Identification and Lifecycle Knowledge Management.

Category	Risk	Origin	Control Strategy	Other
Appearance	Safety	Manufacturing Process	(b) (4)	N/A
Host cell protein	Safety and immunogenicity	Derived from host cell line		N/A
Host cell DNA	Safety	Derived from host cell line		N/A
Protein concentration	General CQA	Formulation		N/A
(b) (4)	Safety and stability	Manufacturing process		N/A
Sequence Variants	Safety, immunogenicity	Manufacturing Process		Resubmission included sufficient information to adequately control sequence variants (CR Comment #5)
Endotoxin	Safety and purity	Manufacturing process		N/A
Bioburden	Safety, purity, and efficacy	Raw materials and		N/A

		manufacturing process	(b) (4)	

- Description:**
TPI-120 is a polyethylene glycol (PEG) modified non-glycosylated form of recombinant human granulocyte colony-stimulating factor (rhG-CSF, filgrastim) with an additional N-terminal methionine added to enforce expression in *E. coli*. The (b) (4) rhG-CSF, which is composed of 175 amino acids and is identical to human G-CSF with the exception of the additional methionine residue at the N-terminus. TPI-120 DS is derived from pegylation of the rhG-CSF (b) (4) by the covalent attachment of linear PEG molecule (~20 kDa) to the N-terminal methionine of rhG-CSF by a secondary amine linkage. The molecular weight of TPI-120 is ~39 kDa.
- Mechanism of Action (MoA):**
The biological activity of G-CSF is initiated by binding of G-CSF to the G-CSF receptor on myeloid progenitor cells and mature neutrophils. This binding initiates transduction signals that lead to the proliferation and differentiation of neutrophil committed progenitor cells, increase of mature neutrophils in the blood, which is a relevant pharmacodynamic (PD) marker for neutropenia, and enhanced neutrophil function.
- Potency Assay:**
An in vitro cell-based assay was adopted from the USP monograph for filgrastim and is executed according to STM-0118 and STM-0127 when used to test IP and DS samples, respectively. The bioassay includes the use of a M-NFS-60 cell line, which is derived from a murine myelogenous leukemia cell and proliferates in response to G-CSF. The cells are incubated with varying concentrations of reference solution and test solution. After a timed incubation, CellTiter-Blue Cell Viability reagent is added to the plates. Viable cells convert a redox dye into a fluorescent end-product which can be measured using a fluorescence-plate reader. The fluorescent values are directly proportional to the number of viable cells in the culture. Biological activity of test sample is determined by comparing its response to that of the reference. The potency of IP lots is expressed as units/milligram, while the potency of DS (and DP) lots is expressed as relative potency percentage in comparison to that of the reference standard. After validation of the bioassay, changes were made to the method. The resubmission included sufficient information to support these changes without the need for additional method validation (CR Comment #8).
- Reference Materials:**
An in-house two-tier system is used for the reference standard campaigns for the IP and the DS/DP. Currently, a Primary Reference Standard (PRS) and Working Reference Standard (WRS) have been qualified for each campaign, and each reference standard was adequately qualified against the previous reference standard. The resubmission included additional information and data to support the qualification of the WRS for the IP campaign (CR Comment #7). In addition, the resubmission included adequate protocols to support requalification of the PRS and WRS for each campaign (CR Comment #6) and qualification of new PRS and WRS for each campaign (Additional Comments #22 and 23).

- Critical starting materials or intermediates:



(b) (4)

- Manufacturing process summary:



(b) (4)

[Redacted] (b) (4)

- **Container closure:**
The IP and DS are filled and stored in a container closure system [Redacted] (b) (4)
- **Dating period and storage conditions:**
[Redacted] (b) (4) the expiry period for the DS is [Redacted] (b) (4). The post-approval stability protocols for testing [Redacted] (b) (4) are adequate.
- **Microbial control:**
Microbial quality is controlled at each step of the manufacturing process [Redacted] (b) (4). Adequate controls are in place to maintain microbiological product quality during maximum hold periods and throughout the manufacturing process.

C. Drug Product Fylnetra Quality Summary:

The following table presents CQAs derived from the DP manufacturing process and general DP attributes, as well as control strategies in place.

Table 5. Drug Product CQA Identification, Risk, and Lifecycle Management

Category	Risk	Origin	Control Strategy	Other
Appearance (color and clarity)	General CQA	Formulation	[Redacted] (b) (4)	N/A
Protein concentration	General CQA	Formulation	[Redacted] (b) (4)	N/A
Osmolality	Product stability, patient discomfort	Formulation	[Redacted] (b) (4)	N/A
pH	Stability	Formulation	[Redacted] (b) (4)	N/A
Polysorbate 20	Safety and stability	Manufacturing process, formulation	[Redacted] (b) (4)	N/A
Visible particles	Immunogenicity and patient safety	Stability, accidental throughout process	[Redacted] (b) (4)	N/A

Sub-visible particles	Safety	Manufacturing process, product degradation	(b) (4)	N/A
Container closure	Negative impact of leachables on product quality. Introduction of container related impurities (e.g., (b) (4))	Filling/storage		N/A
Volume of injection	Efficacy	Manufacturing process		N/A
Syringe break loose and glide force determination	Safety	Manufacturing process		N/A (refer to CDRH review)
Sterility	Safety and product stability	Manufacturing process, container closure integrity failure		N/A
Endotoxin	Safety	Manufacturing process, contamination		Resubmission included adequate information to address Additional Comments #36 – #39.
Container Closure Integrity	Safety (maintenance of sterility during shelf-life)	Container closure breaches during storage. May be impacted by storage conditions.		Resubmission included adequate information to address Additional Comment #40.

- Potency and Strength:**
TPI-120 is supplied in a pre-filled syringe for subcutaneous administration at a strength of 6 mg/0.6 mL. TPI-120 was developed to have the same strength as US-licensed Neulasta.
- Summary of Product Design:**
TPI-120 was developed as a biosimilar to US-licensed Neulasta and targets the quality profile of US-licensed Neulasta.
- List of Excipients:**
TPI-120 has the same formulation as used in US-licensed Neulasta and consists as a sterile, clear, colorless to slightly yellow liquid in a preservative-free liquid formulation (pH 4.0). Components in addition to the active ingredient (pegfilgrastim-pbbk) include acetate (0.35 mg), polysorbate 20 (0.02 mg), sodium (0.02 mg), and sorbitol (30 mg).

- Reference Materials:
The same reference standards are used for DS and DP.

- Manufacturing process summary:



- Container closure:
The container closure system is a single-dose prefilled syringe consisting of a 1 mL Long (b) (4) glass syringe barrel with a staked 27-gauge inch needle and a rigid needle shield and an elastomeric plunger stopped (b) (4)

- Dating period and storage conditions:
The expiry period of the DP is $5 \pm 3^{\circ}\text{C}$ for 24 months.

- List of co-package components, if applicable:
Not applicable.

D. Biopharmaceutics Considerations:
Not applicable

E. Novel Approaches/Precedents:
None

F. Any Special Product Quality Labeling Recommendations:
None

G. Establishment Information:

Overall Recommendation:					
DRUG SUBSTANCE					
Function	Site Information	DUNS/FEI Number	Preliminary Assessment	Inspectional Observations	Final Recommendation
Manufacture and storage of cell banks; DS manufacture and storage; in-process, release and stability testing of DS; raw material testing; and storage of DS and DP	Kashiv BioSciences, LLC (San Diego, CA)	3011289655 (FEI)	Facility Adequate	Yes (5-item Form FDA-483 issued)	Approve
Microorganisms identification	(b) (4)		Facility Adequate	N/A	Approve
Cell bank characterization, end of production cell bank testing			Facility Adequate	N/A	Approve
Cell bank storage			Facility Adequate	N/A	Approve
(b) (4) testing for release and raw material testing			Facility Adequate	N/A	Approve
Host cell DNA and kanamycin testing			Facility Adequate	N/A	Approve
Microbiological in-process and release testing (b) (4)			Facility Adequate	N/A	Approve
Raw material testing			Facility Adequate	N/A	Approve
Raw material testing			Facility Adequate	N/A	Approve
Raw material Storage			Facility Adequate	N/A	Approve

		(b) (4)			
Extractables and Leachables Testing for DS		(b) (4)	Facility Adequate	N/A	Approve
DRUG PRODUCT					
Function	Site Information	DUNS/FEI Number	Preliminary Assessment	Inspectional Observations	Final Recommendation
DP release and stability testing (except polysorbate 20), pre-filled syringe assembly, packaging and cartooning; storage and shipping of packaged DP	Kashiv BioSciences LLC (Chicago, IL)	3011289655 (FEI)	Facility Adequate	Yes (5-item Form FDA-483 issued)	Approve
DP manufacture; raw material testing, storage, and release; DP in-process testing; DP protein concentration release testing; and shipping of DP to Kashiv		(b) (4)	Inadequate	Yes (3-item Form FDA-483 issued)	Approve
Polysorbate 20 release and stability testing of DP			Facility Adequate	N/A	Approve
Particulate matter testing for DP release and stability			Facility Adequate	N/A	Approve
Sterility testing for DP release			Facility Adequate	N/A	Approve
Sterility testing for DP stability			Facility Adequate	N/A	Approve
Microorganisms identification			Facility Adequate	N/A	Approve

Raw material testing	(b) (4)	Facility Adequate	N/A	Approve
Extractables and leachables testing for DP		Facility Adequate	N/A	Approve

H. Facilities:

Kashiv BioSciences LLC (FEI# 3011289655) in Chicago, Illinois is responsible for the manufacture of the TPI-120 IP and DS. Due to U.S. government and/or Agency-wide restrictions on travel, an inspection of the Kashiv BioSciences was not performed during the first review cycle due to the COVID-19 pandemic. However, inspection of this facility was performed during the second review cycle (January 10, 2022 to January 14, 2022) by CDER/OPQ under eNSpect Operation ID 192719. The inspection concluded with the issuance of a five-item FDA Form 483 and a field recommendation of approve for BLA 761084. The compliance review of the firm’s response in CMS WA 437154 concurred with the field recommendation and recommended approval for BLA 761084. All other facilities supporting BLA 761084 have acceptable compliance statuses and are approved in support of BLA 761084.

I. Lifecycle Knowledge Management:

a. Drug Substance:

i. Protocols approved:

1. Qualification of new cell banks (Section 3.2.S.2.3)
2. At-scale chromatography resins/membrane lifetime study (Section 3.2.S.2.5)
3. Requalification of primary and working reference standards (Section 3.2.S.5)
4. Qualification of new primary and working reference standards (Section 3.2.S.5)
5. Ongoing stability studies and post-approval annual stability protocol (Section 3.2.P.8.2)

ii. Outstanding assessment issues/residual risk:

1. None

iii. Future inspection points to consider:

1. Third party audit report (Section 1.2)

b. Drug Product

i. Protocols approved:

1. Ongoing stability studies and post-approval annual stability protocol (Section 3.2.P.8.2)

ii. Outstanding assessment issues/residual risk:

1. None

- iii. Future inspection points to consider:
 - 1. Third party audit report (Section 1.2)

APPENDIX 1

CR Letter Comments (First review cycle):

Facilities CR Comment (provided by OPMA)

1. Following an evaluation of an inspection performed at Kashiv BioSciences (FEI 3011289655) manufacturing facility at Chicago, IL, our field investigator observed objectionable conditions at the facility and conveyed that information to the representative of the facility at the close of the inspection. Satisfactory resolution of the remaining objectionable conditions, and verification by FDA, is required before this application may be approved. We recommend you contact your manufacturing facility if more information is needed.

We will continue to monitor the public health situation as well as travel restrictions. We are actively working to define an approach for scheduling outstanding inspections, once safe travel may resume and based on public health need and other factors. For more information, please see the FDA guidances related to COVID 19. These guidances can be found at <https://www.fda.gov/emergency-preparedness-and-response/coronavirus-disease-2019-covid-19/covid-19-relatedguidance-documents-industry-fda-staff-and-other-stakeholders>

Microbiology CR Comments (provided by OPMA)

2. Information regarding media fill studies is inadequate. Please update Section 3.2.P.3.5 of the BLA with the following:
 - a. Summarized results (media fill date, container closure, filled volume, duration, number of units filled/incubated/rejected, positive) from the three initial media fill validation runs and the latest requalification run that was performed to validate the syringe line filling process relevant to the drug product.
 - b. Description of the hold periods (date, temperature, duration) simulated in each media fill run.
 - c. Description of confirmatory growth promotion test. Include a list of microorganisms used in the test.
3. The bacterial retention study for the sterilizing-grade filter was performed using the drug substance, which is not adequate. Please update BLA section 3.2.P.3.5 with the following:
 - a. Protocol and data from the validation studies using three different lots of the sterilizing filter intended for commercial production using the final drug product solution.
 - b. Study/report # and the date of the study.
 - c. Comparison of validation test parameters with those used during routine operation (i.e., temperature, filtration time, filtration pressure, flow volume, and flow rate, etc.)
 - d. Description of the challenge microorganism, membrane lot numbers, pore size rating, pre- and post-filtration bubble point, challenge (CFU/cm²).
 - e. Demonstration of viability of the challenge organism in the presence of drug product.

Product Quality CR Comments (provided by OBP)

Manufacture and Control

4. Insufficient information and data were provided in the submission, including in the response to the Agency's information request (sent on 05/24/2021, received on 06/03/2021, seq. 0025), to support that the filling line operations for the manufacture of the drug product on the (b) (4) are appropriately controlled and can result in a product with consistent protein concentration.

a. Limited process validation data were provided for the downstream drug product filling operations (Section 3.2.P.3.5, Report CMO-0174). (b) (4)

(b) (4)

Provide individual data points from additional process validation runs by analyzing multiple individual syringes at the beginning, middle, and end of fill to support the homogeneity of the filling operation to ensure the label claim is met for all syringes filled.

b. The fill step includes (b) (4)

(b) (4)

Provide data and information to support that the control strategy allows for the consistent and accurate fill volume for the drug product. In your response, provide data to support that drug product lots that were filled at the lowest allowed volume will ensure the appropriate extractable volume for all syringes.

5. Your control strategy (b) (4)

(b) (4)

Develop an appropriate control strategy (b) (4)

(b) (4) to ensure that all sequence variants in TPI-120 are adequately controlled at levels that do not exceed clinical experience using appropriate methods that are validated for their intended purpose. As part of your response, provide a robust characterization of peaks detected by RP-HPLC and CEX-HPLC for the presence of sequence variants to assist in determining your control strategy. Provide information and data to show a clear understanding of the source of each sequence variant (b) (4) and the potential impact on potency.

6. Inadequate stability protocols were provided to support the requalification of Primary Reference Standards (PRS) and Working Reference Standards (WRS) that are used for testing against intermediate product, drug substance, and drug product samples. The deficiencies observed in the protocols are described below:

(b) (4)

Because of the above issues, we do not agree that the forementioned stability protocols are adequate to requalify PRS and WRS that are intended to be used to test against intermediate product, drug substance, and drug product samples. To address the above issues, provide adequate trending analysis strategies for the EC50 values of the reference standards, and when applicable, provide quantitative, appropriately justified, acceptance criteria to control for (b) (4)

7. Insufficient data were provided to support the qualification of the WRS (b) (4) Data provided in the

qualification report (PTL-2306-R) shows out of specification results or results that are not comparable, such as the following:

[Redacted] (b) (4)

Because of the above issues, we do not agree that the current in-house WRS [Redacted] (b) (4) [Redacted] has been qualified appropriately. To address the above issues, provide an updated qualification report for an adequately qualified in-house WRS.

8. Inadequate information was provided to support recent changes made to the cell-based potency assays [Redacted] (b) (4)

[Redacted] In response to an information request (received on 01/08/2021, Seq. 0010), you provided additional information stating that the potency assays have been revised according to change control procedures (CC-20-036 and CC-20-045). However, inadequate information to support the proposed changes to the potency assays was provided. To ensure that the proposed changes have no impact on the potency assay method validation and test article data, provide adequate information to support the proposed changes.

9. Inadequate method validation data were provided to support the intended use of the CEX-HPLC method (STM-0282) [Redacted] (b) (4)

[Redacted]. The method validation exercise did not include adequate samples to allow for the validation of the method [Redacted] (b) (4)

[Redacted]

Provide additional method validation data to clearly demonstrate that the CEX-HPLC method is precise, accurate, linear, and robust in the

[Redacted] (b) (4)

Additional Comments (provided by OBP)

[Redacted] (b) (4)



Brian
Janelsins

Digitally signed by Brian Janelsins
Date: 5/31/2022 03:35:40PM
GUID: 54b8280e000855ed28b059d11c54a788



Rachel
Novak

Digitally signed by Rachel Novak
Date: 5/31/2022 03:41:41PM
GUID: 52e163f70002ba9e745b4e39384c76d7

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

Recommendation: Complete Response

BLA/NDA Number: 761084
Assessment Number: 1
Assessment Date: July 23, 2021

Drug Name/Dosage Form	(b) (4) (pegfilgrastim-pbbk) injection
Strength/Potency	6 mg/0.6 mL (10 mg/1 mL)
Route of Administration	Subcutaneous
Rx/OTC dispensed	Rx
Indication	All indications for US-licensed Neulasta
Applicant/Sponsor	Kashiv BioSciences, LLC
US agent, if applicable	N/A

Product Overview:

(b) (4) (pegfilgrastim-pbbk) is a proposed biosimilar to US-licensed Neulasta (pegfilgrastim). Pegfilgrastim is a pegylated form of filgrastim (recombinant human methionyl granulocyte colony stimulating factor, G-CSF) with a 20 kDa polyethylene glycol (PEG) molecule covalently bound to the α -amino group of the N-terminal methionine residue. Pegfilgrastim retains the same biological activity as filgrastim upon binding to G-CSF receptor on the cell surface. Binding of the G-CSF portion of pegfilgrastim to the G-CSF receptor on precursor neutrophilic granulocytes initiates signal transduction that leads to the survival, proliferation, and differentiation of neutrophil progenitor cells into mature neutrophils. Pegylation increases the size of G-CSF, reducing renal clearance, and therefore, extending the median serum half-life.

Pegfilgrastim-pbbk is produced in genetically engineered *E. coli* cells (b) (4)

(b) (4) Pegfilgrastim-pbbk drug product (DP), (b) (4) is manufactured to have the same strength, dosage form, and route of administration as US-licensed Neulasta. The formulation and presentation are the same between these products as well. (b) (4) is a sterile, preservative-free, clear, colorless to pale yellow liquid that consists of 0.6 mg of pegfilgrastim-pbbk in 0.6 mL (10 mg/mL) and is supplied in single-use pre-filled syringes for subcutaneous administration.

Quality Assessment Team:

Discipline	Assessor	Branch/Division
Drug Substance	Anshu Rastogi	OBP/DBRRI
Drug Product	Anshu Rastogi	OBP/DBRRI
Comparative Analytical Assessment	Anshu Rastogi	OBP/DBRRI
Immunogenicity Assays	Anshu Rastogi	OBP/DBRRI
Labeling	James Barlow	OBP

Facility	Michael Shanks (DS), Yarery Smith (DP)	OPMA/DBM/BMB2
Microbiology	Michael Shanks (DS), Yarery Smith (DP)	OPMA/DBM/BMB2
OPMA Team Lead	Candace Gomez-Broughton (Microbiology), Peter (Zhihao) Qiu (Facility)	OPMA/DBM/BMB2 (Microbiology), OPMA/DBM (Facility)
Application Team Lead	Brian Janelsins	OBP/DBRRI
Tertiary Reviewer	Rachel Novak	OBP/DBRRI
RBPM	Andrew Shiber	OPRO

Multidisciplinary Assessment Team:

Discipline	Assessor	Office/Division
RPM	May Zuwannin	OND/ORO/DROCHEN
Cross-disciplinary Team Lead	Kathy Robie Suh	OND/OCHEN/DNH
Medical Officer	Hyon-Zu Lee, Kathy Robie Suh (TL)	OND/OCHEN/DNH
Pharmacology/Toxicology	Fred Alavi, Todd Bourcier (TL)	OND/OCHEN/DPTCHEN
Clinical Pharmacology	Kunal Jhunjhunwala, Anusha Ande (TL)	OTS/OCP/DCEP
Statistics	Lola Luo, Yeh-Fong Chen (TL)	OTS/OB/DBIX

1. Names:

- a. Proprietary Name: (b) (4)
- c. Non-Proprietary Name/USAN: Pegfilgrastim
- d. CAS Name: 208265-92-3
- e. Common Name: TPI-120
- f. INN Name: Pegfilgrastim
- h. OBP systematic name: CONJ: RPROT P09919 (CSF3_HUMAN); PEG [TPI120]

Submissions Assessed:

Submission(s) Assessed	Document Date
761084/0001 (Original Submission)	08/11/2020
761084/0002 (Response to OBP IR)	10/01/2020
761084/0005 (Response to OBP IR)	10/29/2020
761084/0006 (Response to OBP IR)	12/08/2020
761084/0010 (Response to OBP IR)	01/08/2021
761084/0012 (Response to OBP IR)	02/19/2021
761084/0014 (Response to OBP IR)	03/09/2021
761084/0015 (Response to OBP IR)	04/23/2021
761084/0016 (Response to OPMA IR)	04/23/2021
761084/0018 (Response to OBP IR)	05/17/2021
761084/0020 (Response to OBP IR)	05/21/2021

761084/0024 (Response to OPMA IR)	06/03/2021
761084/0025 (Response to OBP IR)	06/03/2021
761084/0028 (Response to OBP IR)	06/07/2021
761084/0029 (Response to OBP IR)	06/16/2021
761084/0031 (Response to OBP IR)	06/21/2021

Quality Assessment Data Sheet:

1. Legal Basis for Submission:

351(k)

2. Related/Supporting Documents:

A. DMFs:

DMF #	DMF Type	DMF Holder	Item referenced	Code ¹	Status ²	Date Assessment Completed	Comments
(b) (4)	3	(b) (4)	(b) (4)	3	Adequate	N/A	
	5			3	Adequate	N/A	
	3			3	Adequate	N/A	
	3			3	Adequate	N/A	
	2			3	Adequate	N/A	

1. Action codes for DMF Table: 1- DMF Assessed; Other codes indicate why the DMF was not assessed, as follows: 2- Assessed previously and no revision since last assessment; 3- Sufficient information in application; 4- Authority to reference not granted; 5- DMF not available; 6- Other (explain under “comments”).

2. Action codes for Status column: Adequate, Adequate with Information Request, Deficient, or N/A (There is not enough data in the application; therefore, the DMF did not need to be assessed).

B. Other documents: IND, Referenced Listed Drug (RLD), or sister application:

Not applicable.

3. Consults:

Discipline/Topic	Date Requested	Status	Recommendation	Assessor
CDRH (pre-filled syringe assembly and needle guard component)	02/01/2021	Completed	CR (review uploaded in ICCR platform on 07/22/2021)	Porsche Bennett, Suzanne Hudak (TL)

4. Environmental Assessment of Claim of Categorical Exclusion:

Kashiv requested categorical exclusion from the environmental assessment requirement under 21 CFR 25.31(a), (c), and (d). These claims of categorical exclusions are acceptable for BLA 761084.

Executive Summary:

I. Recommendations:

A. Recommendation and Conclusion on Approvability:

The Office of Pharmaceutical Quality (OPQ), CDER, has completed assessment of STN 761084 for (b) (4) manufactured by Kashiv BioSciences. Sufficient analytical data are provided to support that (b) (4) is highly similar to US-licensed Neulasta, notwithstanding minor differences in clinically inactive components, and that the strength, dosage form, and route of administration of (b) (4) are the same as that of US-licensed Neulasta. However, data submitted in this application are not sufficient to support a conclusion that the manufacture of (b) (4) is well-controlled and will lead to a product that is safe, pure, and potent for the duration of the shelf-life. From a CMC standpoint, OPQ is recommending a Complete Response (CR) letter be issued to Kashiv BioSciences to outline the deficiencies noted below and the information and data that will be required to support approval. In addition, CDRH, which was consulted by the Office of Biotechnology Products (OBP), recommends a CR based on deficiencies identified related to the device perspective.

B. Summary of Complete Response Issues:

The Office of Pharmaceutical Manufacturing Assessment (OPMA) and OBP identified several product quality deficiencies during the review of Module 3 of BLA 761084. The deficiencies do not support that the manufacture of (b) (4) is well-controlled and will lead to a product that is safe, pure, and potent for the duration of the shelf-life. These deficiencies include (i) inadequate protocols for assessing the requalification of reference standards (b) (4); (ii) inadequate qualification of the working reference standard (b) (4); (iii) inadequate information and data to support the cell-based potency assay (b) (4); (iv) inadequate validation of the DP filtration and filling operations at (b) (4); (v) inadequate strategy to control sequence variants in TPI-120; and (vi) inadequate validation of the purity by CEX-HPLC method (STM-0282) (b) (4).

An inspection of the Kashiv BioSciences LLC drug substance facility (FEI #3011289655, Chicago, IL), which is the manufacturing site for the TPI-120 IP and DS, is required before this application can be approved as the FDA must assess the ability of that facility to conduct the listed manufacturing operations in compliance with cGMPs. Due to U.S. Government and/or Agency-wide restrictions on travel, an inspection of the Kashiv BioSciences LLC facility was not able to be conducted during the current review cycle, and the application cannot be approved until the required FDA inspection is conducted and the findings are assessed with regard to this application. Considering the inspection history of the facility, which resulted in unresolved observations, this will be considered a deficiency.

C. Complete Response Letter Draft Language:

Facilities CR Comment (provided by OPMA)

1. Following an evaluation of an inspection performed at Kashiv BioSciences (FEI 3011289655) manufacturing facility at Chicago, IL, our field investigator observed objectionable conditions at the facility and conveyed that information to the representative of the facility at the close of the inspection. Satisfactory resolution of the remaining objectionable conditions, and verification by FDA, is required before this application may be approved. We recommend you contact your manufacturing facility if more information is needed.

We will continue to monitor the public health situation as well as travel restrictions. We are actively working to define an approach for scheduling outstanding inspections, once safe travel may resume and based on public health need and other factors. For more information, please see the FDA guidances related to COVID 19. These guidances can be found at <https://www.fda.gov/emergency-preparedness-and-response/coronavirus-disease-2019-covid-19/covid-19-relatedguidance-documents-industry-fda-staff-and-other-stakeholders>

Microbiology CR Comments (provided by OPMA)

2. Information regarding media fill studies is inadequate. Please update Section 3.2.P.3.5 of the BLA with the following:
 - a. Summarized results (media fill date, container closure, filled volume, duration, number of units filled/incubated/rejected, positive) from the three initial media fill validation runs and the latest requalification run that was performed to validate the syringe line filling process relevant to the drug product.
 - b. Description of the hold periods (date, temperature, duration) simulated in each media fill run.
 - c. Description of confirmatory growth promotion test. Include a list of microorganisms used in the test.
3. The bacterial retention study for the sterilizing-grade filter was performed using the drug substance, which is not adequate. Please update BLA section 3.2.P.3.5 with the following:
 - a. Protocol and data from the validation studies using three different lots of the sterilizing filter intended for commercial production using the final drug product solution.
 - b. Study/report # and the date of the study.
 - c. Comparison of validation test parameters with those used during routine operation (i.e., temperature, filtration time, filtration pressure, flow volume, and flow rate, etc.)
 - d. Description of the challenge microorganism, membrane lot numbers, pore size rating, pre- and post-filtration bubble point, challenge (CFU/cm²).
 - e. Demonstration of viability of the challenge organism in the presence of drug product.

Product Quality CR Comments (provided by OBP)

Manufacture and Control

4. Insufficient information and data were provided in the submission, including in the response to the Agency's information request (sent on 05/24/2021, received on 06/03/2021, seq. 0025),

to support that the filling line operations for the manufacture of the drug product on the (b) (4) are appropriately controlled and can result in a product with consistent protein concentration.

- a. Limited process validation data were provided for the downstream drug product filling operations (Section 3.2.P.3.5, Report CMO-0174). (b) (4)

(b) (4)

Provide individual data points from additional process validation runs by analyzing multiple individual syringes at the beginning, middle, and end of fill to support the homogeneity of the filling operation to ensure the label claim is met for all syringes filled.

- b. The fill step includes (b) (4)

(b) (4)

Provide data and information to support that the control strategy allows for the consistent and accurate fill volume for the drug product. In your response, provide data to support that drug product lots that were filled at the lowest allowed volume will ensure the appropriate extractable volume for all syringes.

5. Your control strategy (b) (4)

(b) (4)

Develop an appropriate control strategy (b) (4) to ensure that all sequence variants in TPI-120 are adequately controlled at levels that do not exceed clinical experience using appropriate methods that are validated for their intended purpose. As part of your response, provide a robust characterization of peaks detected by RP-HPLC and CEX-HPLC for the presence of sequence variants to assist in determining your control strategy. Provide information and data to show a clear understanding of the source of each sequence variant (b) (4) and the potential impact on potency.

6. Inadequate stability protocols were provided to support the requalification of Primary Reference Standards (PRS) and Working Reference Standards (WRS) that are used for testing against intermediate product, drug substance, and drug product samples. The deficiencies observed in the protocols are described below:



Because of the above issues, we do not agree that the forementioned stability protocols are adequate to requalify PRS and WRS that are intended to be used to test against intermediate product, drug substance, and drug product samples. To address the above issues, provide adequate trending analysis strategies for the EC50 values of the reference standards, and when applicable, provide quantitative, appropriately justified, acceptance criteria to control for



7. Insufficient data were provided to support the qualification of the WRS  (b) (4)
 Data provided in the qualification report (PTL-2306-R) shows out of specification results or results that are not comparable, such as the following:



[REDACTED]

Because of the above issues, we do not agree that the current in-house WRS [REDACTED] (b) (4) [REDACTED] has been qualified appropriately. To address the above issues, provide an updated qualification report for an adequately qualified in-house WRS.

8. Inadequate information was provided to support recent changes made to the cell-based potency assays [REDACTED] (b) (4)

[REDACTED] In response to an information request (received on 01/08/2021, Seq. 0010), you provided additional information stating that the potency assays have been revised according to change control procedures (CC-20-036 and CC-20-045). However, inadequate information to support the proposed changes to the potency assays was provided. To ensure that the proposed changes have no impact on the potency assay method validation and test article data, provide adequate information to support the proposed changes.

9. Inadequate method validation data were provided to support the intended use of the CEX-HPLC method (STM-0282) [REDACTED] (b) (4)

[REDACTED] The method validation exercise did not include adequate samples to allow for the validation of the method [REDACTED] (b) (4)

[REDACTED]

[REDACTED] Provide additional method validation data to clearly demonstrate that the CEX-HPLC method is precise, accurate, linear, and robust in the [REDACTED] (b) (4)

Additional Comments (provided by OBP)

Manufacture and Control (IP and DS)

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D. Benefit/Risk Considerations:

(b) (4) is a proposed biosimilar to US-licensed Neulasta and was developed to have the same dosage form, route of administration, and strength as for US-licensed Neulasta. The formulation and presentation are also identical between the products. Kashiv BioSciences requested (b) (4) be approved for all indications approved for US-licensed Neulasta.

The manufacturing process and control strategy of (b) (4) are not sufficient to ensure that the manufacturing process is well-controlled and leads to a product of acceptable quality to ensure drug safety and effectiveness for patients. Due to US government and Agency-wide restrictions on travel, the Agency was not able to conduct an inspection of the DS manufacturing facility (Kashiv BioSciences) during the current review cycle. An inspection of Kashiv BioSciences is required before the applicant can be approved.

A comparative analytical assessment was performed to demonstrate that (b) (4) is highly similar to US-licensed Neulasta. The totality of the analytical data supports that (b) (4) is highly similar to US-licensed Neulasta.

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

There are no recommended post-marketing commitments, requirements, agreements, and/or risk management steps at this time.

II. Comparative Analytical Assessment

I. Analytical Assessment Overview and Conclusions

Kashiv BioSciences provided results to support a demonstration that TPI-120 is highly similar to US-licensed Neulasta, notwithstanding minor differences in clinically inactive components. The comparative analytical assessment was consistent with the FDA Guidance for Industry, Development of Therapeutic Protein Biosimilar: Comparative Analytical Assessment and Other Quality-Related Considerations, May 2019. As part of the assessment, an extensive analytical characterization of TPI-120 and US-licensed Neulasta was performed, which included comparisons of primary structure, pegylation characteristics, higher order structure, purity and product-related variants, size-variant characterization, bioactivity (potency and receptor binding), and protein concentration. Attributes were selected based on the established quality target product profile for US-licensed Neulasta and the defined critical quality attributes (CQAs) for TPI-120. Orthogonal methods were implemented and were designed to be capable

of detecting analytical differences between the products if present. The methods used in the assessment appear suitable for their intended purposes and will be further assessed on inspection during the next review recycle.

The attributes were ranked by criticality and assessed accordingly by quantitative and qualitative approaches. Attributes of very high risk (i.e., protein concentration and potency by cell-based bioassay) were evaluated using a combination of quality ranges and equivalence testing, while attributes of high risk were evaluated using quality ranges. Attributes of low risk or those that are not amendable to quantitative analysis were assessed using qualitative and/or graphic evaluations. In some cases, the Agency determined its own quality ranges for low risk attributes that are amendable to quantitative analysis when sufficient data were provided.

A total of 13 lots of US-licensed Neulasta and 12 lots of TPI-120 drug product were originally used in the assessment. All lots for each product were used in the assessment of very high and high risk quality attributes, while only a subset of lots were used in the analysis of low risk quality attributes. The assessment included analysis of independent TPI-120 drug product lots generated from clinical and commercial (i.e., PPQ lots) processes. The selected US-licensed Neulasta lots span the shelf-life of the product (24 months) and TPI-120 lots ranged from <1 – 17 months. Kashiv BioSciences updated the assessment for certain evaluations with additional lots of TPI-120 (1) and US-licensed Neulasta (5) to support the original dataset. For evaluations that consisted of reporting results relative to a reference standard, two reference standards (a US-licensed Neulasta lot and an in-house primary reference standard lot) were used, and data were provided to adequately qualify and bridge the reference standards.

In addition, the comparative analytical assessment included comparative stability studies, which evaluated the stability profile (i.e., degradation pathways and rates) of TPI-120 and US-licensed Neulasta under accelerated, stressed, and forced degradation conditions by orthogonal stability-indicating methods. The quality attributes assessed, and storage conditions selected were supported by development studies and risk assessments.

In conclusion, Kashiv BioSciences performed an adequate comparative analytical assessment that evaluated a comprehensive array of quality attributes relevant to both products by appropriate methods, used an appropriate data analysis plan, and selected appropriate lots for the assessment. The results from this assessment support a demonstration that TPI-120 is highly similar to US-licensed Neulasta, notwithstanding minor differences in clinically inactive components.

II. Results of the Comparative Analytical Assessment

The results from the comparative analytical assessment that support a demonstration that TPI-120 is highly similar to US-licensed Neulasta are summarized in Table 1 below. While not illustrated in the table below, the stability profile of TPI-120 was demonstrated to be similar to that of US-licensed Neulasta.

Table 1. Quality Attributes Analyzed in the Comparative Analytical Assessment

Physico-chemical/Functional Characteristics	Quality Attribute Assessed	Test Method	Supports a Demonstration of Highly Similar
<i>Primary Structure</i>	<i>Amino acid sequence</i>	<i>Peptide mapping LC-UV</i>	<i>Yes</i>

Physico-chemical/Functional Characteristics	Quality Attribute Assessed	Test Method		Supports a Demonstration of Highly Similar
	<i>Intact Mass</i>	<i>LC-MS, electrospray ionization</i>		<i>Yes</i>
	<i>Disulfide mapping</i>	<i>LC-MS/MS</i>		<i>Yes</i>
	<i>Free Thiol</i>	<i>Thiol fluorometric detector</i>		<i>Yes</i>
<i>Pegylation Characteristics</i>	<i>Pegylation site</i>	<i>MALDI-TOF, LC-MS/MS</i>		<i>Yes</i>
	<i>Polydispersity</i>	<i>MALDI-TOF MS</i>		<i>Yes</i>
	<i>Free PEG</i>	<i>CAD</i>		<i>Yes</i>
		<i>SDS-PAGE (Titrisol staining)</i>		<i>Yes</i>
<i>Higher Order Structure</i>	<i>Secondary structure</i>	<i>Far-UV CD</i>		<i>Yes</i>
		<i>FTIR spectroscopy</i>	<i>α-helix content</i>	<i>Yes</i>
			<i>β-sheet content</i>	<i>Yes</i>
	<i>Tertiary structure</i>	<i>Intrinsic fluorescence</i>		<i>Yes</i>
		<i>Near-UV CD</i>		<i>Yes</i>
		<i>DSC</i>		<i>Yes</i>
		<i>NMR</i>		<i>Yes</i>
<i>Purity and product-related variants</i>	<i>Size variants</i>	<i>SE-HPLC</i>	<i>Monomer</i>	<i>Yes</i>
			<i>Total size variants</i>	<i>Yes</i>
			<i>Di-pegylated variants</i>	<i>Yes</i>
			<i>Aggregates</i>	<i>Yes</i>
			<i>Free rhG-CSF</i>	<i>Yes</i>
	<i>Charge variants</i>	<i>CEX-HPLC</i>	<i>Main species</i>	<i>Yes</i>
			<i>Acidic variants</i>	<i>Yes</i>
			<i>Basic variants</i>	<i>Yes</i>
	<i>Hydrophobic variants</i>	<i>RP-HPLC</i>	<i>Main species</i>	<i>Yes</i>
			<i>Total variants</i>	<i>Yes</i>
			<i>Post-main variants</i>	<i>Yes</i>
			<i>Pre-main variants</i>	<i>Yes</i>
			<i>Oxidized variants</i>	<i>Yes</i>
			<i>Deamidated variants</i>	<i>Yes</i>
	<i>Truncated variants</i>	<i>CE-SDS (non-reduced)</i>		<i>Yes</i>
	<i>Aggregate characterization</i>	<i>AUC</i>		<i>Yes</i>
		<i>DLS</i>		<i>Yes</i>
<i>Bioactivity</i>	<i>Cell proliferation</i>	<i>In vitro cell-based bioassay</i>		<i>Yes</i>
	<i>Receptor binding</i>	<i>ELISA (relative potency)</i>		<i>Yes</i>
		<i>SPR (binding affinity and kinetics)</i>	<i>KD (M)</i>	<i>Yes</i>
			<i>Ka (1/Ms)</i>	<i>Yes</i>
			<i>Kd (1/s)</i>	<i>Yes</i>
<i>Drug Product Attributes</i>	<i>Protein concentration</i>	<i>UV Absorbance Spectroscopy</i>		<i>Yes</i>

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

Not applicable.

IV. Assessment of Analytical Study Results

Several quality attributes were noted as not meeting the pre-determined similarity acceptance criteria or showed variable results in qualitative assessments; however, adequate justification has been provided that the differences observed do not preclude a demonstration that TPI-120 is highly similar to US-licensed Neulasta.

Size variants by SE-HPLC

TPI-120 levels of monomer were slightly higher than the quality range of US-licensed Neulasta, while TPI-120 levels of di-pegylated variants, free rhG-CSF, and total size variants were slightly lower than the quality ranges of US-licensed Neulasta. With respect to aggregates, most TPI-120 lots were within the US-licensed Neulasta quality range (10 of 13), while 3 lots showed levels slightly higher than the quality range (no more than 0.12% over the upper limit). Considering that the levels are very low for di-pegylated variants (no more than 1.38% in US-licensed Neulasta and 0.74% in TPI-120), aggregates (no more than 0.19% in US-licensed Neulasta and 0.36% in TPI-120), free rhG-CSF (no more than 0.14% in US-licensed Neulasta and 0.06% in TPI-120), and total size variants (no more than 1.80% in US-licensed Neulasta and 1.03% in TPI-120), and the differences in mean values between both products are minor for di-pegylated variants (0.7%), aggregates (0.07%), free rhG-CSF (0.06%), total size variants (0.79%), and monomer (0.79%), these differences are not expected to impact potency or have a clinical impact. For example, the comparative analytical assessment results show similar bioactivity between TPI-120 and US-licensed Neulasta. No new size variant species were identified in TPI-120, and orthogonal assessments (AUC and DLS) demonstrate that TPI-120 contains the same high molecular weight types with similar characteristics and levels as those detected in US-licensed Neulasta. In conclusion, there is no residual uncertainty regarding the evaluation for purity and size-variants as performed by SE-HPLC. The small differences in size variants do not preclude a demonstration of highly similar.

Charge variants by CEX-HPLC

TPI-120 levels for main species were slightly higher than the quality range (97.49% - 99.24%) for 5 out of the 13 evaluated lots, but these lots did not exceed the upper limit beyond 0.08%. TPI-120 levels of acidic variants, which consist of mainly di-pegylated, acetylated, and deamidated variants, were slightly lower than the US-licensed Neulasta quality range for all 13 TPI-120 lots. The levels of acidic variants are low in TPI-120 (not higher than 0.2%) and US-licensed Neulasta (0.41% to 0.73%), and the difference is minor. These differences are not expected to impact potency or have a clinical impact. For example, the comparative analytical assessment results show similar bioactivity between TPI-120 and US-licensed Neulasta. In conclusion, there is no residual uncertainty regarding the evaluation for purity and charge variants as performed by CEX-HPLC. The small differences in charge variants do not preclude a demonstration that TPI-120 is highly similar to US-licensed Neulasta.

Hydrophobic variants by RP-HPLC

TPI-120 levels of main peak were within the quality range (94.77% - 97.58%) with the exception of 2 lots that were slightly higher than the quality range by no more than 0.14%. TPI-120 levels for pre-main variants, which consist of oxidized and sequence variants, resulted in 4 lots within the quality range (1.32% - 1.97%), 6 lots below the quality range, and 1 lot above the quality range. The 1 lot above the quality range for pre-main variants was only 0.32% higher than the maximum value reported for US-

licensed Neulasta and only 0.19% higher than the quality range upper limit. The mean levels of pre-main variants are low (1.35% in TPI-120 and 1.65% in US-licensed Neulasta) and the difference between the product is minimal (0.30%). In addition, TPI-120 levels for the main RP-HPLC variant (oxidized Met-127), which is included in the calculation of pre-main variants, were below the quality range for all 11 evaluated lots. All other oxidized variants in TPI-120 and US-licensed Neulasta were below or near LOQ and showed no apparent difference between the products. The mean levels of oxidized Met-127 variant are low (0.97% in TPI-120 and 1.61% in US-licensed Neulasta) and the difference between the product is minimal (0.64%). These differences are not expected to impact potency or have a clinical impact. For example, the comparative analytical assessment results show similar bioactivity between TPI-120 and US-licensed Neulasta.

Characterization of hydrophobic variants (pre- and post-main peaks) revealed that sequence variants (non-terminal, single amino acid misincorporations) are present in TPI-120 and US-licensed Neulasta at very low levels (Table 2, see below). Only one sequence variant (F114 → I/L114) was detected in TPI-120 that was not detected in US-licensed Neulasta by RP-HPLC. While the level of that variant is unclear compared to the levels of the other variants in the peak, the overall peak amount is very low. Furthermore, while there appears to be a general trend of higher percentages of sequence variants in TPI-120, these differences and the overall difference (i.e., 0.38%) reported between the two products is minimal. Given the size (1 amino acid change), location of the misincorporation (not identified as critical for binding), and amount present (no more than 0.54%), the differences in sequence variants do not preclude a demonstration that TPI-120 is highly similar to US-licensed Neulasta.

Table 2. Sequence Variants by RP-HPLC

Peak Number	Sequence Variant	TPI-120 (min-max)	US-licensed Neulasta (min-max)
RP-HPLC P2a	W119 → R119	ND – <LOQ	ND – 0.11%
RP-HPLC P2b	T103 → S103	<LOQ – 0.18%	ND – <LOQ
RP-HPLC P3c	E123 → D123	ND – 0.12%	ND – <LOQ
RP-HPLC P8	D113 → E113 D110 → E110 F114 → I/L114 (only in TPI-120)	ND – 0.54%	0.25 – 0.44%
RP-HPLC P10ab	D105 → E105	ND – 0.38%	ND – 0.29%

*LOD = 0.04%, LOQ = 0.10%

In conclusion, there is no residual uncertainty regarding the evaluation of purity and hydrophobic variants (e.g., oxidized, deamidated, acetylated, truncated, and sequence) as performed by RP-HPLC. The small differences in hydrophobic and sequence variants do not preclude a demonstration that TPI-120 is highly similar to US-licensed Neulasta. However, considering that the source and impact on the product (i.e., potency) of the sequence variants is unknown, the control strategy for sequence variants is inadequate, as discussed in Section I.B of the IQA. To ensure that TPI-120 will remain highly similar to US-licensed Neulasta with the respect of sequence variants, the need to propose an adequate control strategy to monitor sequence variants will be communicated in the Complete Response letter.

Receptor binding by SPR

The data generated from the receptor binding by SPR assessment included values for KD (equilibrium dissociation constant), which is an indication of binding affinity, and Kd (dissociation constant) and Ka

(association constant), which are indications of binding kinetics, from 3 TPI-120 lots and 3 US-licensed Neulasta lots. While no quality ranges were used by Kashiv BioSciences in their evaluation, likely because of inherent method variability and the limited dataset, the Agency generated quality ranges (mean ± 3SD) to assess similarity with respect to receptor binding affinity and kinetics. All 3 TPI-120 lots reported values for KD and Kd within the quality ranges; however, 1 TPI-120 lot reported a value for Ka that was slightly higher than the quality range upper limit. Additional data was provided by Kashiv BioSciences to show higher variability of Ka values for the US-licensed Neulasta lots (CV of 7.7%) compared to TPI-120 lots (CV of 3.5%). Qualification data for the method was also provided to show that there is higher variability for Ka values with respect to Kd values (8.9% vs. 3.8%, respectively). Changes in receptor binding affinity and kinetics could be an indication of structural changes that may impact receptor binding; however, data generated from the higher order structure assessments demonstrated that TPI-120 is similar to US-licensed Neulasta. Receptor binding by ELISA and the cell-based bioassay assessments, which report relative potency, also showed similar results for TPI-120 and US-licensed Neulasta. The difference in Ka values from the receptor binding by SPR assessment is likely the result of method variability. Therefore, the observed difference in Ka values does not preclude a demonstration that TPI-120 is highly similar to US-licensed Neulasta.

A. Same Strength(s)

TPI-120 has the same dosage form and route of administration as US-licensed Neulasta. Kashiv BioSciences is seeking licensure of 0.6 mg/0.6 mL TPI-120 in a pre-filled syringe. US-licensed Neulasta is licensed in this strength (0.6 mg/0.6 mL) in a pre-filled syringe.¹ Kashiv BioSciences is seeking approval of TPI-120 for the same strength as US-licensed Neulasta. Comparative protein concentration (10 mg/mL) was assessed as part of the comparative analytical assessment. The deliverable volume (0.6 mL) and fill weight data were assessed (b) (4). The proposed presentation of TPI-120 has the same total content of drug substance in units of mass in a container and the same concentration of drug substance in units of mass per unit volume as US-licensed Neulasta (6 mg/0.6 mL). The strength of TPI-120 pre-filled syringe is the same as that of US-licensed Neulasta. However, insufficient information was provided to ensure the consistency of the drug product filling process in terms of protein concentration. While deliverable volume (0.6 mL) and fill weight data were assessed (b) (4) Kashiv BioSciences did not provide sufficient information to demonstrate that all syringes that are filled will be of consistent protein concentration based on the sampling strategy employed during process validation studies. This issue will be addressed in the CR letter as a manufacturing deficiency.

III. Summary of Quality Assessments:

A. CQA Identification, Risk and Lifecycle Knowledge Management

Table 3. Active Pharmaceutical Ingredient CQA Identification, Risk and Lifecycle Knowledge Management

<u>CQA (type)</u>	<u>Risk</u>	<u>Origin</u>	<u>Control Strategy</u>	<u>Other</u>

¹ U.S. Prescribing Information, U.S.-Licensed Neulasta. Accessed 7/7/21 from <https://www.accessdata.fda.gov/scripts/cder/daf/>

Potency (in vitro cell-based bioassay)	Efficacy	Intrinsic to molecule	(b) (4)	Insufficient data were provided to support changes to the validated potency method (CR Comment #9).
Receptor binding by ELISA	Efficacy	Intrinsic to molecule		N/A
Primary Sequence (Identity)	Efficacy	Intrinsic to molecule		N/A
Higher order structure	Efficacy	Intrinsic to molecule		N/A
Free PEG	Safety	Manufacturing Process/Storage conditions		N/A
Low molecular weight variants (Free rhG-CSF and other truncated species)	Efficacy and safety	Manufacturing process and storage conditions		N/A
High molecular weight variants (e.g., dimer, aggregates, and dipegylated species (only for DS and DP))	Efficacy, PK, and safety	Manufacturing process and storage conditions		N/A
Charge variants (e.g., acidic and basic variants)	Efficacy, PK, and safety	Manufacturing process and storage conditions		Insufficient data were provided to support the suitability of the method for its intended use to test DS and DP (CR Comment #9).
Hydrophobic variants (e.g., oxidized, deamidated, acetylated, and sequence variants)	Efficacy and safety	Manufacturing process and storage conditions		N/A

B. Drug Substance pegfilgrastim-pbbk Quality Summary

The following table presents CQAs derived from the DS manufacturing process and general DS attributes, as well as control strategies in place.

Table 4. Drug Substance CQA Process Risk Identification and Lifecycle Knowledge Management.

Category	Risk	Origin	Control Strategy	Other
Appearance	Safety	Manufacturing Process	(b) (4)	N/A
Host cell protein	Safety and immunogenicity	Derived from host cell line		N/A
Host cell DNA	Safety	Derived from host cell line		N/A
Protein concentration	General CQA	Formulation		N/A
(b) (4)	Safety and stability	Manufacturing process		N/A
Sequence Variants	Safety, immunogenicity	Manufacturing Process		See CR Comment #5
Endotoxin	Safety and purity	Manufacturing process		N/A
Bioburden	Safety, purity, and efficacy	Raw materials and manufacturing process		N/A

• **Description:**

TPI-120 is a polyethylene glycol (PEG) modified non-glycosylated form of recombinant human granulocyte colony-stimulating factor (rhG-CSF, filgrastim) with an additional N-terminal methionine added to enforce expression in *E. coli*. The (b) (4) rhG-CSF, which is composed of 175 amino acids and is identical to human G-CSF with the exception of the additional methionine residue at the N-terminus. TPI-120 DS is derived from pegylation of the rhG-CSF (b) (4) by the covalent attachment of linear PEG molecule (~20 kDa) to the N-terminal methionine of rhG-CSF by a secondary amine linkage. The molecular weight of TPI-120 is ~39 kDa.

• **Mechanism of Action (MoA):**

The biological activity of GCSF is initiated by binding of GCSF to the GCSF receptor on myeloid progenitor cells and mature neutrophils. This binding initiates transduction signals that lead to the proliferation and differentiation of neutrophil committed progenitor cells,

increase of mature neutrophils in the blood, which is a relevant pharmacodynamic (PD) marker for neutropenia, and enhanced neutrophil function.

- Potency Assay:

An in vitro cell-based assay was adopted from the USP monograph for filgrastim and is executed according to STM-0118 and STM-0127 when used to test IP and DS samples, respectively. The bioassay includes the use of a M-NFS-60 cell line, which is derived from a murine myelogenous leukemia cell and proliferates in response to G-CSF. The cells are incubated with varying concentrations of reference solution and test solution. After a timed incubation, CellTiter-Blue Cell Viability reagent is added to the plates. Viable cells convert a redox dye into a fluorescent end-product which can be measured using a fluorescence-plate reader. The fluorescent values are directly proportional to the number of viable cells in the culture. Biological activity of test sample is determined by comparing its response to that of the reference. The potency of IP lots is expressed as units/milligram, while the potency of DS (and DP) lots is expressed as relative potency percentage in comparison to that of the reference standard. After validation of the bioassay, changes were made to the method without supporting information (CR Comment #8).

- Reference Materials:

An in-house two-tier system is used for the reference standard campaigns for the IP and the DS/DP. Currently, a Primary Reference Standard (PRS) and Working Reference Standard (WRS) have been qualified for each campaign, and each reference standard was adequately qualified against the previous reference standard with the exception of the WRS for the IP campaign (CR Comment #7). Protocols to support requalification of the PRS and WRS for each campaign are deficient (CR Comment #6).

- Critical starting materials or intermediates:



• Manufacturing process summary:

(b) (4)



• Container closure:

The IP and DS are filled and stored in a container closure system (b) (4)



• Dating period and storage conditions:

The expiry period of the IP is (b) (4) while the expiry period for the DS is at (b) (4). The post-approval stability protocols for testing of IP and DS are adequate.

• Microbial control:

Microbial quality is controlled at each step of the manufacturing process (b) (4)



Adequate

controls are in place to maintain microbiological product quality during maximum hold periods and throughout the manufacturing process.

C. Drug Product ^{(b) (4)} Quality Summary:

The following table presents CQAs derived from the DP manufacturing process and general DP attributes, as well as control strategies in place.

Table 5. Drug Product CQA Identification, Risk, and Lifecycle Management

Category	Risk	Origin	Control Strategy	Other
Appearance (color and clarity)	General CQA	Formulation	(b) (4)	N/A
Protein concentration	General CQA	Formulation		N/A
Osmolality	Product stability, patient discomfort	Formulation		N/A
pH	Stability	Formulation		N/A
Polysorbate 20	Safety and stability	Manufacturing process, formulation		N/A
Visible particles	Immunogenicity and patient safety	Stability, accidental throughout process		N/A
Sub-visible particles	Safety	Manufacturing process, product degradation		N/A
Container closure	Negative impact of leachables on product quality. Introduction of container related impurities (e.g., ^{(b) (4)})	Filling/storage		N/A
Volume of injection	Efficacy	Manufacturing process		N/A
Syringe break loose and glide force determination	Safety	Manufacturing process		Refer to CDRH review
Sterility	Safety and product stability	Manufacturing process, container closure integrity failure		N/A
Endotoxin	Safety	Manufacturing process, contamination		See Additional Comments #36 - #39
Container Closure Integrity	Safety (maintenance of sterility during shelf-life)	Container closure breaches during storage. May be		See Additional

		impacted by storage conditions.	integrity is included in the DP stability program.	Comment #40

- Potency and Strength:

TPI-120 is supplied in a pre-filled syringe for subcutaneous administration at a strength of 0.6 mg/0.6 mL. TPI-120 was developed to have the same strength as US-licensed Neulasta.

- Summary of Product Design:

TPI-120 was developed as a biosimilar to US-licensed Neulasta and targets the quality profile of US-licensed Neulasta.

- List of Excipients:

TPI-120 has the same formulation as used in US-licensed Neulasta and consists as a sterile, clear, colorless to slightly yellow liquid in a preservative-free liquid formulation (pH 4.0). Components in addition to the active ingredient (pegfilgrastim-pbbk) include acetate (0.35 mg), polysorbate 20 (0.02 mg), sodium (0.02 mg), and sorbitol (30 mg).

- Reference Materials:

The same reference standards are used for DS and DP.

- Manufacturing process summary:

(b) (4)

- Container closure:

The container closure system is a single-dose prefilled syringe consisting of a 1 mL Long (b) (4) glass syringe barrel with a staked 27-gauge inch needle and a rigid needle shield and an elastomeric plunger stopped (b) (4)

- Dating period and storage conditions:

The expiry period of the DP is 5 ± 3°C for 24 months. However, the shelf-life is not supported because stability data for volume of injection at shelf-life was not provided

(Additional Comment #28). The post-approval stability protocol does not include sufficient testing time-points for container closure integrity testing (CCIT). It will be requested that Kashiv BioSciences revises the stability protocol to include CCIT for stability samples every 12 months until expiry (Additional Comment #40).

- List of co-package components, if applicable:
Not applicable.

D. Biopharmaceutics Considerations:
Not applicable

E. Novel Approaches/Precedents:
None

F. Any Special Product Quality Labeling Recommendations:
Labeling review was not concluded given that the BLA will receive a CR.

G. Establishment Information:

Overall Recommendation:					
DRUG SUBSTANCE					
Function	Site Information	DUNS/FEI Number	Preliminary Assessment	Inspectional Observations	Final Recommendation
Manufacture and storage of cell banks; DS manufacture and storage; in-process, release and stability testing of DS; raw material testing; and storage of DS and DP	Kashiv BioSciences, LLC (San Diego, CA)	3011289655 (FEI)	Not performed due to travel restrictions	N/A	Inspection is required for approval
Microorganisms identification	(b) (4)	(b) (4)	Facility Adequate	N/A	Approve
Cell bank characterization, end of production cell bank testing			Facility Adequate	N/A	Approve
Cell bank storage			Facility Adequate	N/A	Approve
(b) (4) testing for release and raw material testing			Facility Adequate	N/A	Approve

Host cell DNA and kanamycin testing	(b) (4)	Facility Adequate	N/A	Approve	
Microbiological in-process and release testing		Facility Adequate	N/A	Approve	
Raw material testing		Facility Adequate	N/A	Approve	
Raw material testing		Facility Adequate	N/A	Approve	
Raw material Storage		Facility Adequate	N/A	Approve	
Extractables and Leachables Testing for DS		Facility Adequate	N/A	Approve	
DRUG PRODUCT					
Function	Site Information	DUNS/FEI Number	Preliminary Assessment	Inspectional Observations	Final Recommendation
DP release and stability testing (except polysorbate 20), pre-filled syringe assembly, packaging and cartooning; storage and shipping of packaged DP	Kashiv BioSciences LLC (Chicago, IL)	3011289655 (FEI)	Not Performed due to travel restrictions	N/A/	Inspection is required
DP manufacture; raw material testing, storage, and release; DP in-process testing; DP protein concentration release testing; and shipping of DP to Kashiv	(b) (4)	Inadequate	Yes (3-item Form FDA-483 issued)	VAI	

Polysorbate 20 release and stability testing of DP	(b) (4)	Facility Adequate	N/A	Approve
Particulate matter testing for DP release and stability		Facility Adequate	N/A	Approve
Sterility testing for DP release		Facility Adequate	N/A	Approve
Sterility testing for DP stability		Facility Adequate	N/A	Approve
Microorganisms identification		Facility Adequate	N/A	Approve
Raw material testing		Facility Adequate	N/A	Approve
Extractables and leachables testing for DP		Facility Adequate	N/A	Approve

H. Facilities:

Kashiv BioSciences LLC (FEI# 3011289655) in Chicago, Illinois is responsible for the manufacture of the TPI-120 IP and DS. Inspection of this site is required before the application can be approved as the FDA must assess the ability of the facility to conduct the listed manufacturing operations in compliance with cGMPs. Due to U.S. Government and/or Agency-wide restrictions on travel, an inspection of the Kashiv BioSciences LLC facility was not able to be conducted during the current review cycle, and the application cannot be approved until the required FDA inspection is conducted and the findings are assessed with regard to this application. This will be included as a CR comment.

(b) (4)

_____ (b) (4) is responsible for the manufacture of the TPI-120 DP. ORA led an inspection of the facility in support of different applications, and at the conclusion of the inspection, a three-item Form FDA-483 was issued to the firm. The responses to the items listed in the Form FDA-483 were inadequate and resulted in an initial OAI classification; however, this classification was later downgraded to VAI.

I. Lifecycle Knowledge Management:

a. Drug Substance:

i. Protocols approved:

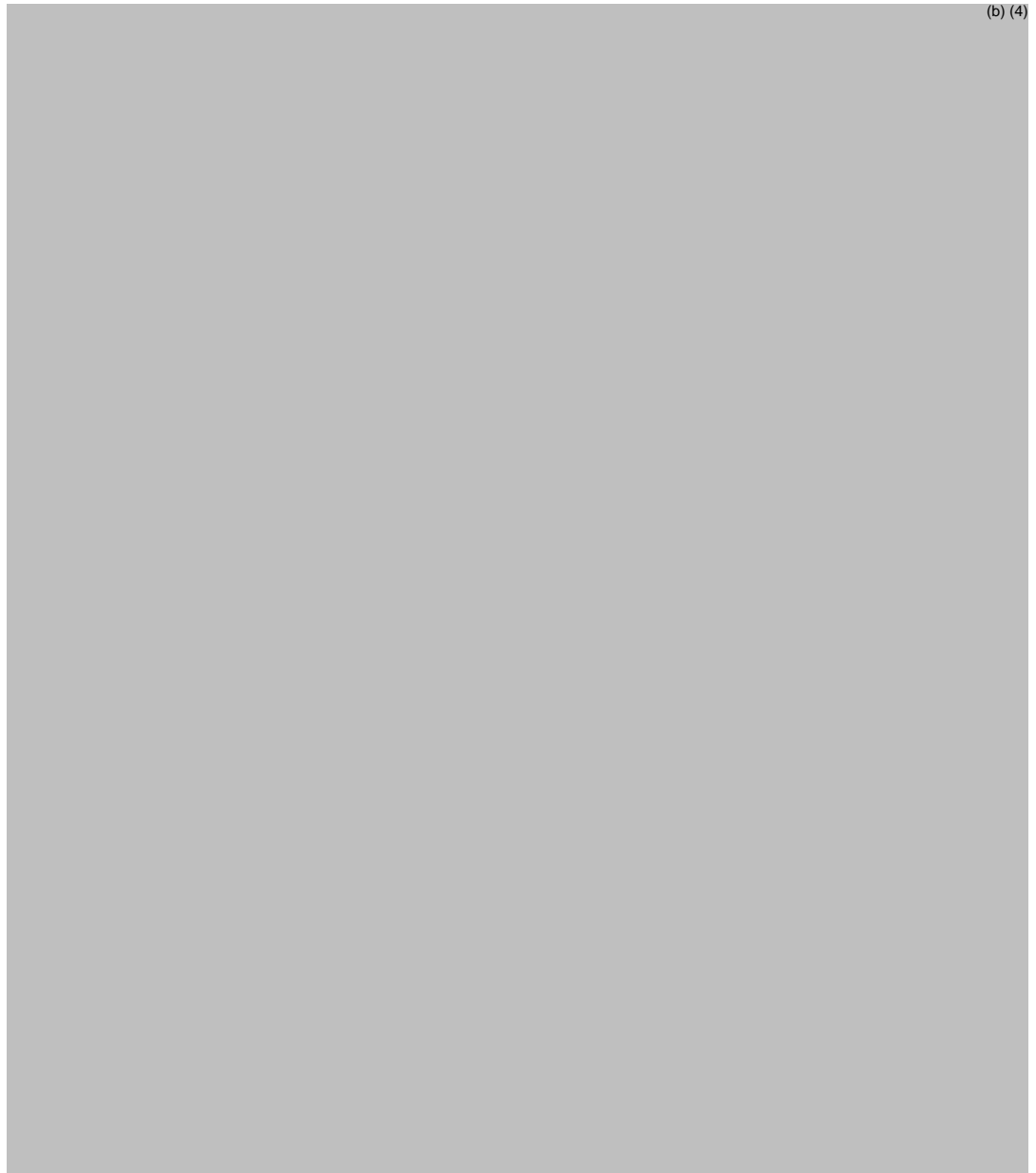
Not applicable

ii. Outstanding assessment issues/residual risk:

See CR comments in Section I.C.

iii. Future inspection points to consider:

The following are issues that were noted during the review of the BLA and should be evaluated on inspection during the resubmission review cycle.



(b) (4)



b. Drug Product

i. Protocols approved:
Not Applicable

ii. Outstanding assessment issues/residual risk:
See CR comments in Section I.C.

iii. Future inspection points to consider:
The following are issues that were noted during the review of the BLA and should be evaluated on inspection during the resubmission review cycle.

(b) (4)



Facilities Inspection Recommendation

APPEARS THIS WAY ON ORIGINAL

**PRODUCT QUALITY MICROBIOLOGY/FACILITY
ASSESSMENT**

Memorandum of Review to the File

Application ID	BLA 761084
Submission Type	Other: Resubmission (Seq. 0036)
Drug Product Name	Pegfilgrastim
Strengths	6 mg/0.6 mL
Dosage Form	injection
Administration Route	subcutaneous
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 clinically significant incidence of febrile neutropenia
Applicant Name	Kashiv BioSciences, LLC
US License Number	2131
Application Type	351 (k)
Primary Reviewer	Yarery Smith, Ph.D.
Secondary Reviewer	Candace Gomez-Broughton, Ph.D.
Goal Date	05/29/2022
Review Date	04/18/2022

Recommendation for Approvability:

- This BLA resubmission was reviewed from a product quality microbiology perspective and sterility assurance perspective and is recommended for Approval with the following post-marketing commitment(s): To conduct a drug product (DP) real-time transport qualification study shipping syringes of Pegfilgrastim (Fylnetra), from the DP manufacturing site to Kashiv BioSciences and to the distribution center. The corresponding qualification report will be submitted after one year upon approval of the BLA.
- Manufacturing Facility Assessment Recommendation: Approval
- Product quality aspects not related to microbial control and facilities should be reviewed by OBP.

List Submissions being assessed (Table):

Document Description (SD #)	Date Received
Resubmission (0036)	11/29/2021

Application Submission Background

Reviewer's Comment: For Information

The 11/29/2021 submission relates to a Complete Response Letter (CRL) for the original BLA 761084 application due to the deficiencies issued on 08/11/2021.

The following deficiencies (in italics) were conveyed to the applicant in the CR letter:

1. *Information regarding media fill studies is inadequate. Please update Section P.3.5 of the BLA with the following:*
 - a. *Summarized results (media fill date, container closure, filled volume, duration, number of units filled/incubated/rejected, positive) from the three initial media fill validation runs and the latest requalification run that were performed to validate the syringe line filling process relevant to the drug product.*
 - b. *Description of the hold periods (date, temperature, duration) simulated in each media fill run.*
 - c. *Description of confirmatory growth promotion test. Include a list of microorganisms used in the test.*

Response: The applicant states that Section P.3.5 has been updated to incorporate the results from the three initial media fill validation runs and the latest requalification run, as well as the hold time /period (date, temperature, duration) simulated in each media fill run.

The following media fills information is provided in the updated Section P.3.5.





Yarery
Smith

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Candace
Gomez-
Broughton

Digitally signed by Candace Gomez-Broughton

Date: 4/18/2022 10:00:26AM

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PRODUCT QUALITY MICROBIOLOGY/FACILITY ASSESSMENT

Memorandum of Review to the File

Table with 2 columns: Field Name and Value. Fields include Application ID, Submission Type, Drug Product Name, Strengths, Dosage Form, Administration Route, Indication, Applicant Name, US License Number, Application Type, Primary Reviewer, Secondary Reviewer, and Goal Date.

Recommendation for Approvability:

- Three bullet points with checkboxes regarding BLA review, Manufacturing Facility Assessment Recommendation, and product quality aspects.

Summary Basis of Recommendation (DS):

Overall, the process is under adequate microbial control (b) (4)



adequate controls are in place to maintain microbiological product quality during maximum hold periods and throughout the manufacturing process. All Drug Substance deficiencies from a microbial control and microbiology product quality perspective were resolved and found approvable in the first round of application review, BLA 761082-ORIG-1.

Adequate descriptions of the facilities, equipment, environmental controls, cleaning and contamination control strategy were provided for Kashiv Biosciences LLC (FEI 3011289655), proposed for Drug Substance manufacture. With the exception of the Drug Substance manufacturing site, Kashiv Biosciences LLC, all other Drug Substance related facilities are acceptable based on their current CGMP compliance status and recent relevant inspectional coverage.



QUALITY ASSESSMENT



Previously, the drug substance manufacturing site, Kashiv Bioscience LLC has had the following pre-license inspections:

The two pre-license inspections of Kashiv Biosciences were conducted 11/13-21/2017 and 01/22-26/2018 during the first review cycle of BLA 761082-ORIG-1 and resulted in a Withhold decision based on the following:

1. Inadequate CAPAs to address issues identified during deviation and OOS investigations.
2. Inadequate written procedures for performance of deviation investigations.
3. Inadequate impact assessment of consequences of deviations and OOS events on product quality and submitted data.
4. There was no evidence that changes to revised and new SOPs written in response to the 483 observations had been conveyed to employees, and that employees had been trained in the new procedures.

Following the 03/04-12/2019 PLI of the Kashiv Biosciences DS site conducted in support of the BLA 761082-ORIG-1-RESUB-38 resubmission, deficiencies were identified for the following:

1. QA oversight of dispositioning and analytical methods.
2. Deviation management.
3. Change control management.
4. Adherence to written procedures.
5. Adequacy of written procedures.
6. Adequacy of analytical methods.
7. Robustness of manufacture.

In this current review cycle, BLA 761084 Resubmission 36, a pre-license inspection was conducted on 01/10/2021 to 01/14/2021 in support of this BLA by CDER/OPQ under eNSpect Operation ID 192719. The inspection concluded with the issuance of a five-item FDA Form 483 and a field recommendation of approve for BLA 761082. The compliance review of the firm's response in CMS WA 437154 concurred with the field recommendation and recommended approval for BLA 761084.

All other facilities supporting this BLA have acceptable compliance statuses and are approved in support of this BLA, 761084.

Drug Substance CQA Process Risk Identification and Lifecycle Knowledge Management:

CQA (type)	Risk	Origin	Control Strategy	Other
Endotoxin	Safety, Purity	Raw materials, manufacturing process	(b) (4)	
Bioburden	Safety, Purity and Efficacy due to degradation or modification of the product by microbial contamination	Raw materials, manufacturing process		

List Submissions being assessed (Table):

Document Description (SD #)	Date Received
BLA 761084-ORIG-01 (0001)	August 11, 2020
BLA 761084-ORIG-01 (0002)	October 1, 2020
BLA 761084-ORIG-01 (0005)	October 29, 2020
BLA 761084-ORIG-01 (0036)	November 29, 2021

Application Submission Background

Reviewer’s Comment: For Information - The original submission and the amendment sequence 0002 received on October 1, 2020, and amendment sequence 0005 on October 29, 2020 were reviewed in this Microbiology and Facility IQA. Additionally, the following amendments submitted in this resubmission, sequence 0036 was reviewed during this review cycle from a Microbial and Facility Standpoint.

MODULE 3.2.S

Module 3.2.S Lifecycle Management Considerations

Lifecycle considerations:	No
Post-approval inspection?	No

S.1 General Information

TPI-120 (Pegfilgrastim) is a modified form of rhG-CSF produced by covalent conjugation of a 20 kDa monomethoxypolyethylene glycol (mPEG) molecule to the N-terminal methionine residue of the polypeptide chain of filgrastim, produce by *Escherichia coli* (*E. coli*), and as a proposed biosimilar to US-licensed Neulasta®.

Reviewer’s Comment: For Information

S.2 Manufacture

S.2.1 Manufacturer(s)

Facility name and address	FEI	Responsibilities
Kashiv BioSciences, LLC 3440 S. Dearborn Street Suite 300, Chicago, IL, USA, 60616	3011289655	Manufacturing and Storage of MCB, WCB and DS; DS In-Process, Release and Stability Testing; Raw Material and components Storage and Testing; DS Shipping (b) (4)
(b) (4)		Extractables and Leachables Testing for DS.
(b) (4)		Microorganisms Identification.
(b) (4)		Master Cell Bank and Working Cell Bank storage.

(b) (4)	Cell Banks characterization and DNA testing• End of production cell bank testing.
	Host Cell DNA and Kanamycin testing. (b) (4)
	Microbiological (Bioburden) testing - In-Process control samples; IP and DS release raw material Testing.
	Testing for (b) (4) as a part of DS release.

Reviewer's Evaluation: The manufacturing facilities of the Drug Substance were adequately described.

Assessment: SATISFACTORY

S.2.2 Description of the Manufacturing Process and Process Controls

Reviewer's Evaluation: There have been no changes in the current resubmission (Sequence 0036, November 29, 2021) regarding the Description of the Manufacturing Process and Process Controls from the previously reviewed original submission (Sequence 0001, August 11, 2020).

Assessment: SATISFACTORY

Batch and Scale Definition

Reviewer's Evaluation: See comment in the Reviewer's Evaluation for the Description of the Manufacturing Process and Process Controls.

Assessment: SATISFACTORY

Description of Process Steps/Process Overview

Reviewer's Evaluation: See comment in the Reviewer's Evaluation for the Description of the Manufacturing Process and Process Controls.

Assessment: SATISFACTORY

Cell Culture and Harvest



Reviewer's Evaluation: See comment in the Reviewer's Evaluation for the Description of the Manufacturing Process and Process Controls.

Assessment: SATISFACTORY

Purification and Formulation

Reviewer's Evaluation: See comment in the Reviewer's Evaluation for the Description of the Manufacturing Process and Process Controls.

Assessment: SATISFACTORY

Bulk Drug Substance (BDS)

Reviewer's Evaluation: See comment in the Reviewer's Evaluation for the Description of the Manufacturing Process and Process Controls.

Assessment: SATISFACTORY

Shipping

Reviewer's Evaluation: See comment in the Reviewer's Evaluation for the Description of the Manufacturing Process and Process Controls.

Assessment: SATISFACTORY

Reprocessing

Reviewer's Evaluation: See comment in the Reviewer's Evaluation for the Description of the Manufacturing Process and Process Controls.

Assessment: SATISFACTORY

Hold Times and Conditions

Reviewer's Evaluation: See comment in the Reviewer's Evaluation for the Description of the Manufacturing Process and Process Controls.

Assessment: SATISFACTORY

S.2.3 Control of Materials

Reviewer's Evaluation: There have been no changes in the current resubmission (Sequence 0036, November 29, 2021) regarding the Control of Materials from the previously reviewed original submission (Sequence 0001, August 11, 2020).

Assessment: SATISFACTORY

S.2.4 Control of Critical Steps and Intermediates

Microbial Controls

Reviewer's Evaluation: *There have been no changes in the current resubmission (Sequence 0036, November 29, 2021) regarding the Control of Critical Steps and Intermediates from the previously reviewed original submission (Sequence 0001, August 11, 2020).*

Assessment: SATISFACTORY

In-Process Microbial Methods

Reviewer's Evaluation: *There have been no changes in the current resubmission (Sequence 0036, November 29, 2021) regarding the Control of Critical Steps and Intermediates from the previously reviewed original submission (Sequence 0001, August 11, 2020).*

Assessment: SATISFACTORY

S.2.5 Process Validation and Evaluation

Process Performance Qualification (PPQ)

Reviewer's Evaluation: *There have been no changes in the current resubmission (Sequence 0036, November 29, 2021) regarding the Process Validation and Evaluation from the previously reviewed original submission (Sequence 0001, August 11, 2020).*

Assessment: SATISFACTORY

Hold Time Qualification

Reviewer's Evaluation: *There have been no changes in the current resubmission (Sequence 0036, November 29, 2021) regarding the Process Validation and Evaluation from the previously reviewed original submission (Sequence 0001, August 11, 2020).*

Assessment: SATISFACTORY

Chromatography (b) (4) *Validation*

Reviewer's Evaluation: *There have been no changes in the current resubmission (Sequence 0036, November 29, 2021) regarding the Process Validation and Evaluation from the previously reviewed original submission (Sequence 0001, August 11, 2020).*

Assessment: SATISFACTORY

Shipping Qualification

Reviewer's Evaluation: *There have been no changes in the current resubmission (Sequence 0036, November 29, 2021) regarding the Process Validation and Evaluation from the previously reviewed original submission (Sequence 0001, August 11, 2020).*

Assessment: SATISFACTORY

S.4 Control of Drug Substance

S.4.1 Specifications

(b) (4)

Reviewer's Evaluation: *There have been no changes in the current resubmission (Sequence 0036, November 29, 2021) regarding the Drug Substance Specification from the previously reviewed original submission (Sequence 0001, August 11, 2020).*

Assessment: SATISFACTORY

S.4.2 Analytical Procedures

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Reviewer's Evaluation: *There have been no changes in the current resubmission (Sequence 0036, November 29, 2021) regarding the Bioburden and Endotoxin Analytical Procedures from the previously reviewed original submission (Sequence 0001, August 11, 2020).*

Assessment: SATISFACTORY

S.4.3 Validation of Analytical Procedures

Reviewer's Evaluation: *There have been no changes in the current resubmission (Sequence 0036, November 29, 2021) regarding the Validation of the Bioburden and Endotoxin Analytical Procedures from the previously reviewed original submission (Sequence 0001, August 11, 2020).*

Assessment: SATISFACTORY

S.4.4 Batch Analyses

Reviewer's Evaluation: *There have been no changes in the current resubmission (Sequence 0036, November 29, 2021) regarding the Batch Analyses from the previously reviewed original submission (Sequence 0001, August 11, 2020).*

Assessment: SATISFACTORY

S.4.5 Justification of Specification

Reviewer's Evaluation: *There have been no changes in the current resubmission (Sequence 0036, November 29, 2021) regarding the Justification of Specification from the previously reviewed original submission (Sequence 0001, August 11, 2020).*

Assessment: **SATISFACTORY**

S.6 Container Closure System

Reviewer's Evaluation: *There have been no changes in the current resubmission (Sequence 0036, November 29, 2021) regarding the Container Closure System from the previously reviewed original submission (Sequence 0001, August 11, 2020).*

Assessment: **SATISFACTORY**

S.7 Stability

Reviewer's Evaluation: *There have been no changes in the current resubmission (Sequence 0036, November 29, 2021) regarding the Stability from the previously reviewed original submission (Sequence 0001, August 11, 2020).*

Assessment: **SATISFACTORY**

FACILITY ASSESSMENT

(b) (4)





Michael
Shanks

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Broughton

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**PRODUCT QUALITY MICROBIOLOGY/FACILITY
ASSESSMENT**

Memorandum of Review to the File

Application ID	BLA 761084
Submission Type	Original BLA
Drug Product Name	(b) (4) (TPI-120, pegfilgrastim-pbbk)
Strengths	6 mg/0.6 mL
Dosage Form	Sterile solution for injection
Administration Route	Subcutaneous
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 clinically significant incidence of febrile neutropenia
Applicant Name	Kashiv BioSciences, LLC
US License Number	2068
Application Type	351 (k)
Primary Reviewer	Yarery Smith, Ph.D.
Secondary Reviewer	Candace Gomez-Broughton, Ph.D..
Submission date	08/11/2020
Goal Date	08/21/2021

Recommendation for Approvability:

- This BLA was reviewed from a sterility assurance perspective and **is NOT recommended for approval.**
- Manufacturing Facility Assessment Recommendation: Withhold (See Drug Substance Review)
- Product quality aspects not related to microbial control and facilities should be reviewed

Deficiencies:

1. Information regarding media fill studies is inadequate. Please update Section P.3.5 of the BLA with the following:
 - a. Summarized results (media fill date, container closure, filled volume, duration, number of units filled/incubated/rejected, positive) from the three initial media fill validation runs and the latest requalification run that were performed to validate the syringe line filling process relevant to the drug product.
 - b. Description of the hold periods (date, temperature, duration) simulated in each media fill run.
 - c. Description of confirmatory growth promotion test. Include a list of microorganisms used in the test.

3. The bacterial retention study for the sterilizing-grade filter was performed using the drug substance, which is not adequate. Please update BLA section P.3.5 with the following:
 - a. Protocol and data from the validation studies using three different lots of the sterilizing filter intended for commercial production using the final drug product solution.
 - b. Study/report # and the date of the study.
 - c. Comparison of validation test parameters with those used during routine operation (i.e., temperature, filtration time, filtration pressure, flow volume, and flow rate, etc.)
 - d. Description of the challenge microorganism, membrane lot numbers, pore size rating, pre- and post-filtration bubble point, challenge (CFU/cm²).
 - e. Demonstration of viability of the challenge organism in the presence of drug product.

Summary Basis of Recommendation (DP):

All sterile drug product-contact equipment and components are sterilized and depyrogenated using validated processes. (b) (4)

[Redacted]

Container closure integrity testing using a validated method is included in the stability program. The applicant needs to provide additional information and data to support the sterility assurance during the validation processes and the commercial manufacturing of the subject drug product.

List Submissions being assessed:

Document Description (SD #)	Date Received
Original-1 (1)	08/11/2020
Original-1-Amend-2 (2)	10/01/2020
Original-1-Amend-2 (16)	04/23/2021

List of DMFs assessed:

DMF	DMF Holder	Subject of DMF	Relevant information reviewed in:	Status
		(b) (4)	D27555M03R01.docx	Adequate
			D21723M10R01.docx and D21723M10R01.docx	Adequate
			D13014M09R01.docx	Adequate

Application Submission Background

Reviewer's Comment: For Information

The subject drug product, TPI-120, is the code name used by Kashiv BioSciences, LLC (formerly Adello Biologics) for the biosimilar molecule to Neulasta. Neulasta is a prescription medication from Amgen approved to reduce the risk of infection in some cancer patients receiving chemotherapy. The applicant has also submitted information for sister application BLA 761082. The drug products for BLA 761084 and 761082 are manufactured at the

(b) (4)

MODULE 1

1.14 LABELING

The drug product (DP) is provided as a single dose in a pre-filled syringe (PFS) presentation, which is assembled with a needle guard.

The instructions in the package insert recommend the following: Store refrigerated between 36°F to 46°F (2 to 8°C) in the carton to protect from light. Do not shake. Discard syringes stored at room temperature for more than 72 hours. Avoid freezing; if frozen, thaw in the refrigerator before administration. Discard syringe if frozen more than once.

Reviewer's Comment: The storage of the drug product in the original primary container closure system recommended in the labeling is deemed adequate. No studies are needed to support the label as written, as the drug product is not reconstituted or diluted for administration.

P.1 Description and Composition of the Drug Product

The drug product is a clear, colorless to slightly yellow, preservative-free solution supplied in a prefilled 1 mL single-dose syringe.

P.2 Pharmaceutical Development

(b) (4)

(b) (4)



P.7 Container Closure System

Reviewer's Evaluation: See Section P.2

Assessment: **SATISFACTORY**

P.8 Stability

	Test method	Testing Interval (Months)						
		0	3	6	9	12	18	24
Endotoxins	STM-0005	x						x
Sterility	STM-0186	x						x
CCIT*	STM-0210					x		

* Container Closure Integrity Test

The DP proposed shelf life of 24 months at 5 °C. The applicant indicates that the proposed shelf life is based on up to 24 months of stability data at the intended storage condition of 5°C.

Reviewer's note: The CCIT was said to be scheduled for testing only at 12-month but not 24-month time-point; [REDACTED] (b) (4)

[REDACTED] deficiency will be conveyed to the applicant for the subject BLA.

Reviewer's Evaluation: The stability protocol includes container closure integrity (CCI) testing only at the 12-month time-point. Revise the stability protocol to include CCIT for stability samples every 12 months until expiry. Please update the BLA accordingly.

Assessment: NOT SATISFACTORY

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**PRODUCT QUALITY MICROBIOLOGY/FACILITY
ASSESSMENT**

Memorandum of Review to the File

Application ID	BLA 761084
Submission Type	Original BLA
Drug Product Name	Pegfilgrastim
Strengths	6 mg/0.6 mL
Dosage Form	injection
Administration Route	subcutaneous
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 clinically significant incidence of febrile neutropenia
Applicant Name	Kashiv BioSciences, LLC
US License Number	2131
Application Type	351 (k)
Primary Reviewer	Michael Shanks
Secondary Reviewer	Candace Gomez-Broughton
Goal Date	08/10/2021

Recommendation for Approvability:

- This BLA was reviewed from a product quality microbiology perspective and sterility assurance perspective and is recommended for Approval.
- Manufacturing Facility Assessment Recommendation: Withhold
- Product quality aspects not related to microbial control and facilities should be reviewed by OBP.

Summary Basis of Recommendation (DS):

Overall, the process is under adequate microbial control. (b) (4)



Adequate controls are in place to maintain microbiological product quality during maximum hold periods and throughout the manufacturing process.

Adequate descriptions of the facilities, equipment, environmental controls, cleaning and contamination control strategy were provided for Kashiv Biosciences LLC (FEI 3011289655), proposed for Drug Substance manufacture. With the exception of the Drug Substance manufacturing site, Kashiv Biosciences LLC, all other Drug Substance related facilities are acceptable based on their current CGMP compliance status and recent relevant inspectional coverage.

Previously, the drug substance manufacturing site, Kashiv Bioscience LLC has had the following pre-license inspections:

The first round of the application review, BLA 761082-ORIG-1 (Sequence 0001), had a Complete Response issued on 05/10/2018, in part, to a withhold decision for the Kashiv Biosciences LLC facility, as well as for Drug Substance deficiencies from a microbial control and microbiology product quality perspective. The two inspections of Kashiv Biosciences conducted 11/13-21/2017 and 01/22-26/2018 during the first review cycle resulted in a Withhold decision based on the following:

1. Inadequate CAPAs to address issues identified during deviation and OOS investigations.
2. Inadequate written procedures for performance of deviation investigations.
3. Inadequate impact assessment of consequences of deviations and OOS events on product quality and submitted data.
4. There was no evidence that changes to revised and new SOPs written in response to the 483 observations had been conveyed to employees, and that employees had been trained in the new procedures.

The second round of application review, BLA 761082-ORIG-1-RESUB-38 (Sequence 0038), had a Complete Response issued on 06/11/2019, in part, to a withhold decision for the Kashiv Biosciences LLC facility. Following the 03/04-12/2019 PLI of the Kashiv Biosciences DS site conducted in support of the subject resubmission, deficiencies were identified for the following:

1. QA oversight of dispositioning and analytical methods.
2. Deviation management.
3. Change control management.
4. Adherence to written procedures.
5. Adequacy of written procedures.
6. Adequacy of analytical methods.
7. Robustness of manufacture.

The third round of the application review, BLA 761082-ORIG-1-RESUB-49 (Sequence 0049), concludes with a withhold decision for the Kashiv Biosciences LLC facility. This decision is based on the Withhold decision from the 03/04-12/2019 Pre-license inspection of the Kashiv Biosciences Drug Substance site conducted in support of the previous resubmission. An inspection of the Kashiv Biosciences LLC DS site (FEI 3011289655), Chicago, Illinois, facility is required before this application can be approved as the FDA must assess the ability of that facility to conduct the listed manufacturing operations in compliance with CGMP. Due to U.S. Government and/or Agency-wide restrictions on travel, we were unable to conduct an inspection of the Kashiv Biosciences LLC facility during the current review cycle, and the application cannot be approved until the required FDA inspection is conducted and the findings are assessed with regard to this application. **In this current review cycle, the Kashiv Biosciences LLC DS site (FEI 3011289655), Chicago, Illinois, facility has a withhold recommendation.**

Drug Substance CQA Process Risk Identification and Lifecycle Knowledge Management:

CQA (type)	Risk	Origin	Control Strategy	Other
Endotoxin	Safety, Purity	Raw materials, manufacturing process	(b) (4)	

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

List Submissions being assessed (Table):

Document Description (SD #)	Date Received
BLA 761084-ORIG-01 (0001)	August 11, 2020
BLA 761084-ORIG-01 (0002)	October 1, 2020
BLA 761084-ORIG-01 (0005)	October 29, 2020

Application Submission Background

Reviewer's Comment: For Information - The original submission and the amendment sequence 0002 received on October 1, 2020, and amendment sequence 0005 on October 29, 2020 were reviewed in this Microbiology and Facility IQA.

MODULE 3.2.S

Module 3.2.S Lifecycle Management Considerations

Lifecycle considerations:	No
Post-approval inspection?	No

S.1 General Information

TPI-120 (Pegfilgrastim) is a modified form of rhG-CSF produced by covalent conjugation of a 20 kDa monomethoxypolyethylene glycol (mPEG) molecule to the N-terminal methionine residue of the polypeptide chain of filgrastim, produce by *Escherichia coli* (*E. coli*), and as a proposed biosimilar to US-licensed Neulasta®.

Reviewer's Comment: For Information

S.2 Manufacture

S.2.1 Manufacturer(s)

(b) (4)



**DIVISION OF DRUG DELIVERY, GENERAL HOSPITAL & HUMAN FACTORS
INTERCENTER CONSULT MEMORANDUM – PRE-FILLED SYRINGES**

Date	4/20/2022		
To:	Andrew Shiber		
Requesting Center/Office	CDER-OPQ	Clinical Review Division	Other
From	Porsche Bennett OPEQ/OHT3/DHT3C		
Through (Team)	Courtney Evans, Team Lead, Injection Devices Team OPEQ/OHT3/DHT3C		
Subject	ICCR: 00805454 ICC: 2100990 Submission: BLA761084 Sponsor: KASHIV BIOSCIENCES, LLC Drug/Device: TPI-120 Indications for Use: Decrease the incidence of infection, as manifested by febrile neutropenia, in patients with non-myeloid malignancies receiving myelosuppressive anti-cancer drugs associated with a clinically significant incidence of febrile neutropenia		
Recommendation	Final Recommendation: ☒ Device Constituent Parts of the Combination Product are Approvable. ☐ Device Constituent Parts of the Combination Product are Approvable with the following Post-Market Requirements/Commitments, ☐ Device Constituent Parts of the Combination Product are Not Approvable with the following CR Deficiencies Comments to Review Team: N/A		

Digital Signature Concurrence Table		
Reviewer	Team Lead (TL)	Division (*Optional)

1. PURPOSE

This review provides an assessment of the needle safety device constituent part **Error! Bookmark not defined.** of the prefilled syringe product.

This review will cover the following review areas:

- Device performance of combination product
- Stability – device performance on stability
- Essential Performance Requirements (EPR) Control strategy

CDRH Quality Systems Assessment / Facilities consult not required per internal MAP 5017.7

It was determined that a device quality systems / facilities assessment is not required for this product because the product is not an emergency (i.e., life-saving and essential¹) treatment that are administered by non-health care professionals.

Note: The initial review (ICC2100789) of this submission found adequacy in device performance, stability and EPRS/control strategy for all areas except the following:

1. Activation force testing at T=0 and over self-life
2. Needle safety override testing over shelf life

Therefore, the focus on this review (ICC2100990) will focus on the inadequacies identified in the CR letter. Updated information included in this memo to assess needle safety has been highlighted in yellow.

2. BACKGROUND

This review is for a resubmission of BLA 761084 that was issued a complete response (CR) on 8/11/2021. The initial CDRH review was ICC2100789, ICCR00027305. FDA issued a CR letter that included 13 deficiencies within the following review areas: microbiology, product quality, **CDRH (CR#9)**, prescribing information, carton and container labeling, proprietary name, and facilities inspection. Additionally, the letter included 8 safety update requests and 31 additional comments within the following review areas: manufacture and control, control of raw materials, specifications, method validation, reference standards, container closure systems, manufacture development and control, stability, shipping validation, filter validation, immunogenicity, and microbiology.

The firm submitted a Type-A/Type 1 meeting request, ICC2100813 with CDRH response completed under ICCR00788539, completed October 2021. Therefore, this review will be a continuation of the initial review, ensuring that the firm submitted the additional data as agreed in the type 1 meeting to respond to the CR deficiency (#9). CR deficiency #9 requests activation force data for T=0 and over shelf as well as needle safety override force data to support shelf life.

Review of responses to all other CR deficiencies and additional data are out of scope of this review.

CR Deficiency 9:

You provided a design verification (DV) plan (DCP-0037), DV protocol (PTL-1712), and T=0, 6, 24, and 36 month reports, as well as a ^{(b)(4)} safety device verification summary (DCP-0025) which were assessed for adequacy of testing to support verification of the device and the proposed shelf life. However, you did not provide adequate data for needle safety performance to support verification of the design and the proposed shelf life for the following reasons:

- 1. In the Prefilled Syringe Design Verification Protocol, PTL-1712, section 6.6 details the deliverable volume and automatic safety device activation. 30 samples were tested for deliverable volume and activation force*

¹ Examples of emergency, life-saving and essential treatments include those used for conditions such as anaphylaxis or cardiac arrest and others in which failure of drug delivery may expose the patient to the reasonable likelihood of serious injury or death.

sequentially and another 30 samples were solely tested for activation force. However, automatic safety device activation was conducted for an attribute method and as such quantitative data was not measured to verify that the activation forces met specification. As such, the acceptance criteria for activation force testing as detailed in section 6.6 is inadequate.

Section 6.9 details how you will test safety device activation force using a variable method. However, in section 6.9.2.2-6.9.2.3 of the protocol, it is stated to (b) (4) " which is not a representative method of forces that would be required to activate the needle guard. As such, the method for testing activation force as detailed in section 6.9 is inadequate.

2. You reference DCP-0025, attachment 1 to demonstrate that needle safety activation force meets a 95% confidence level / 99% reliability criteria. While your firm's vendor, (b) (4) appears to have tested activation force to demonstrate a confidence and reliability of 95/99%, the testing does not appear to have been conducted with the to-be-marketed configuration of TPI-120 containing the TPI-120 drug solution. Since the activation force may be impacted by the pre-filled syringe glide force, this testing must be conducted on the final finished combination product. Additionally, AQL testing represents T=0 and does not assess performance of aged product. As such, you have not provided sufficient data to support a confidence and reliability of 95/99% for needle safety activation force to support verification of the design and the proposed shelf life.

3. You reference (b) (4) testing to demonstrate control of needle safety override force over shelf life. However, while (b) (4) conducted testing on the needle safety feature, which includes compression force testing, the testing appears to only be conducted for T=0. Additionally, while (b) (4) performs AQL testing before releasing each lot in which Kashiv performs verification of Certificate of Conformance, lot release testing also represents only T=0 timepoint. Thus, you have not provided adequate data to support needle safety override performance over the proposed shelf life.

Per FDA's Guidance for Industry and FDA Staff "Medical Devices with Sharps Injury Prevention Features" bench testing should be conducted to assess the force to activate and deactivate the safety feature (i.e., activation force and override force). As such, the following data is required to support design verification and the proposed shelf life of TPI-120:

1. Provide design verification and shelf life testing to demonstrate needle safety activation force performance meets specification. Please ensure forces are evaluated after sequential shelf-life, shipping and drop/freefall, with final finished combination product, utilizing a variable method that is representative of forces that a user would experience to activate the needle guard (i.e., measured after depressing the drug product out of the syringe), meeting a confidence and reliability of 95/99%.
2. Provide test data for needle safety override force over shelf life.

Reviewed material includes the following:

- Sequence 036
 - Section 1.2 Cover letter
 - Section 1.11.4 Multiple Module Information Amendment
 - Multiple Module Information Amendment- 0036
 - Section 3.2.P.7 Container Closure System
 - PTL- 2467: Retrospective Evaluation of Device Functional Verification of TPI-120 Drug Product Assembled with Ultrasafe Safety Device

- PTL-R- 2467: Retrospective Evaluation Report of Device Functional Verification of TPI-120 Drug Product Assembled with Ultrasafe Safety Device

3. DEVICE DESCRIPTION

TPI-120 is intended to be a biosimilar to Neulasta. The TPI-120 combination product is a single-use prefilled syringe (PFS) filled with 6mg/0.6mL drug product. The combination product is individually packaged in a blister pack. The primary container is a (b) (4) glass syringe barrel with a staked needle. The 1mL clear, (b) (4) glass syringe barrel and rubber plunger stopper are both off-the-shelf components.



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4. DEVICE PERFORMANCE REVIEW

4.1. Design Verification & Validation

Performance Requirement	Specification	Verification Method Acceptable (Y/N)	Shelf-life (Y/N)	Shipping/Transportation (Y/N)	Drop/Free Fall Testing (Y/N)
Needle Safety Activation force	(b) (4)			ASTM 4169 Y	11608-5
Needle safety lockout force/override force/safe mode challenge				ASTM 4169 Y	Y-Refer to IR#6-adequately addressed
Needle safety Access in safe mode				N/A	N/A
Safety device separation force				ASTM 4169 Y	N-Refer to IR#6
Dose Accuracy				ASTM 4169 Y	11608-5
Break loose Force					11608-5
Glide Force					11608-5
Cap Removal Force				ASTM 4169 Y	N-Refer to IR#6-adequately addressed
Packaging visual inspection				ASTM 4169 Y	N-Refer to IR#6

	(b) (4)		
Device visual inspection		ASTM 4169 Y	N-Refer to IR 6- adequately addressed
CCI		ASTM 4169 Y	11608-5

Validation Method: Note that specifications were validated for meeting users needs by simulated clinical use (human factors studies). HF studies will be assessed by DMEPA.

ISO23908 -2011 states the following shall be included in the test report:

- identity of the device;
- test equipment description;
- the safety mechanism activation force;
- the force used to challenge the safe mode (overriding force);
- test results for verifying access to the sharps in safe mode.

The samples were stored for at least 4 hours under conditions prior to evaluation per ISO23908.

This review covered biocompatibility assessment of the plunger rod and safety device components as highlighted in the tables below. The firm intends to leverage the supplier (b) (4) cytotoxicity, sensitization, and irritation biocompatibility assessments. The referenced (b) (4) 510K does not include biocompatibility assessment of a (b) (4) plunger rod, but a (b) (4) green plunger rod. As such, refer to Information Request # 5. The information request has been resolved.

Table 14. Biocompatibility Assessment of PFS Components

Component	Material	Biocompatibility Compliance	Assessment or Testing By
(b) (4) Syringe Barrel Assembly	(b) (4)	NAMSA Material Assessment	NAMSA
		ISO 10993-5, 10993-10, 10993-11, 10993-6	
		NAMSA Material Assessment	
		ISO 10993-5, 10993-11, 10993-10, 10993-6, 10993-4	
		ISO 10993-5, 10993-11, 10993-10	
Plunger	(b) (4)	NAMSA Material Assessment	(b) (4)
		USP <381> (b) (4) closure	
Plunger Rod	(b) (4)	ISO 10993-1:2009 skin surface, limited exposure	(b) (4)
Safety Device	(b) (4)	ISO 10993-1:2009 skin surface, limited exposure	(b) (4)

Table 15. Manufacturer Reference Files for Verification of Component Requirements

Component	Supplier	Reference File
Syringe Barrel Assembly	(b) (4)	(b) (4)
Plunger Stopper		
Plunger Rod		
Safety Device		

Reviewer Comments:

Summary of testing (per DCP-0037):

The sponsor has conducted T=0 testing and T=6, 12, 24, and 36 months per the flow charts below to support performance testing and a proposed shelf life of 24 months.



DIVISION OF DRUG DELIVERY, GENERAL HOSPITAL & HUMAN FACTORS
INTERCENTER CONSULT MEMORANDUM – MEETING REQUEST

Date	10/18/2021		
To:	Kelly Ballard		
Requesting Center/Office	CDER/OPQ	Clinical Review Division	Other
From	Porsche Bennett OPEQ/OHT3/DHT3C		
Through (Team)	Suzanne Hudak, Team Lead, Injection Team OPEQ/OHT3/DHT3C		
Through (Division) *optional	CPT Alan Stevens, Assistant Director, Injection Team OPEQ/OHT3/DHT3C		
Subject	BLA761084, TPI-120 ICC2100813 ICCR00788539		
Recommendation	Recommendation Date: 10/18/2021 ☒ CDRH has provided responses to the Sponsor questions ☒ CDRH has additional comments for the Sponsor ☐ CDRH has comments for the review division		

Digital Signature Concurrence Table		
Reviewer	Team Lead (TL)	Division (*Optional)

1. SUBMISSION OVERVIEW

Submission Information	
Submission Number	BLA761084
Sponsor	KASHIV BIOSCIENCES, LLC
Drug/Biologic	TPI-120
Indications for Use	TPI-120, decrease the incidence of infection, as manifested by febrile neutropenia, in patients with non-myeloid malignancies receiving myelosuppressive anti-cancer drugs associated with a clinically significant incidence of febrile neutropenia
Device Constituent	Pre-Filled Syringe
Related Files	ICC2000789, CR letter issued 8/11/2021 (DAARTS: Communication PK 4839703)

Review Team		
Lead Device Reviewer	<i>Porsche Bennett</i>	
Discipline Specific Consults	Reviewer Name (Center/Office/Division/Branch)	CON #
N/A	N/A	N/A

Important Dates	
Discipline-Specific Review Memos Due	N/A
Final Lead Device Review Memo Due	10/11/2021
Interim Due Dates	Meeting/Due Date
Internal Meeting	09/30/2021
Pre-Meeting Feedback Due	10/07/2021
Sponsor Meeting or WRO due	10/07/2021

2. FINAL COMMENTS/RECOMMENDATION

2.1. Preliminary Feedback for the Sponsor



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3. PURPOSE/BACKGROUND

3.1. Scope

KASHIV BIOSCIENCES, LLC is requesting approval of TPI-120. The device constituent of the combination product is a Pre-Filled Syringe.

CDER/OPQ has requested the following [consult](#) for review of the device constituent of the combination product:

A Type A meeting request was submitted in response to a CR. Question 9 on the meeting package needs CDRH input. The internal meeting is on 9/30 and the sponsor meeting is on 10/7.

The goal of this memo is to provide a response to the following questions in the meeting package:

CR 9 deficiency response.

This review will not cover the following questions:

CR 2, 3, 4, 5 and additional comments 5, 6, 18, 23 deficiency responses.

3.2. [Prior Interactions](#)

A complete response letter was issued to the firm on 8/11/2021. The letter included 13 deficiencies, 8 safety updates and 31 additional comments. CDRH issued 1 CR deficiency, CR #9. The firm now provided responses to the CR letter. The goal of this memo is to provide a response to the firm’s CR deficiency 9 response.

Prior to issuance of CR#9, CDRH issued 1- 74 day letter Information Request (IR), and 9 post mid-cycle IRs. IR 1, 7, 8 and 9 responses were inadequate and resulted in a CR#9.

V9.23.2019

Refer to ICC2000879, ICCR00027305 for complete CDRH review memo. The complete memo can also be found in the Appendix of this memo.

3.2.1. Related Files

ICC2000879, ICCR00027305

3.3. Indications for Use

Combination Product	Indications for Use
TPI-120	TPI-120, decrease the incidence of infection, as manifested by febrile neutropenia, in patients with non-myeloid malignancies receiving myelosuppressive anti-cancer drugs associated with a clinically significant incidence of febrile neutropenia
Pre-Filled Syringe	Delivery of the Drug Product

3.4. Materials Reviewed

Materials Reviewed	
Sequence	Module(s)
0035 (\\CDSESUB1\evsprod\bla761084\0035\m1\us\16-meetings\meeting-materials\meet-backgrd-materials-0035.pdf)	1.2 Cover letter
0035	1.6.1 Meeting Request
0035	1.6.2 Meeting Background Materials

4. DEVICE DESCRIPTION

4.1. Device Description

Refer to ICC2000789 (attached as an appendix to this memo).

4.1.1. *Essential Performance Requirements*

Refer to ICC2000789 (attached as an appendix to this memo).

4.2. Steps for Using the Device

Refer to ICC2000789 (attached as an appendix to this memo).

5. REVIEW OF QUESTIONS

CR Deficiency 9:

You provided a design verification (DV) plan (DCP-0037), DV protocol (PTL-1712), and T=0, 6, 24, and 36 month reports, as well as a ^{(b) (4)} safety device verification summary (DCP-0025) which were assessed for adequacy of testing to

support verification of the device and the proposed shelf life. However, you did not provide adequate data for needle safety performance to support verification of the design and the proposed shelf life for the following reasons:

- a. In the Prefilled Syringe Design Verification Protocol, PTL-1712, section 6.6 details the deliverable volume and automatic safety device activation. 30 samples were tested for deliverable volume and activation force sequentially and another 30 samples were solely tested for activation force. However, automatic safety device activation was conducted for an attribute method and as such quantitative data was not measured to verify that the activation forces met specification. As such, the acceptance criteria for activation force testing as detailed in section 6.6 is inadequate.
Section 6.9 details how you will test safety device activation force using a variable method. However, in section 6.9.2.2-6.9.2.3 of the protocol, it is stated to “ (b) (4) ” which is not a representative method of forces that would be required to activate the needle guard. As such, the method for testing activation force as detailed in section 6.9 is inadequate.
- b. You reference DCP-0025, attachment 1 to demonstrate that needle safety activation force meets a 95% confidence level / 99% reliability criteria. While your firm’s vendor, (b) (4) appears to have tested activation force to demonstrate a confidence and reliability of 95/99%, the testing does not appear to have been conducted with the to-be-marketed configuration of TPI-120 containing the TPI-120 drug solution. Since the activation force may be impacted by the pre-filled syringe glide force, this testing must be conducted on the final finished combination product. Additionally, AQL testing represents T=0 and does not assess performance of aged product. As such, you have not provided sufficient data to support a confidence and reliability of 95/99% for needle safety activation force to support verification of the design and the proposed shelf life.
- c. You reference (b) (4) testing to demonstrate control of needle safety override force over shelf life. However, while (b) (4) conducted testing on the needle safety feature, which includes compression force testing, the testing appears to only be conducted for T=0. Additionally, while (b) (4) performs AQL testing before releasing each lot in which Kashiv performs verification of Certificate of Conformance, lot release testing also represents only T=0 timepoint. Thus, you have not provided adequate data to support needle safety override performance over the proposed shelf life.

Per FDA’s Guidance for Industry and FDA Staff “Medical Devices with Sharps Injury Prevention Features” bench testing should be conducted to assess the force to activate and deactivate the safety feature (i.e. activation force and override force). As such, the following data is required to support design verification and the proposed shelf life of TPI-120:

- a. Provide design verification and shelf life testing to demonstrate needle safety activation force performance meets specification. Please ensure forces are evaluated after sequential shelf-life, shipping and drop/freefall, with final finished combination product, utilizing a variable method that is representative of forces that a user would experience to activate the needle guard (i.e. measured after depressing the drug product out of the syringe), meeting a confidence and reliability of 95/99%.
- b. Provide test data for needle safety override force over shelf life.

Question # 9a:

(b) (4)



Anshu
Rastogi

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Brian
Janelsins

Digitally signed by Brian Janelsins
Date: 4/15/2022 10:03:03PM
GUID: 54b8280e000855ed28b059d11c54a788



**DIVISION OF DRUG DELIVERY, GENERAL HOSPITAL & HUMAN FACTORS
INTERCENTER CONSULT MEMORANDUM – PRE-FILLED SYRINGES**

Date	7/22/2021		
To:	Charlene Wheeler		
Requesting Center/Office	CDER-OND/ORO	Clinical Review Division	Other
From	Porsche Bennett OPEQ/OHT3/DHT3C		
Through (Team)	Suzanne Hudak, Team Lead, Injection Devices Team OPEQ/OHT3/DHT3C		
Through (Division) *Optional	CAPT Alan Stevens, Assistant Director, Injection Devices Team OPEQ/OHT3/DHT3C		
Subject	ICCR: 00027305 ICC: 2000789 Submission: BLA761084 Sponsor: KASHIV BIOSCIENCES, LLC Drug/Device: TPI-120 Indications for Use: Decrease the incidence of infection, as manifested by febrile neutropenia, in patients with non-myeloid malignancies receiving myelosuppressive anti-cancer drugs associated with a clinically significant incidence of febrile neutropenia		
Recommendation	Final Recommendation: ☐ Device Constituent Parts of the Combination Product are Approvable. ☐ Device Constituent Parts of the Combination Product are Approvable with the following Post-Market Requirements/Commitments, ☒ Device Constituent Parts of the Combination Product are Not Approvable with the following CR Deficiencies		
	Comments to Review Team: N/A		
	PMC/PMR or CR Deficiencies: CR Deficiency 1: You provided a design verification (DV) plan (DCP-0037), DV protocol (PTL-1712), and T=0, 6, 24, and 36 month reports, as well as a (b) (4) safety device verification summary (DCP-0025) which were assessed for adequacy of testing to support verification of the device and the proposed shelf life. However, you did not provide adequate data for needle safety performance to support verification of the design and the proposed shelf life for the following reasons: In the Prefilled Syringe Design Verification Protocol, PTL-1712, section 6.6 details the deliverable volume and automatic safety device activation. 30 samples were tested for deliverable volume and activation force sequentially and another 30 samples were solely tested for activation force. However, automatic safety device activation was conducted for an attribute method and as such quantitative data was not measured to verify that the activation forces met specification. As such, the acceptance criteria for activation force testing as detailed in section 6.6 is inadequate. Section 6.9 details how you will test safety device activation force using a variable method. However, in section 6.9.2.2-6.9.2.3 of the protocol, it is stated to “ (b) (4)		

(b) (4), which is not a representative method of forces that would be required to activate the needle guard. As such, the method for testing activation force as detailed in section 6.9 is inadequate.

2. You reference DCP-0025, attachment 1 to demonstrate that needle safety activation force meets a 95% confidence level / 99% reliability criteria. While your firm's vendor, (b) (4) appears to have tested activation force to demonstrate a confidence and reliability of 95/99%, the testing does not appear to have been conducted with the to-be-marketed configuration of TPI-120 containing the TPI-120 drug solution. Since the activation force may be impacted by the pre-filled syringe glide force, this testing must be conducted on the final finished combination product. Additionally, AQL testing represents T=0 and does not assess performance of aged product. As such, you have not provided sufficient data to support a confidence and reliability of 95/99% for needle safety activation force to support verification of the design and the proposed shelf life.

3. You reference (b) (4) testing to demonstrate control of needle safety override force over shelf life. However, while (b) (4) conducted testing on the needle safety feature, which includes compression force testing, the testing appears to only be conducted for T=0. Additionally, while (b) (4) performs AQL testing before releasing each lot in which Kashiv performs verification of Certificate of Conformance, lot release testing also represents only T=0 timepoint. Thus, you have not provided adequate data to support needle safety override performance over the proposed shelf life.

Per FDA's Guidance for Industry and FDA Staff "Medical Devices with Sharps Injury Prevention Features" bench testing should be conducted to assess the force to activate and deactivate the safety feature (i.e. activation force and override force). As such, the following data is required to support design verification and the proposed shelf life of TPI-120:

1. Provide design verification and shelf life testing to demonstrate needle safety activation force performance meets specification. Please ensure forces are evaluated after sequential shelf-life, shipping and drop/freefall, with final finished combination product, utilizing a variable method that is representative of forces that a user would experience to activate the needle guard (i.e. measured after depressing the drug product out of the syringe), meeting a confidence and reliability of 95/99%.
2. Provide test data for needle safety override force over shelf life.

Digital Signature Concurrence Table

Reviewer	Team Lead (TL)	Division (*Optional)
Porsche P. Bennett -S Digitally signed by Porsche P. Bennett -S Date: 2021.07.22 14:37:46 -04'00'		Suzanne J. Hudak -S Digitally signed by Suzanne J. Hudak -S Date: 2021.07.22 15:38:17 -04'00'

1. PURPOSE

This review provides an assessment of the needle safety device constituent part **Error! Bookmark not defined.** of the prefilled syringe product.

This review will cover the following review areas:

- Device performance
- Stability – device performance on stability
- Essential Performance Requirements (EPR) Control strategy

CDRH Quality Systems Assessment / Facilities consult not required per internal MAP 5017.7

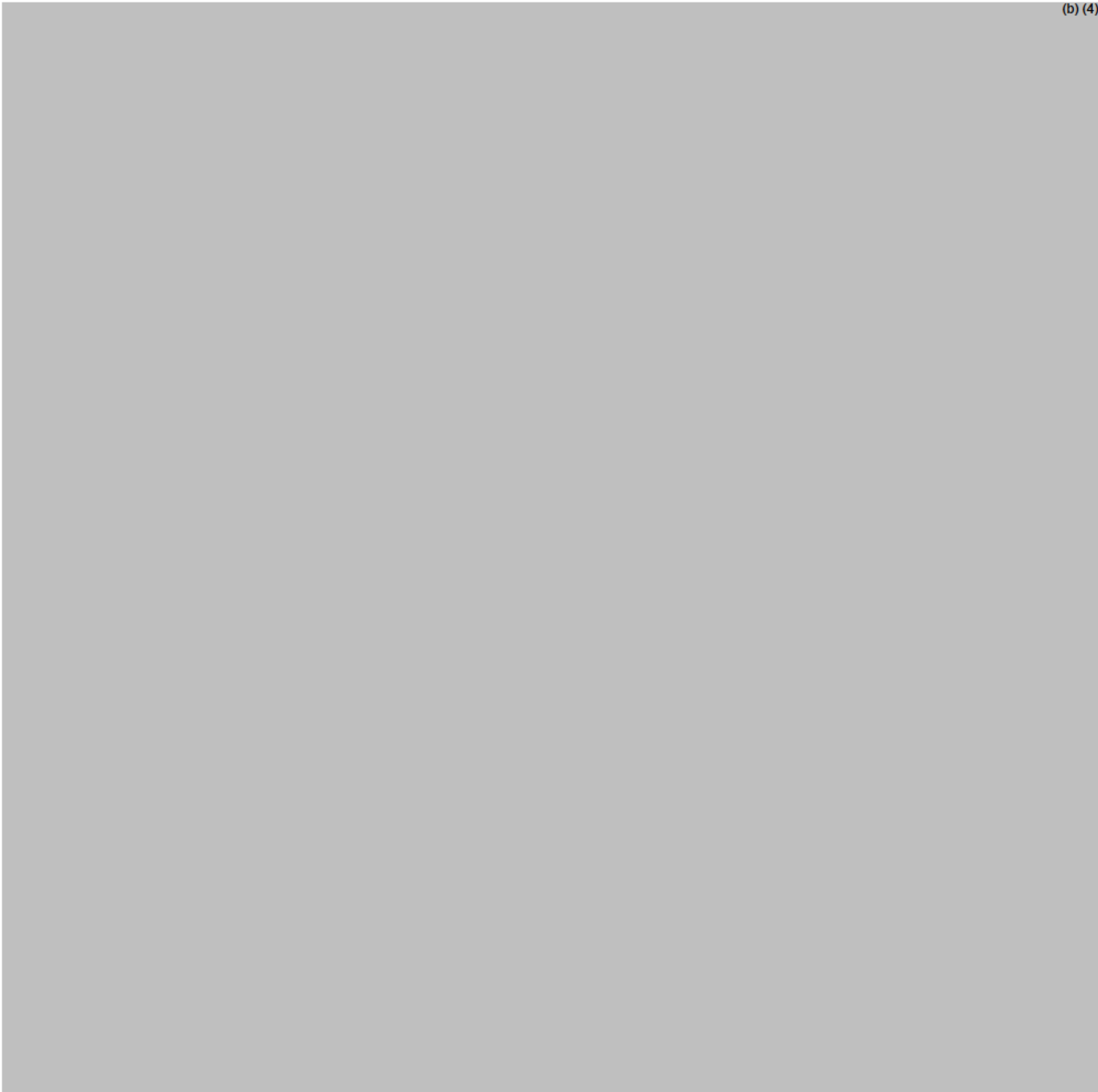
It was determined that a device quality systems / facilities assessment is not required for this product because the product is not an emergency (i.e., life-saving and essential¹) treatment that are administered by non-health care professionals.

2. DEVICE DESCRIPTION

TPI-120 is intended to be a biosimilar to Neulasta. The TPI-120 combination product is a single-use prefilled syringe (PFS) filled with 6mg/0.6mL drug product. The combination product is individually packaged in a blister pack. The primary container is a ^{(b) (4)} glass syringe barrel with a staked needle. The 1mL clear, ^{(b) (4)} glass syringe barrel and rubber plunger stopper are both off-the-shelf components.

3 Page(s) of Draft Labeling have been Withheld in Full as b4 (CCI/TS) immediately following this page

¹ Examples of emergency, life-saving and essential treatments include those used for conditions such as anaphylaxis or cardiac arrest and others in which failure of drug delivery may expose the patient to the reasonable likelihood of serious injury or death.
v08.06.2019



Inte profe disease state that impact device use)	environment or by a health care professional in a clinic or hospital setting. The device is single use and disposable. TPI-120 is intended for patients undergoing chemotherapy who may suffer from cognitive impairment affecting verbal memory, depression, and stroke, thus possibly impairing ability to follow and recall instructions. Patients might suffer from musculoskeletal weakness and/or neuropathy, leading to poor manual dexterity.
Injection Site	Front of thighs, lower stomach area, upper outer buttocks, and upper outer arms
Injection tissue and depth of injection	Subcutaneous tissue
Needle connection (e.g. luer, slip tip, staked)	Staked needle 27G 1/2"

Needle safety type (active or passive)	Passive needle guard
Delivered Dose Volume	6mg/0.6mL
Shelf-life/Storage, including excursions (e.g., 36months, 5C)	The proposed shelf life is 24 months. Testing included up to 36 months at 5C.
Hypodermic Needle: (length, gauge)	Stainless steel ½ inch, 27 gauge
Safety Features (e.g. Needle safety component/device)	^{(b) (4)} UltraSafe Plus clear passive needle guard that consists of a polycarbonate body with a stainless-steel spring

3. DEVICE PERFORMANCE REVIEW

3.1. Design Verification & Validation

Performance Requirement	Specification	Verification	Shelf-life	Shipping/Transportation (Y/N)	Drop/Free Fall Testing (Y/N)
Needle Safety Activation force	^{(b) (4)}			ASTM 4169 Y	11608-5
Needle safety lockout force/override force/safe mode challenge				ASTM 4169 Y	N-Refer to IR#6
Needle safety Access in safe mode				N/A	N/A
Safety device separation force				ASTM 4169 Y	N-Refer to IR#6
Dose Accuracy				ASTM 4169 Y	11608-5
Break loose Force					11608-5
Glide Force					11608-5

Cap Removal Force	(b) (4)	ASTM 4169 Y	N-Refer to IR#6
Packaging visual inspection		ASTM 4169 Y	N-Refer to IR#6
Device visual inspection		ASTM 4169 Y	N-Refer to IR 6
CCI		ASTM 4169 Y	11608-5

Validation Method: Note by simulated clinical use (human factors studies). HF studies will be

ISO23908 -2011 states the following shall be included in the test report:

- identity of the device;
- test equipment description;
- the safety mechanism activation force;
- the force used to challenge the safe mode (overriding force);
- test results for verifying access to the sharps in safe mode.

The samples were stored for at least 4 hours under conditions prior to evaluation per ISO23908.

This review cover biocompatibility assessment of the plunger rod and safety device components as highlighted in the tables below. The firm intends to leverage the supplier (b) (4) cytotoxicity, sensitization, and irritation biocompatibility

assessments. The referenced (b)(4) 510K does not include biocompatibility assessment of a white plunger rod, but a lime green plunger rod. As such, refer to Information Request # 5. The information request has been resolved.

Table 14. Biocompatibility Assessment of PFS Components

Component	Material	Biocompatibility Compliance	Assessment or Testing By
(b)(4) Syringe Barrel Assembly	(b)(4)	NAMSA Material Assessment	(b)(4)
		ISO 10993-5, 10993-10, 10993-11, 10993-6	
		NAMSA Material Assessment	
		ISO 10993-5, 10993-11, 10993-10, 10993-6, 10993-4	
		ISO 10993-5, 10993-11, 10993-10	
		NAMSA Material Assessment	
Plunger		USP <381> Type I closure	
Plunger Rod		ISO 10993-1:2009 skin surface, limited exposure	
Safety Device		ISO 10993-1:2009 skin surface, limited exposure	

Table 15. Manufacturer Reference Files for Verification of Component Requirements

Component	Supplier	Reference File
Syringe Barrel Assembly	(b)(4)	(b)(4)
Plunger Stopper		
Plunger Rod		
Safety Device		

Reviewer Comments:

Summary of testing (per DCP-0037):

The sponsor has conducted T=0 testing and T=6, 12, 24, and 36 months per the flow charts below to support performance testing and a proposed shelf life of 24 months.

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BLA 761084
Non-proprietary Name: pegfilgrastim-pbbk
A proposed biosimilar to US-licensed Neulasta
Applicant: Kashiv BioSciences

Anshu Rastogi, Ph.D., Primary Reviewer
Brian Janelins, Ph.D., Secondary Reviewer, ATL

Division of Biotechnology Review and Research I
Office of Biotechnology Products (OBP)
Office of Pharmaceutical Quality (OPQ)
Center for Drug Evaluation and Research (CDER)

OBP Product Quality Assessment Data Sheet

1. BLA: 761084

2. Assessment Date: June 25, 2021

3. Primary Assessment Team:

Medical Officer: Hyon-Zu Lee, Kathy Robie Suh
 Pharm/Tox: Fred Alavi, Todd Bourcier
 Product Quality Team: Anshu Rastogi, Brian Janelins, Rachel Novak
 Microbiology: Michael Shanks, Yarery Smith, Dupeh Palmer
 Facilities: Michael Shanks, Yarery Smith, Peter Qiu
 Clinical Pharmacology: Kunal Jhunjunwala, Anusha Ande
 Statistics: Lola Luo, Yeh-Fong Chen
 CDRH Consult (facilities and engineering): Porsche Bennet, Courtney Evans, Rumi Young
 OBP Labeling: James Barlow
 OPRO RBPM: Andrew Shiber
 Clinical RPM: May Zuwannin

4. Major GRMP Deadlines:

Filing Meeting: September 28, 2020
 Mid-cycle meeting: February 11, 2021 (2/24/2021 applicant)
 Late-cycle meeting: May 19, 2021, (6/7/2021 applicant)
 Primary assessment due: June 14, 2021
 Secondary assessment due: July 12, 2021
 BSUFA action date: August 11, 2021

5. Communications with Sponsor and OND:

Communication/Document:	Date Sent:
Filing Information Request	September 25, 2020
Teleconference with Kashiv	October 8, 2020
Filing Letter	October 9, 2020
Information Request 1	October 21, 2020
Information Request 2	November 24, 2020
Email Correspondence Re: additional DP lots	December 22, 2020
Information Request 3	February 4, 2021
Information Request 4	February 19, 2021
Information Request 5	March 30, 2021
Information Request 6	April 22, 2021
Information Request 7	May 7, 2021
Information Request 8	May 24, 2021
Addendum to IR 8	June 3, 2021
Information Request 9	June 11, 2021

6. Submission Assessed:

Submission:	Date Received:	Assessment Completed (yes or no)
761084/0001 Original Submission	August 11, 2020	Yes
761084/0002 Response to Filing IR	October 1, 2020	Yes
761084/0005 Response to IR 1	October 29, 2020	Yes
761084/0006 Response to IR 2 (part 1)	December 8, 2020	Yes
761084/0010 Response to IR 2 (part 2)	January 8, 2021	Yes
761084/0012 Response to IR 3	February 19, 2021	Yes
761084/0014 Response to IR 4	March 9, 2021	Yes
761084/0015 Response to IR 5	April 23, 2021	Yes
761084/0017 Audit Report	April 29, 2021	Yes*
761084/0018 Response to IR 6	May 17, 2021	Yes
761084/0020 Response to IR 7	May 21, 2021	Yes
761084/0025 Response to IR 8 (part 1)	June 3, 2021	Yes
761084/0028 Response to IR 8 Addendum	June 7, 2021	Yes
761084/0029 Response to IR 9	June 16, 2021	Yes
761084/0031 Response to IR 8 (part 2)	June 21, 2021	Yes

*A summary audit report was provided in the submission (Module 1.2, Attachment 2). The cover letter of this submission indicates that a final audit report will be provided at a later time. The audit findings will be reviewed in the next review cycle and will be used to assist in the preparation for the inspection.

7. Drug Product Name/Code/Type:

Proprietary Name: (b) (4) (proposed)
 Non-Proprietary Name/USAN: pegfilgrastim-pbbk
 CAS Name: 208265-92-3
 Common Name: TPI-120
 INN Name: Pegfilgrastim
 OBP systematic Name: CONJ: RPROT P09919 (CSF3_HUMAN); PEG [TPI-120]
 Other names: TPI-120

8. Pharmacological Category: Leukocyte Growth Factor

9. Dosage Form: Injection

10. Strength/Potency: 6 mg/0.6 mL

11. Route of Administration: Subcutaneous

12. Referenced Drug Master Files (DMF):

DMF#	DMF Holder	Item Referenced	Letter of Cross-Reference	Comments (status)

(b) (4)	Yes	Not Reviewed. DMF was reviewed previously without revision since last review, and the DMF status is adequate. Sufficient information provided in the BLA from OBP's perspective.
	Yes	
	Yes	
	Yes	
	Yes	
	Yes	

nce facility (FEI #3011289655, Chicago, IL), which is the manufacturing site for the TPI-120 intermediate product and drug substance, is required before this application can be approved as the FDA must assess the ability of that facility to conduct the listed manufacturing operations in compliance with cGMPs. Due to U.S. Government and/or Agency-wide restrictions on travel, an inspection of the Kashiv Biosciences LLC facility was not able to be conducted during the current review cycle, and the application cannot be approved until the required FDA inspection is conducted and the findings are assessed with regard to this application. In addition, ORA led a preannounced GMP and pre-license inspection (PLI) of the (b) (4) (b) (4) which is the manufacturing site for the TPI-120 drug product, in May 2021. The PAI was performed in support of (b) (4) (b) (4) At the conclusion of the inspection, a three-item Form FDA-483 was issued to the firm, which resulted in a recommendation of Official Action Indicated (OAI). Details of the inspection are covered in the EIR. OPMA is recommending facility withhold based on the unresolved deficiencies noted during inspection.

14. Consults Requested by OBP:

CDRH: Porsche Bennet, Courtney Evans, Rumi Young

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

17. Administrative:

Name and Title	Signature and Date
Anshu Rastogi, Ph.D. Biologist (Primary Review) Office of Biotechnology Products Division of Biotechnology Review and Research I	See Panorama
Brian Janelins, Ph.D. Lead Microbiologist (Secondary Review, ATL) Office of Biotechnology Products Division of Biotechnology Review and Research I	See Panorama

Summary of Quality Assessments

I. Primary Assessor Summary Recommendation

Sufficient analytical data are provided to support that (b) (4) is highly similar to US-licensed Neulasta, notwithstanding minor differences in clinically inactive components, and that the strength, dosage form, and route of administration of (b) (4) is the same as that of US-licensed Neulasta. However, data submitted in this application are not sufficient to support a conclusion that (i) the manufacture of (b) (4) is well-controlled and will lead to a product that is safe, pure, and potent for the duration of the shelf-life, and (ii) (b) (4) will remain highly similar to US-licensed Neulasta. From a CMC standpoint, OPQ is recommending a Complete Response letter be issued to Kashiv Biosciences to outline the deficiencies noted below and the information and data that will be required to support approval. These deficiencies include (i) inadequate protocols for assessing the requalification of reference standards (b) (4) (b) (4) (ii) inadequate qualification of the working reference standard (b) (4) (b) (4) (iii) inadequate information and data to support the cell-based potency assay (b) (4) (iv) inadequate validation of the drug product filling operation at (b) (4) (v) inadequate strategy to control sequence variants in TPI-120; and (vi) inadequate validation of the purity by CEX-HPLC method (STM-0282) (b) (4) (b) (4)

II. List of Deficiencies to be Communicated

Refer to the Complete Response Letter for a complete list of deficiencies and additional comments.

III. List of Post-Marketing Commitments/Requirements

Not applicable

IV. Assessment of Common Technical Document- Quality Module 1
Environmental Assessment of Claim of Categorical Exclusion

In Section 1.12.14, Kashiv requested categorical exclusion from the environmental assessment requirement under 21 CFR 25.31(a), (c), and (d). These claims of categorical exclusions are acceptable for BLA 761084.

V. Primary Container Labeling Assessment

The OBP review of the drug product labeling was not completed since the submission will not be approved.

VI. Assessment of Common Technical Document- Quality Module 3.2

See below (pages 8 – 240)

VII. Assessment of Immunogenicity Assays- Module 5.3.1.4

See Below (pages 240 – 259)

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DESCRIPTION OF DRUG SUBSTANCE AND DRUG PRODUCT

TPI-120 is a proposed biosimilar to US-licensed Neulasta, which acts on hematopoietic cells by binding to specific cell surface receptors, thereby stimulating proliferation, differentiation, commitment, and end cell functional activation.

3.2.S. DRUG SUBSTANCE

3.2.S.1 General Information

3.2.S.1.1 Nomenclature

The company name for the product is TPI-120. Pegfilgrastim is the assigned INN and USAN for the product, which are the same for US-licensed Neulasta.

3.2.S.1.2 Structure

TPI-120 is a polyethylene glycol (PEG) modified non-glycosylated form of recombinant human granulocyte colony-stimulating factor (rhG-CSF; filgrastim) with an additional N-terminal methionine to enforce expression in *E. coli*. Linear PEG is covalently attached to filgrastim, which is composed of 175 amino acid residues with one free cysteine (Cys) residue at position 18 and two intramolecular disulfide linkages (Cys-37 and Cys-43, and Cys-65 and Cys-75); the disulfide bonds form a loop-like structure that maintain the biologically active conformation of the protein.

Assessor comment: Additional information about the structure of TPI-120 is detailed in Section 3.2.R.

(b) (4)

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3.2.P.6 Reference Standards or Materials

The reference standard used to evaluate DP is the same as used for DS and us reviewed in Section 3.2.S.5

3.2.P.7 Container/Closure System

The DP is provided as an individually blistered single-use prefilled syringe (PFS) containing 6 mg/0.6 mL of product. The syringe consists of a (b) (4) glass barrel with staked 27 gauge needle, and rigid needle shield, and the plunger stopper and plunger rod. The syringe component materials are compendial. Primary packaging in the CCS (filling and stoppering syringes) is performed by (b) (4) while secondary packaging (plunger rod and safety device assembly), labeling, blistering, and cartoning is performed by Kashiv.

Assessor comment: The adequacy of the functional attributes of the CCS are deferred to CDRH, while the microbiological attributes are deferred to OPMA. The assessment of extractables and leachables from the PFS is contained in Section 3.2.P.2.4.

3.2.P.8 Stability

3.2.P.8.1 Stability Summary and Conclusion

Stability testing was performed on DP (5 lots unpackaged and 3 lots packaged) manufactured using a process representative of the proposed commercial manufacturing process. Stability studies were performed at long-term ($5 \pm 3^\circ\text{C}$; up to 36 months), accelerated ($25 \pm 2^\circ\text{C}$; 6 months), and stressed ($40 \pm 2^\circ\text{C}$; 6 months) storage conditions. All samples were stored horizontally. Specifications for stability studies are provided in Section 3.2.P.5.1.

Assessor comment: The storage conditions selected are representative of the long term storage conditions for DP, while the stressed and accelerated conditions are appropriate to evaluate potential degradation of both materials. The adequacy of stability specifications is discussed in Section 3.2.S.5.1.

3.2.P.8.2 Post-Approval Stability Commitment

One lot each of DP will be placed on stability at intended long-term storage condition of $5^\circ\text{C} \pm 3^\circ\text{C}$ in each calendar year starting from the year of commercialization. The proposed timepoints and analytical methods for stability testing are provided in Table 2 for DS.

Table 2. TPI-120 Drug Product (DP)-Packaged Annual Stability Study Design (Intended Storage Condition, 2-8°C)

Test	Test Method	Testing Interval (Months) ¹						
		0 ²	3	6	9	12	18	24
Appearance	STM-0017	x	x	x	x	x	x	x
pH	STM-0015	x	x	x	x	x	x	x
Concentration by UV Absorbance	STM-0108	x	x	x	x	x	x	x
Free PEG by SDS-PAGE with Titrisol Staining (non-reduced)	STM-0125	x	x	x	x	x	x	x
SE-HPLC	STM-0099	x	x	x	x	x	x	x
RP-HPLC	STM-0081	x	x	x	x	x	x	x
CEX-HPLC	STM-0282	x	x	x	x	x	x	x
CE-SDS (Non - Reduced)	STM-0317	x	x	x	x	x	x	x
Potency (M-NFS-60 cell proliferation assay)	STM-0127	x	x	x	x	x	x	x
Polysorbate 20	SOP-15583 ⁶	x				x		x
Particulate Matter Testing	STM-0143	x				x		x
Container Closure Integrity ³	STM-0210					x		
Sterility ³	STM-0186	x						x
Endotoxin ⁴	STM-0005	x						x
Volume of Injection in Syringe ⁵	STM-0101	x				x		x
Syringe Break Loose and Glide Force Determination ⁴	STM-0224	x						x

¹ An "X" designates testing will be performed at this time point.

² [Redacted]

³ [Redacted]

⁴ [Redacted]

⁵ [Redacted]

⁶ Test method for Element, external laboratory.

(b) (4)

Assessor comment: Testing is acceptable according to OBP’s perspective. Evaluation from microbiology and device perspectives are deferred to OPMA and CDRH, respectively.

3.2.P.8.3 Stability Data

Data is provided for packaged (5 lots) and unpackaged (3 lots), clinical and PPQ DP lots. At the long-term storage condition of 5°C, DP remains stable up to 36 months; no apparent trends are observed for the product quality attributes tested. At the 25°C accelerated condition, a slight increase is observed in free theragrastim (e.g., <LOQ to 0.4%; result of “fail” by SDS-PAGE), aggregates (e.g., 0.3% to 0.6%), and the sum of all impurities by SE-HPLC (e.g., 0.8% to 1.3%) by 6 months, with a slight decrease in monomer content (e.g., 99.1% to 98.7%); the data are within acceptance criteria with the exception of free theragrastim. Main peak purity by RP-HPLC also slightly decreases (e.g., 96.6% to 93.5%) with an increase in any single impurity peak (e.g., 0.9% to 3.2%) and the sum of all impurities (e.g., 3.0% to 6.1%); data are outside of the acceptance criteria by 6 months at 25°C. At 40°C, the same trends are observed by 3 months (compared to 1 month time point): monomer content decreases (e.g., 97.5% to 92.5%), while increases are observed in di-PEG variants (e.g., 0.5% to 0.7%), free theragrastim (e.g., 0.4% to 1.0%), aggregates (e.g., 1.6% to 5.8%), and the sum of all impurities (e.g., 2.5% to 7.5%) by SE-HPLC; data are outside of acceptance criteria with the exception of di-PEG variants. Main peak by RP-HPLC also decreases (e.g., 93.4% to 87.8%), while any single impurity peak and the sum of all impurities

increase (e.g., 2.3% to 5.3% and 6.2% to 12.0%, respectively); the levels are all outside of the acceptance criteria. Free PEG evaluated by SDS-PAGE also has a result of “fail” by 3 months at 40°C. Protein concentration, pH, and potency remain stable up to 3 months at 40°C in 2 lots of DP. Data is available from one lot of DP (lot 500-17011) which shows that by 3 months at 40°C, protein concentration decreased and by 6 months at 40°C, potency also decreased (compared to 3 month time point: protein concentration (b) (4); and potency (b) (4)); protein concentration levels were outside of acceptance criteria at 3 and 6 months, while potency remained within acceptance criteria.

***Assessor comment:** The adequacy of bioburden, endotoxin, and container closure integrity testing is deferred to OPMA. Data was not provided for syringe break loose and glide force; this is deferred to CDRH. Data was also not provided for volume of injection for any lot at any time point, in any storage condition.*

As mentioned in Section 3.2.P.3.6 and 3.2.P.5.4, osmolality (not performed on stability), purity by CEX-HPLC, PS20, and non-reduced CE-SDS test methods were implemented after the release of the PPQ lots. However, these test methods, excluding osmolality, were incorporated in the stability protocol and data were provided between 12 – 36 months of long-term storage. As discussed in Section 3.2.P.5.1, the values for these attributes do not change during long-term stability and limits for the purity tests are set with very low impurity limits. This is acceptable.

It is noted that protein concentration was evaluated by two different methods, UV spectroscopy and RP-HPLC, at different time points for several lots, including clinical and PPQ lots, under the various storage conditions. This issue is discussed in Section 3.2.S.7.3 and was ultimately resolved.

At the long-term storage condition of 5°C, the DP remains stable up to 36 months, and a difference is not observed between unpackaged DP and packaged DP. Overall, the data provided supports that the product is stable at 5°C for up to 36 months, which supports the proposed shelf-life of 24 months at 5°C. However, in the February 7, 2017 teleconference for the Type 4 meeting to discuss the content of format of the BLA, it was stated that data for expellable volume testing should be included to support the end of the proposed shelf life. Given this omission, it is unclear whether the volume of injection is sufficient during long-term storage conditions up to shelf-life. This will be included as an additional comment in the CR letter. An evaluation of the degradation pathways and rates of the product is included in Section 3.2.R (CAA).

In-use Stability

The in-use conditions for the DP include storage of syringe at ambient conditions for no more than 72 hours and allow for freeze-thaw; a study was performed to support these in-use conditions (Report PTL-2260-R). DP was stored at 30 ± 2°C in the presence of 300 lux. Dark control samples were also evaluated. In addition, a freeze-thaw study was performed, whereby DP was kept at -80°C for 48 hours then thawed at 4°C. A single freeze-thaw was evaluated in the study. DP lot 3-FIN-2932 was selected for use since it was near the end of shelf-life. For both studies, product quality was assessed (protein concentration, pH, appearance, potency, visible particles, sub-visible particles, and purity by CEX-HPLC, SE-HPLC, and RP-HPLC).

***Assessor Comment:** The product quality attributes during storage at 72 hours exposed to 300 lux did not show significant changes. The light intensity chosen appears to be reasonable, albeit on the low end of what may be expected during clinical conditions. Test results were not provided for the DP lot prior to*

freeze; therefore, it is unclear how the impact of a freeze-thaw cycle impacts product quality. Considering that the product quality was within specification for a DP lot near shelf-life, one freeze-thaw cycle is supported. However, insufficient data has been provided to support that multiple freeze-thaw cycles, does not impact product quality. Considering that the label does not specify the number of freeze-thaw cycles allowed, non-used DP could potentially be frozen and re-used multiple times. An additional comment will be included in the CR for the applicant to provide additional data to support the lack of multiple freeze-thaw cycles on the product quality of DP or revise the label to restrict the allowed freeze-thaw cycle to one cycle.

(b) (4)

(b) (4)

(b) (4)

3.2.R Regional Information

Comparative Analytical Assessment (Document PTL-1868-R)

TPI-120 DP is manufactured using the same formulation as described for US-licensed Neulasta, the reference product. Both products are presented at 6 mg per 0.6 mL in a single-use pre-filled syringe. TPI-120 DP and US-licensed Neulasta lots were evaluated in a CAA to support a determination that TPI-120 is highly similar to US-licensed Neulasta. Both products were evaluated in a Phase I clinical study (TPI-CL-109-A) to determine the PK/PD of TPI-120 compared to US-licensed Neulasta, as well as a comparative study (ADL-CL-112) to assess the immunogenicity and safety of both products. Lots were analyzed for their physiochemical properties (e.g., primary and higher-order structure, purity, product-related impurities, PEGylation characteristics, etc.), biological activities (e.g., cell proliferation and receptor binding assays), and strength (protein concentration). Additionally, lots were evaluated in

comparative stability studies (i.e., stressed and accelerated storage conditions) and forced degradation studies (e.g., thermal stress, light stress, oxidative stress, etc.) to assess the stability profiles (i.e., degradation rates and pathways) between TPI-120 DP and US-licensed Neulasta.

Evaluation of Quality Attributes

The list of attributes evaluated in the CAA and the methodology used to assess each attribute are provided in Table 6. The attributes were ranked by criticality and placed into a tier assignment based on their risk ranking. Furthermore, Table 6 shows the data analysis plan (e.g., similarity acceptance criteria for quantitative and qualitative evaluations) assigned for each test based on the criticality risk ranking of the attribute tested.

Table 6: Tier Assignment and Similarity Acceptance Criteria

Tier	Quality Attributes	Test Method	Methodology	Similarity Acceptance Criteria
Tier 1	Strength	STM-0108	Protein Concentration by UV Absorbance Spectroscopy	$-1.5 \sigma_R < 90\% \text{ C.I. of } (\mu_T - \mu_R) < 1.5 \sigma_R$
	Functional Potency and Bioactivity	STM-0127	Functional potency by rhG-CSF Adapted M-NFS-60 Cells	$-1.5 \sigma_R < 90\% \text{ C.I. of } (\mu_T - \mu_R) < 1.5 \sigma_R$
Tier 2	Receptor Binding	STM-0083	Binding by ELISA	90% of the test results within $\hat{\mu}_R \pm 3.0 \hat{\sigma}_R$
	Size Variants	STM-0099	SE-HPLC	90% of the test results within $\hat{\mu}_R \pm 3.0 \hat{\sigma}_R$ for monomer, Di-Pegylated variants and total size variants
	Product-related Impurities: Charge Species	STM-0282	CEX-HPLC	90% of the test results within $\hat{\mu}_R \pm 3.0 \hat{\sigma}_R$ for % main peak, total acidic, total basic, and total charge variants.
	Product-related Impurities: reduced, deamidated, oxidized species	STM-0081	RP-HPLC	90% of the test results within $\hat{\mu}_R \pm 3.0 \hat{\sigma}_R$ for % total impurities, % main peak, % total pre-main peak, and % total post-main peak impurities
Tier 3	Binding Activity	STM-0319	Receptor Binding kinetics by SPR	Sensorgrams and KD value of TPI-120 and Neulasta should be comparable.
	Secondary and Higher Order Structure	STM-0104	Thermal Unfolding Melting Point (DSC)	Thermogram similar for TPI-120 and Neulasta by visual comparison
	Secondary Structure	STM-0071	Far-UV Circular Dichroism	Far-UV CD spectra similar for TPI-120 and Neulasta by visual comparison
Tier 3	Secondary Structure	CRO (b) (4)	FTIR	FTIR spectra similar for TPI-120 and Neulasta by visual comparison
	Higher Order Structure	CRO	NMR	The 1D-1H NMR spectrum of TPI-120 and Neulasta should be comparable in the amide, aromatic, and aliphatic regions.
	Tertiary Structure	STM-0029	Intrinsic Fluorescence Emission	Fluorescence spectra similar for TPI-120 and Neulasta by visual comparison
		CRO (b) (4)	Near-UV Circular Dichroism	CD spectra similar for TPI-120 and Neulasta by visual comparison
	PEGylation	STM-0300	Polydispersity	The index should be around 1.1 and similar for TPI-120 and Neulasta
		STM-0299	PEGylation site by MALDI	Dominant PEGylation site is on the N-terminal methionine residue. Similar for TPI-120 and Neulasta
		STM-0320	Free PEG quantitation (CAD)	The level of Free-PEG detected in TPI-120 and Neulasta should be comparable.
	Primary Structure: Peptide Mapping	STM-0056**	Peptide Mapping by UHPLC-UV	The peptide profile in TPI-120 should match that in Neulasta by visual comparison
	Primary Structure: Disulfide Bond Integrity	STM-0182	Peptide mapping with LC-MS/MS of disulfide bonds in non-reduced condition	The location and number of disulfide bonds should be similar for TPI-120 and Neulasta
Primary Structure: Free Thiol	STM-0298	Reactivity with a fluorescent probe	The amount of free thiol should be close to the theoretical value and should be similar for TPI-120 and Neulasta	

Identity	STM-0125	SDS-PAGE with Titrisol Staining	NMT 1% of free PEG present and the intensity and profile should be similar for TPI-120 and Neulasta
	STM-0082	Western Blot	The principal band position should be similar for TPI-120 and Neulasta
	STM-0296	Intact mass by LC/MS	The intact mass should not be more than 1kD different from the theoretical mass and should be similar for TPI-120 and Neulasta
Sub-visible Particles	CRO (b) (4)	Flow microscopy	Report results
	CRO (b) (4)	Light obscuration	Report results
Aggregate characterization	CRO (b) (4)	AUC	The sedimentation coefficient of TPI-120 should be comparable with that of Neulasta. No new variants with different sedimentation coefficient should be observed in TPI-120 which were not observed in Neulasta.
	CRO (b) (4)	DLS	The average z-average values of TPI-120 should be comparable with that of Neulasta.
Aggregate Characterization	STM-0099*	SEC-MALS	The chromatographic profile of TPI-120 observed with MALS detector should be comparable with that of Neulasta. No new impurity should be observed in TPI-120 which was not observed in Neulasta.
Truncated variants/size-variants	STM-0317	CE-SDS (non-reduced)	The qualitative chromatographic profile of TPI-120 should be similar to that of Neulasta and no new impurity should be observed in TPI-120 which was not observed in Neulasta
*Chromatographic method followed was same as that in STM-0099, except the column and detector. Details are given in RPT-1283.			
**STM-0314 was used for MS parameters.			

Assessor comment: *The quality attributes selected are adequate to fully characterize US-licensed Neulasta and TPI-120 and are therefore appropriate to evaluate the similarity between TPI-120 and US-licensed Neulasta. Although sub-visible particles are not required to be evaluated in the CAA, the data from this assessment will be reviewed for informational purposes only. The methods used for evaluation included validated release test methods as well as methods that were qualified for suitability in the CAA. The QC methods that were used in the CAA, which are reviewed in Section 3.2.S.4.2/3.2.4.3, are acceptable for their intended purpose. The applicant provided the qualification reports for all methods that are not validated QC methods, and updated Section 3.2.R in response to an IR (sent 2/4/19, received 2/29/21). The adequacy of these methods will be assessed during the resubmission review cycle and on inspection. The adequacy of the criticality assessment of attributes and data analysis plan is discussed below.*

Criticality Assessment

A ranking system was established based on the impact/risk of each quality attribute evaluated in the CAA on clinical performance and patient safety. Each attribute was first ranked based on impact on clinical safety, efficacy, and immunogenicity, and then multiplied by a knowledge score (i.e., based on prior knowledge and published information of the product) and a method sensitivity value (i.e., based on the method used, its quantitation capability, and sensitivity). In addition, the ranking of attributes also considered the mechanism of action, literature, and in-house characterization studies. The overall

criticality assessment ranged from ≥ 90 for very high, 50-89 for high and < 50 for low (Table 5). The criticality score was then used to establish a Tier assignment and acceptance criteria for each quality attribute evaluated. Attributes assigned to Tiers 1 and 2 analyses included evaluations using quality ranges (QRs, 90% of the test results within $\hat{\mu}_R \pm X \hat{\sigma}_R$), where $\hat{\mu}_R$ is the sample mean, $\hat{\sigma}_R$ is the sample standard deviation based on the reference product lots, and X is the multiplier). Additionally, very high risk quality attributes (i.e., protein concentration and potency by cell-based bioassay) were evaluated using equivalence testing as supportive Tier 1 analysis (i.e., $-1.5 \sigma_R < 90\% \text{ C.I. of } (\mu_T - \mu_R) < 1.5 \sigma_R$, where μ_T and μ_R are the mean responses of biosimilar and reference product lots, respectively, and σ_R is the variability of the reference product lots). Attributes assigned to Tier 3 analyses were assessed using qualitative and graphic evaluations.

***Assessor comment:** The criticality risk ranking performed for quality attributes evaluated in the CAA is appropriate; higher risk attributes and attributes pertaining to the mechanism of action (i.e., strength and potency) were evaluated more rigorously. While the type of analysis for each Tier is also appropriate, justification was not provided for the multiplier used for the QRs. An IR was sent to the applicant (11/24/20) to provide appropriate justification (e.g., method variability, risk of attribute, level and nature of attribute, etc.) for the multiplier used in each quantitative assessment in which a QR was evaluated. The multiplier should be scientifically justified for each attribute, and not be a fixed multiplier according to a “Tier” assignment. In response (12/8/20), the applicant stated that the 1.5 multiplier was used to evaluate Tier 1 attributes with a “very high” criticality score (i.e., protein concentration and cell-based bioactivity assay), while a 3 multiplier was used to evaluate the Tier 2 attributes with a “high” criticality score. The evaluations performed with the 3 multiplier were based on method orthogonality, low level of variants, and method variability. Overall, the multipliers used for the QR analysis are acceptable with the exception of the 3 multiplier chosen for the assessment of receptor binding by ELISA, which should be performed with a smaller multiplier according to their plan based on its “very high” criticality score. Potency assays for receptor binding and inhibition of proliferation are not considered orthogonal assays, which was proposed by Kashiv to support the use of the 3 multiplier. This is ultimately not an issue, which is further discussed below.*

Lot Selection for CAA

A total of 13 lots of US-licensed Neulasta and 12 lots of TPI-120 DP were originally used in the CAA. All of these lots for each product were used in the assessment of Tier 1 and Tier 2 quality attributes, while only a subset of lots were used in the qualitative analysis of Tier 3 attributes. The CAA included analysis of TPI-120 DP lots derived from small scale, clinical, process validation, and commercial material (Table 1, below), and US-licensed Neulasta lots that were commercially available and span the shelf life of the product (36 months). The age of lots at the time of testing ranged from 0 – 17 months for TPI-120 and 1 – 24 months for US-licensed Neulasta.

Table 1: Genealogy of TPI-120 Lots Used for Analytical Similarity Studies

Lot Number of PEG used	IP Lot Number	DS Lot Number	Derived DP Lot Number	DOM for DP	Used For
G15614	15-0069-IP 15-0070-IP	100-16002	150-16008	1-Apr-2016	Immunogenicity lot
G15614	15-0070-IP 15-0071-IP	100-16020	150-16021	16-Jun-2016	PK/PD lot
G15614	15-0069-IP	100-16006-ENG-RW	3-NFF-0387	29-Aug-2016	Engineering batch
G15614	15-0071-IP 16-0011-IP 16-0013-IP 16-0015-IP 16-0019-IP	100-16026	3-FIN-2741	13-Oct-2016	Engineering batch
G15614 G14501	16-0011-IP 16-0013-IP	100-16027 100-16028	3-FIN-2742	28-Oct-2016	PPQ-1
G15614 G14501 G15D04	16-0015-IP 16-0019-IP	100-16030 100-16029	3-FIN-2744	18-Nov-2016	PPQ-3
G15D04	17-0003-IP 17-0012-IP	100-17007	3-FIN-2932	19-May-2017	DS PPQ-5
G15D04	16-0017-IP	100-16031	150-16043*	13-Dec-2016	Similarity batches
G15D04	16-0017-IP	100-16032	150-16044*	15-Dec-2016	Similarity batches
G15D04 G16N05	17-0021-IP	100-18001	190107	23-Apr-2019	Similarity batches
G15D04 G16N05	17-0023-IP	100-18002	190108	24-Apr-2019	Similarity batches
G16N05 G18205	15-18001	100-19001	190109	07-May-2019	Similarity batches

* Small-Scale batches (b) (4). Refer [PTL-1645-R](#) for comparability of (b) (4)
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(b) (4) which further distinguishes lots from each other and confers lot independence, therefore, this is acceptable.

Additionally, although the shelf-life of US-licensed Neulasta is publicly listed as 36 months, the applicant calculated the age of the lots based on a 24 month shelf life (i.e., date of manufacture was calculated as 24 months prior to the expiration date; age of the lot was based on the date of manufacture). Therefore, it appears that the ages of US-licensed Neulasta lots listed are incorrect. Considering the 36 month shelf-life, lots that were stated to be tested at 24 months of age are likely to be tested at 36 months of age. Therefore, we can consider lots were tested up to the end of shelf life, though perhaps not in the 0-12 month age range, which is adequate to capture the variability in the product to the point where the CAA is not impacted. Therefore, the age of the US-licensed Neulasta lots is acceptable to generate the quality ranges to assess the similarity of TPI-120 to US-licensed Neulasta.

In response to an IR (1/8/21) regarding RP-HPLC analysis of hydrophobic variants (further discussed below), the applicant provided data for additional DP lots of TPI-120 and US-licensed Neulasta (i.e., 5 additional lots of US-licensed Neulasta and 1 additional independent lot of TPI-120). The review of the data (below) is inclusive of the additional lots; the number of lots used in each analysis is specified in each section. Overall, the lots used in the CAA, which includes clinical and commercial lots of varying age of TPI-120, is acceptable.

Reference Standards

Information on the use of reference standards (RS) in the CAA was not provided. It is stated in Module 3.2.S.2.5 that US-licensed Neulasta lots 1053071, 1053082, and 1070909, as well as TPI-120 primary reference standard lot (b) (4) were used as RS for all testing which required a RS in the CAA.

Assessor Comment: An IR was sent to the applicant (11/24/20) to indicate which RS was used for each lot evaluated in the CAA that required a comparison to a RS to generate results (i.e., potency by cell-based bioassay and receptor binding by ELISA), and if more than one RS was used for a particular analysis, to describe how the data were interpreted for the CAA. Additionally, the applicant was asked to provide additional information that supports the bridging and qualification of these RS lots to demonstrate that they are acceptable for their intended purpose. In response (12/8/20), the applicant stated that for the cell-based potency and ELISA receptor binding assay US-licensed Neulasta lot 1053071 was used as the RS for the evaluation of 9 lots of TPI-120 and 10 lots of US-licensed Neulasta for each test method, while TPI-120 primary reference standard (b) (4) was used in the evaluation of 3 lots

of each product in each test method (TPI-120 lots 190107, 190108, and 190109 and US-licensed Neulasta lots 1093751, 1094104, and 1095683). The applicant was then asked (2/4/19) to provide a justification, and any accompanying data, to support the selection of this lot (1053071) as the RS for this purpose. The applicant stated (2/19/21) that this US-licensed Neulasta lot was one of the lots used during early development for head-to-head testing with TPI-120 material and was available in sufficient quantities. Additionally, this lot was tested with two other lots of US-licensed Neulasta, which were all found to be similar, including for potency by the cell-based bioassay and receptor binding assay (data provided in Table 1 of the response; tested against the first (developmental) in-house (b) (4)). This is acceptable.

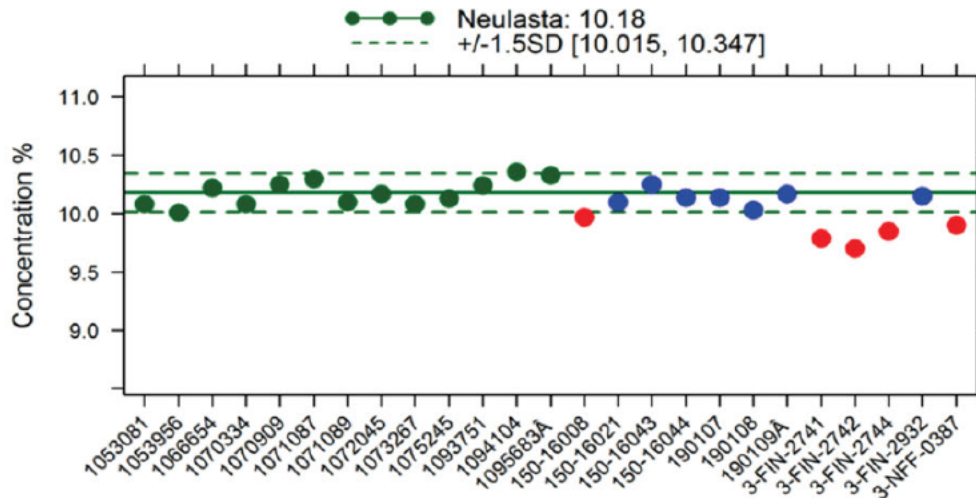
The TPI-120 PRS (b) (4) was then qualified against the US-licensed Neulasta RS (lot 1053071), where potency of the PRS was assigned as 100%. For qualification of the PRS, 12 independent determinations were used for potency assignment of the PRS against the US-licensed Neulasta RS in the cell-based potency assay. The potency of PRS was calculated to be 103% relative to the US-licensed Neulasta RS, and the upper and lower 95% confidence interval was determined to be 100% to 106%, which is inclusive of the assigned 100% potency. This is acceptable. For receptor binding by ELISA, however, only three replicates were used for the determination of relative binding activity of the PRS against US-licensed Neulasta RS, which was determined to be 91%. While receptor binding by ELISA is not a proposed QC test and doesn't require additional replicates and requirements for potency assignment to support new RS, additional replicate data would have been more supportive. To determine whether this impacts the CAA, an independent analysis was performed in which the data generated from the three lots of each product that were evaluated using the PRS for the receptor binding by ELISA assessment were re-calculated by adding 9% to the results for those lots. Considering that the adjusted data from these lots resulted in a sufficient number of TPI-120 lots within the QR when an appropriate multiplier was used, the lack of additional data to support the 100% potency assignment (receptor binding by ELISA) for the PRS does not impact the CAA for the receptor binding by ELISA evaluation (discussed further below).

Results of Comparative Analytical Assessment

Protein Concentration

The protein concentration of TPI-120 and US-licensed Neulasta was evaluated by Tier 1 analysis. Originally, 12 lots of TPI-120 and 13 lots of US-licensed Neulasta were used in the evaluation of protein concentration by UV 280. The mean protein concentration for US-licensed Neulasta lots was 10.18 mg/mL (range: 10.01 – 10.36 mg/mL), while for TPI-120 lots it was 10.02 g/mL (range: 9.7 – 10.25 mg/mL), with a 1.1% CV for US-licensed Neulasta and 1.7% CV for TPI-120 lots (Tables 17 and 18). The QR calculated from US-licensed Neulasta lots was 10.015 – 10.347 mg/mL; only 7 out of 12 TPI-120 lots were within the QR (58%), which does not meet the criteria of at least 90% of TPI-120 lots within the QR (Figure 1). In addition, the criteria for equivalency testing was not met. The lots that were out of the QR (shown in red in Figure 1) were then excluded from the assessment based on the reasoning that changes were made to the process control strategy to address the aberrant protein concentration results after the production of these lots, which resulted in all further TPI-120 lots (n = 6) to fall within the QR (Figure 2).

Figure 1: Distribution of TPI-120 and Neulasta A280 Protein Concentration, and Neulasta Quality Range Limits with Mean difference Statistical Evaluation

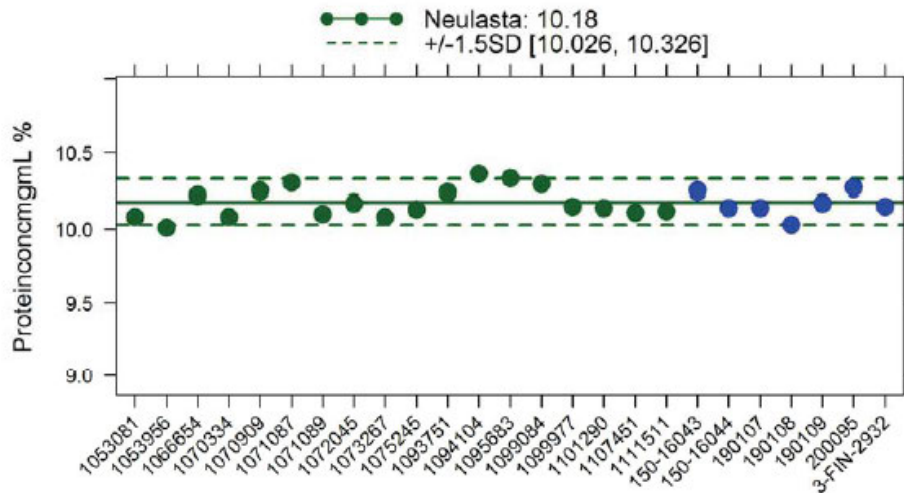


Assessor comment: The applicant proposed to use only data from the latest six manufactured DP lots of TPI-120, which all fall within the QR, as these lots were manufactured after changes were made to the manufacturing process to ensure tighter control and more consistent product. An IR was sent to the applicant (11/24/20) to provide protein concentration test results from additional lots of TPI-120 DP that have been manufactured after the change to the process control strategy, if available, in order to support that the process control strategy changes ensure that protein concentration remains within the QR. The applicant was also asked to provide information that supports that they have identified the root cause of the aberrant results for protein concentration and provide additional details regarding the changes that

	n
(b) (4)	(b) (4)
(b) (4)	(b) (4)
(b) (4)	(b) (4)
(b) (4)	5

Seven lots of TPI-120 and 18 lots of US-licensed Neulasta were used in the final evaluation of protein concentration. The mean protein concentration for was 10.18 mg/mL for US-licensed Neulasta (range: 10.01– 10.36 mg/mL) and 10.16 mg/mL for TPI-120 (range: 10.03 – 10.27 mg/mL; Table 19). The QR calculated from US-licensed Neulasta lots was 10.026 – 10.326 mg/mL; 7 out of 7 TPI-120 lots were within the QR (Figure 2); the additional lot of TPI-120 included in the analysis (lot 200095, manufactured February 2020) had a protein concentration of 10.27 mg/mL, which is within QR and the range of US-licensed Neulasta. In addition, the criteria for equivalency testing was met.

Figure 2: Distribution of Last 7 Manufactured Lots of TPI-120 A280 Protein Concentration and Neulasta A280 Protein Concentration Quality Range Limits



Assessor comment: Three lots of DP that were manufactured prior to the process change but are among the latest 7 lots of DP manufactured were included in the re-evaluation. Since the change in the process is only an update to ensure tighter control (b) (4) the inclusion of these lots in the evaluation is acceptable. The TPI-120 lots are found to be within the QR range established from US-licensed Neulasta lots, which is tighter than the previously established QR. The update in the DP manufacturing control strategy further ensures that the protein concentration of DP will remain within the range.

However, it was unclear whether a theoretical or experimental extinction coefficient (EC) was used to calculate protein concentration for the evaluations performed and whether the EC is acceptable. To measure protein concentration, the theoretical EC of US-licensed Neulasta should be determined, and not back-calculated based on the label claim of US-licensed Neulasta. An IR was sent to the applicant (11/24/20) to clarify whether a theoretical or experimental EC was used, and to provide data to support that the theoretical EC is confirmed experimentally with justification for the experimental method(s) used. In response (12/8/20), the applicant stated that the EC for Theragrastim, which was based on the published EC for its reference product US-licensed Neupogen (i.e., $0.86 \text{ mL} \times \text{mg}^{-1} \times \text{cm}^{-1}$), was used as the EC in the calculation of protein concentration for TPI-120 and US-licensed Neulasta lots. They also calculated the theoretical and experimental EC for Theragrastim (i.e., $0.84 \text{ mL} \times \text{mg}^{-1} \times \text{cm}^{-1}$ for both) and noted that they are in close agreement to the experimentally calculated EC for US-licensed Neupogen (i.e., $0.83 \text{ mL} \times \text{mg}^{-1} \times \text{cm}^{-1}$). The difference between the EC back calculated from the label claim of US-licensed Neulasta is similar to that of the EC theoretically and experimentally determined for US-licensed Neupogen. The applicant stated that the Theragrastim EC was used for TPI-120 because the PEGylation would not cause any changes in primary, secondary, or tertiary structure of Theragrastim, therefore, it would not impact the EC for the non-pegylated molecule (i.e., Theragrastim). An IR was communicated to the applicant (2/4/21) to provide data to support that the PEG portion of TPI-120 does not impact the EC. In response (2/19/21), the applicant provided sufficient data comparing the structure primary, secondary, and tertiary structure of Theragrastim and TPI-120 to show that they were similar and that PEGylation does not change the structural attributes of the product in an unexpected manner. This is

adequate to support the use of the EC of Theragrastim to calculate the protein concentration of TPI-120. Therefore, the protein concentration evaluation is acceptable and supports that TPI-120 is highly similar to US-licensed Neulasta.

Potency by Cell-Based Bioassay

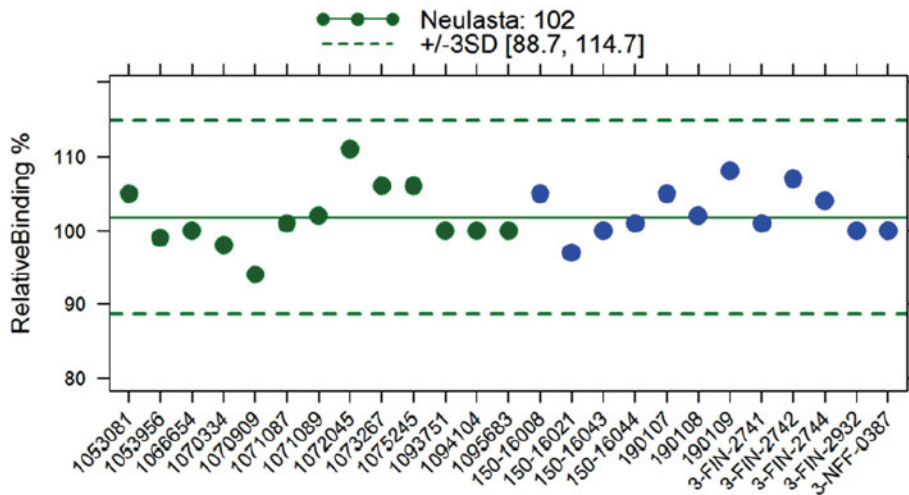
The biological potency of TPI-120 and US-licensed Neulasta was evaluated by Tier 1 quality analysis in a cell-based bioassay. M-NFS-60 cells were used in a cell proliferation assay and the potency of 13 lots of TPI-120 and 16 lots of US-licensed Neulasta was reported relative to US-licensed Neulasta RS lot 1053071 or PRS (b) (4). The mean potency for US-licensed Neulasta lots was 97% (range: 87% – 106%), while for TPI-120 lots it was 100% (range: 90% – 105%), with a 4.7% CV for both US-licensed Neulasta and TPI-120 lots (Tables 20 and 21). The QR calculated from US-licensed Neulasta lots was 88.6% – 104.7; 11 out of 13 TPI-120 lots were within the QR, however, the 2 lots that were outside of the range (105% each) were at the border and would be considered to meet the QR if the upper limit was rounded up to 105%. In terms of equivalence testing, the 90% confidence interval of the mean difference between US-licensed Neulasta and TPI-120 for relative potency (-0.4, 6.2) is within the equivalence acceptance criterion of (-8.0, 8.0, Figure 3). The dose response curves between TPI-120 and US-licensed Neulasta was also similar (Figure 4).

Assessor comment: *All of the TPI-120 lots were within the minimum and maximum range of potency values of US-licensed Neulasta lots. Therefore, the potency of TPI-120 lots and US-licensed Neulasta lots is determined to be similar based on the cell-based proliferation assay. An IR was sent to the applicant (2/4/21) to clarify the number of lots that were used in the potency assessment; an updated CAA report was provided (which corrected the discrepancies in the text) in the response (2/19/21) which confirmed that all 13 lots of TPI-120 were used in the evaluation of potency by the cell-based bioassay by equivalency and quality range testing. Additionally, the applicant updated the information provided in this section to reflect the use of PRS (b) (4) to analyze TPI-120 lots 190107, 190108, 190109, and 200095 and US-licensed Neulasta lots 1093751, 1094104, 1095683, 1101290, 1099977, and 1099084 for the evaluation of potency by bioassay. There was no obvious bias when using the different reference standards.*

Receptor Binding by ELISA

The receptor binding capabilities of TPI-120 and US-licensed Neulasta lots were analyzed by an *in vitro* ELISA by Tier 2 analysis. The receptor binding activity of both products (%relative binding) was compared against either US-licensed Neulasta RS 1053071 or the PRS (b) (4) for 12 TPI-120 lots and 13 US-licensed Neulasta lots. The mean receptor binding activity for US-licensed Neulasta lots was 102% (range: 94% – 111%), and for TPI-120 lots it was also 102% (range: 97% – 108%), with a 4.3% CV for US-licensed Neulasta lots and 3.3% CV for TPI-120 lots (Tables 22 and 232). The QR calculated from US-licensed Neulasta lots was 88.7% – 114.7%; 12 out of 12 TPI-120 lots were within the QR (mean $\pm$ 3 σ ; Figure 5). The ELISA dose response curves between TPI-120 and US-licensed Neulasta was also similar (Figure 6).

Figure 5: Distribution of TPI-120 and Neulasta ELISA Relative Binding, and Neulasta Quality Range Limits



Assessor comment: The results from the receptor binding by ELISA evaluation show that lots of TPI-120 and US-licensed Neulasta are similar in terms of their receptor binding activity, all 12 lots of TPI-120 were within the min-max range (and therefore the QR) of US-licensed Neulasta. However, as discussed above, the qualification of the TPI-120 PRS ^{(b) (4)} led to a 91% potency result and it was unclear whether the statistical analysis of that data supports a potency assignment of 100%. While this is not a requirement to qualify new RS, there could be a potential difference in potency between the RS that was used to evaluate different lots of US-licensed Neulasta and TPI-120 in terms of this potency-related attribute, which could lead to TPI-120 lots being outside of the min-max and QR of US-licensed Neulasta. Additionally, the receptor binding assay was evaluated in terms of a QR with a multiplier of 3. This is not appropriate since receptor binding is a very high risk quality attribute, which typically would result in evaluation with a smaller multiplier (e.g., 1.5 or 2). Therefore, the data provided for the receptor binding assay was re-evaluated by the Assessor using multipliers of 1.5, 2, and 2.5 to calculate the QR to determine whether a sufficient number of TPI-120 lots are within the QR when the additional 9% potency was factored into the current results for US-licensed Neulasta and TPI-120 lots evaluated with the PRS (lot ^{(b) (4)}). The results of this assessment are summarized in the table below, which demonstrate a sufficient number of TPI-120 lots are within the QR based on an appropriate multiplier (e.g., 2) and this is irrespective of a potential potency difference in the RS. Therefore, it can be concluded that the data provided adequately supports that TPI-120 is highly similar to US-licensed Neulasta with respect to receptor binding by ELISA.

Multiplier	Quality Range (original data)	TPI-120 lots within range (original data)	Quality Range (based on adjusted data)	TPI-120 lots within range (based on adjusted data)
3	88.7 - 114.7	12/12	88.2 - 119.3	12/12 (100%)
2.5	90.8 - 112.6	12/12	90.8 - 116.7	11/12 (91.6%)
2	93.0 - 110.4	12/12	93.4 - 114.1	11/12 (91.6%)
1.5	95.2 - 108.2	12/12	96.0 - 111.5	10/12 (83.3%)

Size Variants by SE-HPLC

Size variants, such as di-PEGylated rhG-CSF, aggregates, non-PEGylated rhG-CSF (free rhG-CSF), and LMWs (unknown peak reported as impurity peak at RRT 0.80 – 0.86), were analyzed in TPI-120 and US-licensed Neulasta lots by SE-HPLC. Thirteen lots of TPI-120 and 18 lots of US-licensed Neulasta were used in the analysis of size variants by Tier 2 analysis (QR using $[\text{mean} \pm 3\sigma]$). Results for main peak, aggregates, di-PEG/dimer, free rhG-CSF, impurity peak at RRT 0.80 – 0.86 and total size variants for both products are reported in Table 24. Quality ranges were established for main peak, aggregates, di-PEG, free rhG-CSF, and total size variants.

Assessor Comment: *While a QR was not established for the unknown LMW peak, the levels in TPI-120 were between no more than 0.06% or not-detected and the levels in US-licensed Neulasta were between 0.10% and 0.16%.*

The mean main peak %area for US-licensed Neulasta lots was 98.46% (range: 98.2% – 98.62%), and for TPI-120 lots it was 99.25% (range: 98.89% – 99.5%), with a 0.1% CV for US-licensed Neulasta lots and 0.2% CV for TPI-120 lots (Table 25). The QR calculated from US-licensed Neulasta lots was 98.156% – 98.762%; 13 out of 13 TPI-120 lots were above the upper quality limit of the QR for main peak (i.e., higher monomer content in TPI-120 lots; Figure 7).

The mean di-PEG %area for US-licensed Neulasta lots was 1.18% (range: 1.04% – 1.38%), and for TPI-120 lots it was 0.48% (range: 0.28% – 0.74%), with a standard deviation of 0.102 for US-licensed Neulasta lots and 0.147 for TPI-120 lots (Table 26). The QR calculated from US-licensed Neulasta lots was 0.873% – 1.486%; 13 out of 13 TPI-120 lots were below the lower quality limit of the QR for di-PEG (i.e., lower di-PEG impurity in TPI-120 lots; Figure 8).

Assessor comment: *TPI-120 lots were outside of the QRs for monomer and di-PEG variants, the TPI-120 lots have lower levels of the di-PEG impurity and higher levels of monomer compared to US-licensed Neulasta. The age difference in the lots does not appear to be a contributing factor to the difference in data. Considering that these differences are minor, these differences are not expected to impact potency or have a clinical impact. For example, the comparative analytical assessment results support similar bioactivity between TPI-120 and US-licensed Neulasta, this is acceptable.*

The mean aggregates %area for US-licensed Neulasta lots was 0.14% (range: 0.09% – 0.19%), and for TPI-120 lots it was 0.21% (range: 0.08% – 0.36%), with a standard deviation of 0.033 for US-licensed Neulasta lots and 0.075 for TPI-120 lots (Table 27). The QR calculated from US-licensed Neulasta lots was 0.039% – 0.242%; 10 out of 13 TPI-120 lots (77%) were within the QR for aggregates, while 3 lots were above the upper quality limit, which does not meet the criteria of at least 90% of TPI-120 lots within the QR (Figure 9).

Assessor comment: *The level of aggregates is slightly higher in some TPI-120 lots compared to US-licensed Neulasta lots, though the overall level of aggregates is fairly low. It is unclear if this difference can be attributed to method variability, as aggregate data for the US-licensed Neulasta lots does not appear to be as variable. An IR was sent to the applicant (11/24/20) to provide justification for the higher level of aggregates observed in TPI-120 material and data from additional lots of TPI-120 material, if available, to determine whether these differences are consistent or minimized. The applicant was also asked to provide data to characterize the aggregate species from the TPI-120 lots that have higher*

aggregate levels (lots 150-16021, 0.24%; 150-16043, 0.36%; 190108, 0.29%; and 3-FIN-2741, 0.29%) and assess their impact on potency, if possible. In response (1/8/21), the applicant provided data from an additional 2 lots of TPI-120, as well as 5 lots of US-licensed Neulasta. While the recalculated QR is slightly wider (0.040 – 0.238), the same 4 TPI-120 lots that were outside of the QR previously, remain outside of the QR (the 2 additional TPI-120 lots were within the QR). Therefore, 11 out of 14 TPI-120 lots were ultimately within the QR (78%). The applicant provided chromatograms for the 4 lots that were outside the QR, which shows a higher slightly aggregate peak in each. Structural characterization (CD, DSC, FTIR and fluorescence) was performed on lot 3-FIN-2741 (one of the lots outside QR), which did not find any structural differences in this lot compared to other TPI-120 and US-licensed Neulasta lots (further discussed in the review section of each respective evaluation), which would lead to a higher aggregate level in this lot. Likewise, lot 190108 (also outside of QR) was further evaluated for the presence of aggregates and HMWs using SE-HPLC MALS, SV-AUC and DLS, which did not detect a difference in this lot compared to other lots of TPI-120 or US-licensed Neulasta. Review of these analyses is contained at the end of this section.

For free rhG-CSF the mean %area for US-licensed Neulasta lots was 0.09% (range: 0.05 – 0.14%), and for TPI-120 lots it was 0.03% (range: 0.01% – 0.06%), with a standard deviation of 0.022 for US-licensed Neulasta lots and 0.015 for TPI-120 lots (Table 28). The QR calculated from US-licensed Neulasta lots was 0.024% – 0.154%; 7 out of 13 TPI-120 lots were below the lower quality limit of the QR for free rhG-CSF (i.e., lower level of free rhG-CSF in TPI-120 compared to US-licensed Neulasta; Figure 10).

The impurity peak at RRT 0.80 – 0.86 was found to either be not detected or up to 0.06% in TPI-120 lots, while it ranged from 0.12 – 0.16% in US-licensed Neulasta lots (Table 24). QR were not generated as impurity peak at RRT 0.80 – 0.86 was not detected in most TPI-120 lots.

For total size variants, the mean %area for US-licensed Neulasta lots was 1.47% (range: 1.28% – 1.8%), and for TPI-120 lots it was 0.68% (range: 0.48% – 1.03%), with a standard deviation of 0.127 for US-licensed Neulasta lots and 0.175 for TPI-120 lots (Table 29). The QR calculated from US-licensed Neulasta lots was 1.091% – 1.855%; 13 out of 13 TPI-120 lots were below the lower quality limit of the QR for total size variants (i.e., lower amount of total size variants in TPI-120 lots; Figure 11).

Assessor comment: *This is acceptable since, overall, TPI-120 lots had a lower level of total impurities compared to US-licensed Neulasta lots and the comparative analytical assessment results show that TPI-120 and US-licensed Neulasta are similar with respect to bioactivity.*

Representative chromatograms for US-licensed Neulasta lots and TPI-120 lots are provided in Figures 12 and 13. The peak profiles show that the di-PEG peaks in US-licensed Neulasta lots are larger than in TPI-120, while some of the aggregate peaks in TPI-120 lots are larger than in US-licensed Neulasta, which corresponds to the quantitative data discussed above. Additionally, a free rhG-CSF peak and an additional peak between the di-PEG and aggregates peaks is visible in US-licensed Neulasta lots, though not in TPI-120 lots.

Assessor comment: *Overall, TPI-120 lots appear to have higher monomer content and lower level of di-PEG, free rh G-CSF, unknown LMW impurity peak at RRT 0.80 – 0.86, and total size variants compared to US-licensed Neulasta lots. These slight differences in values do not preclude a demonstration of similarity as the levels of the impurities are very low and the potency of TPI-120 and US-licensed Neulasta*

is similar. In addition, the chromatograms support that both products contain the same types of size variants with the exception of a small peak (di-PEG variant that elutes as three acidic variants via CEX-HPLC) that is detected in US-licensed Neulasta and not in TPI-120.

While aggregates and other HMWs were evaluated by SE-HPLC, data from orthogonal methods (e.g., SE-HPLC MALS, SV-AUC, and DLS) was not included in the CAA to further evaluate HMWs (e.g., aggregates, dimers, and di-PEG G-CSF) in TPI-120 and US-licensed Neulasta lots. An IR was sent to the applicant (11/24/20) to update the CAA report to include this data. In response (1/8/21), the applicant provided data from the evaluation of three lots each of TPI-120 (lots 190107, 190108, and 200095) and US-licensed Neulasta (lots 1099977, 1107451, and 1093751) using SE-HPLC MALS, SV-AUC, and DLS for the assessment of aggregates and HMWs (reviewed below). The CAA report was updated to include these analyses, and reports 1222/20086 (Analysis of PEG-GSCF by SV-AUC and DLS) and RPT-1283 (SEC-MALS Analysis and Assessment of PEG Moiety between TPI-120 and Neulasta Drug Product) were included in the IR response and updated CAA report.

SV-AUC analysis

Each lot evaluated was found to contain three HMW variant species, which are the same in both products based on the sedimentation coefficient: HMWs 1 corresponds to a sedimentation coefficient (SC) of ~1.67, HMWs 2 to an SC of ~3, and HMWs 3 to an SC of ~6.7. HMWs 1 is detected at 3.9% – 5.9% in US-licensed Neulasta lots and 5% – 6.1% in TPI-120 lots. HMWs 2 is detected at 2.1% – 2.8% in US-licensed Neulasta lots and 2.4% – 2.8% in TPI-120 lots, while HMWs 3 detected at 0.7% – 0.8% in US-licensed Neulasta lots and 0.8 – 0.9% in TPI-120 lots (Tables 30 and 31). An overlay of the normalized SC data is provided in Figure 14.

DLS Analysis

The radius of species 1 (main species) detected in both products was 2.2 – 2.5 nm in US-licensed Neulasta lots, and 2.4 – 2.6 nm in TPI-120 lots, while the z-average radius ranged from 6.2 ± 0.1 to 19 ± 7.6 in US-licensed Neulasta lots and 4.7 ± 0.4 to 8.6 ± 0.5 in TPI-120 lots (Table 32).

SE-HPLC MALS

The content of aggregates was not significant enough in US-licensed Neulasta and TPI-120 lots in order to characterize them in terms of their hydrodynamic radii and percentage content by MALS analysis. Data are provided for molar mass, polydispersity, and hydrodynamic radius for both products (undigested). The molar mass was observed to be 38017 – 40399 g/mol for US-licensed Neulasta lots and 36238 – 39821 g/mol for TPI-120 lots. Polydispersity was found to be 1.011 – 1.021 for US-licensed Neulasta lots and 1.015 – 1.029 for TPI-120 lots, while the radius of the main peak was observed to be 8.15 – 8.35 nm for US-licensed Neulasta lots and 7.93 – 8.43 nm for TPI-120 lots (data provided in IR response dated 1/8/21).

Assessor comment: *Analysis of US-licensed Neulasta and TPI-120 lots by orthogonal methods shows that the products are similar in terms of aggregates and HMWs observed in both products. Further, TPI-120 lot 190108, which was one of the lots observed to have a higher overall level of aggregates compared to US-licensed Neulasta lots as evaluated by SE-HPLC (QR analysis), was found to be similar to the other TPI-120 lots and US-licensed Neulasta lots evaluated by orthogonal methods.*

Charge Variants by CEX

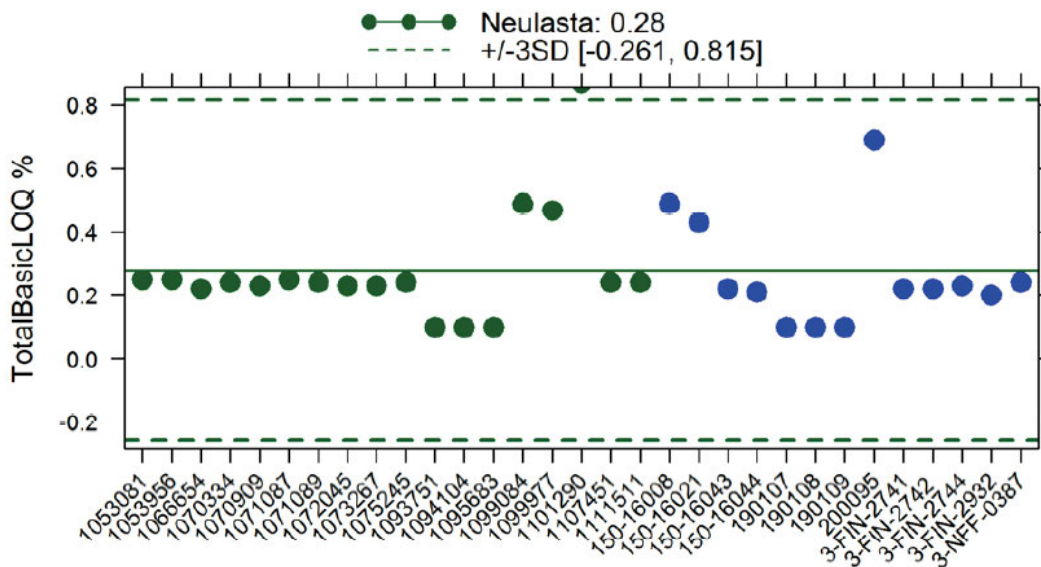
Charge variants were analyzed in TPI-120 and US-licensed Neulasta lots by CEX. Thirteen lots of TPI-120 and 18 lots of US-licensed Neulasta were used in the analysis of charge variants by Tier 2 analysis (QR using $[\text{mean} \pm 3\sigma]$). Results for main peak, acidic variants, basic variants, and total charge impurities for both products are reported in Table 33.

The mean main peak %area for US-licensed Neulasta lots was 98.37% (range: 97.62% – 98.77%), and for TPI-120 lots it was 99.05% (range: 98.69% – 99.32%), with a 0.3% CV for US-licensed Neulasta and 0.2% CV for TPI-120 lots (Table 34). The QR calculated from US-licensed Neulasta lots was 97.492% – 99.239%; 8 out of 13 TPI-120 lots were within the QR for main peak and 5 lots were above the upper quality limit (Figure 15).

The mean acidic variants %area for US-licensed Neulasta lots was 0.6% (range: 0.4% – 0.7%), and for TPI-120 lots it was < LOQ (0.2%) (range: < LOQ), with a standard deviation of 0.13 for US-licensed Neulasta lots and 0.00 for TPI-120 lots (Table 35). The QR calculated from US-licensed Neulasta lots was 0.17% – 0.97%; 13 out of 13 TPI-120 lots were below the lower quality limit of the QR for acidic variants (Figure 16), though the limit of quantitation (LOQ) is 0.2, therefore the actual values are not known.

The mean basic variants %area for US-licensed Neulasta lots was 0.23% (range: 0.20% – 0.87%), and for TPI-120 lots it was 0.315% (range: 0.20% – 0.69%), with a standard deviation of 0.182 for US-licensed Neulasta lots and 0.166 for TPI-120 lots (Table 36). The QR calculated from US-licensed Neulasta lots was -0.261% – 0.815%; 13 out of 13 TPI-120 lots were within the QR for basic variants (Figure 17).

Figure 17: Distribution of TPI-120 and Neulasta CEX %Total Basic, and Neulasta Quality Range (LL-UL)

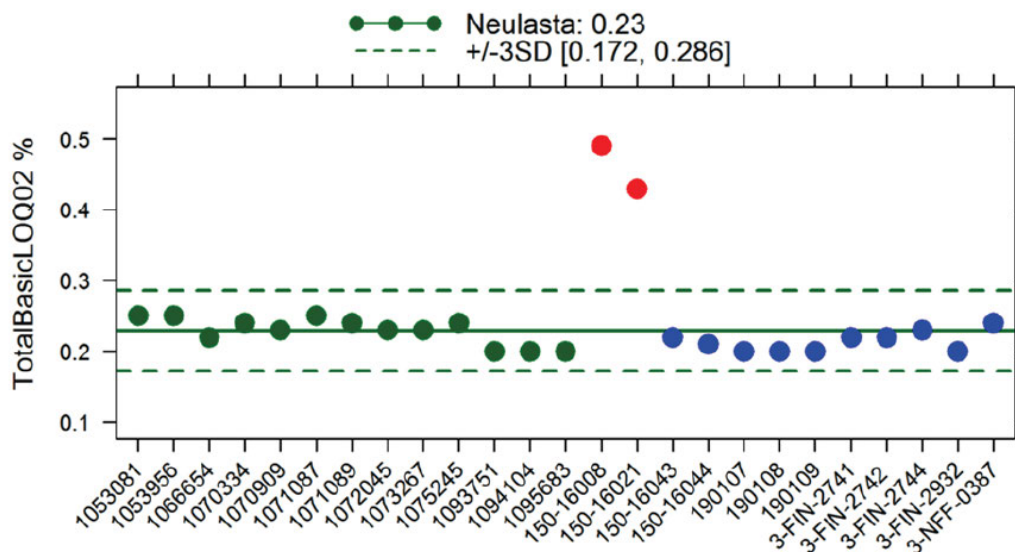


Assessor comment: Five additional lots of US-licensed Neulasta were included in the CAA evaluation, and were analyzed for charge variants by CEX (the data described above reflects the additional lots), therefore, the QR was re-calculated; all lots of TPI-120 are within the re-calculated QR. However, one of the additional US-licensed Neulasta lots (1101290) generated a basic variant value that was much higher compared to the other US-licensed Neulasta lots analyzed (0.87% compared to 0.20 – 0.49%; Table 33

and Figure 17). There is a concern that this difference may be related to an issue with chromatographic peak integration, as this was an issue for the analysis of two of the TPI-120 lots (described below). An IR was sent to the applicant (2/4/21) to provide an explanation for how the basic variant peaks were integrated for US-licensed Neulasta lots used to establish the similarity acceptance criteria. In response (2/19/21), the applicant stated that the integration parameters used for all CEX evaluations were consistent for all samples. The higher level of basic variants in lot 1101290 was due to a higher content of basic peak 7 (0.54%) in comparison to the other lots (e.g., 0.19% and 0.26%). An overlay of chromatograms of lot 1101290 and two of the other lots is provided which supports this data.

Conversely, in terms of the original evaluation of basic variants before additional lots of US-licensed Neulasta and TPI-120 were added, 2 lots of TPI-120 were above the upper quality limit of the QR (lots 150-16008 and 150-06021, shown in Figure 15 below). These two lots were early lots of TPI-120 material, and the higher level of basic variants comes from the contribution of a post-shoulder peak eluting at RRT ~1.3 and from free G-CSF.

Figure 15: Distribution of TPI-120 and Neulasta CEX %Total Basic, and Neulasta Quality Range (LL-UL)



An IR was sent to the applicant (11/24/20) to provide an explanation for the two TPI-120 lots that are high in basic variants and characterization data for any additional basic variants present in these lots (i.e., post-shoulder peak eluting at RRT ~1.3 and for free-G-CSF) and impact on potency, and to clarify whether these additional variants or very high levels of basic variants have been observed in TPI-120 lots (at release or on stability) after the manufacture of these two early lots. In response (12/8/20), the applicant stated that upon further assessment, an additional basic peak is not present in these two lots, but the differences observed are due to peak integration. In lot 150-16008, a post-peak tail was integrated as a separate basic peak, which was not integrated as a basic peak in other lots, and for lot 150-16021, basic peak-2 elutes as a split peak, but it was integrated as a single peak, making the total peak area 0.21% rather than <LOQ if they had been integrated as individual peaks, in which case they would not be used in the calculation of total basic variants. Chromatograms were provided in the response; however, they were not clear and not labeled. An IR was sent to the applicant (2/4/21), to provide clarification as to how the auto-integration parameters, which would be the same between evaluation of different lots, would result in samples being integrated differently. Additionally, the applicant was asked to provide

clear, labeled, zoomed-in chromatograms that support that there is no difference in the peak profile between the two lots of TPI-120 (150-16008 and 150-16021) compared to additional lots of TPI-120. In response (2/19/21) stated that the auto-integration parameters were the same for all lots evaluated, which included “detect shoulder”, due to which peak tail was integrated as a shoulder peak (basic peak 1) in lot 150-16008, and in lot 150-16021 the split peaks within basic peak 2 were integrated as a single peak. Clearly labeled chromatograms were also provided which support that lots 150-16008 and 150-16021 do not have additional basic peaks; there is no visible difference in peak profiles compared to the chromatograms provided for three additional lots of TPI-120. The integration events should be looked at on inspection for the CEX-HPLC method to ensure that peaks are properly integrated, and levels are accurately analyzed.

The mean for total charge variants %area for US-licensed Neulasta lots was 0.83% (range: 0.63% – 1.51%), and for TPI-120 lots it was 0.27% (range: 0.10% – 0.69%), with a standard deviation of 0.234 for US-licensed Neulasta lots and 0.172 for TPI-120 lots (Table 37). The QR calculated from US-licensed Neulasta lots was 0.125% – 1.532%; 10 out of 13 TPI-120 lots were within the QR (Figure 18).

Assessor comment: *Three lots of TPI-120 were outside of the QR, due to a lower level of total charge variants in TPI-120 lots. Since the minimum value reported was 0.10%, the difference is very little, at $\leq 0.025\%$ from the QR. Therefore, this does not preclude a demonstration of similarity.*

Representative chromatograms for US-licensed Neulasta lots and TPI-120 lots are provided in Figures 19 and 20. The peak profiles show that the acidic peaks resulting from the total population of di-PEG variants are larger in US-licensed Neulasta lots than in TPI-120, albeit there is one di-PEG variant with an additional PEG-aldehyde at Lys-41 that is detected at a larger quantity in TPI-120 lots than in US-licensed Neulasta lots; overall this variant was detected at levels less than the LOQ of the method (0.2%).

Assessor comment: *Representative CEX-HPLC chromatograms for TPI-120 lots 190109 and 190108 appear to show a small basic peak (below LOQ) eluting after the main peak at ~16.5 minutes, which is not present in US-licensed Neulasta lots (Figure 20). It is unclear whether this peak is referenced as basic peak 2 in the IR response (2/19/21), and if so, why the peak doesn’t appear in US-licensed Neulasta lots in Figure 20. An IR was sent to the applicant (5/7/21) to provide an explanation and additional information and data to support the identification and characterization of the species (e.g., impact on potency) for the additional variant basic peak observed in TPI-120. In response (5/21/21), the applicant clarified that the small basic peak corresponds to basic peak 2, which is observed at or below levels corresponding to the LOQ of the method (i.e., 0.04% to 0.2%), but not higher than LOQ, and does not increase on stability. Clear identification of the peak was not feasible, as the peak could not be purified or enriched, due to which potency could not be evaluated as well. However, since impurity characterization identified only sequence variants as unique impurities in TPI-120 that are not present in US-licensed Neulasta, it is hypothesized the peak is from a sequence variant. However, this was not confirmed. The applicant was asked whether any sequence variants have been identified during characterization of CEX-HPLC variant peaks in TPI-120 and US-licensed Neulasta; in response the applicant stated that no sequence variants have been identified through CEX-HPLC, only through RP-HPLC (discussed under review of the CAA evaluation by RP-HPLC).*

TPI-120 lots and US-licensed Neulasta lots slightly differ in terms of the levels of different charge variants detected in each product. Overall, the level of acidic and basic variants is lower in TPI-120 lots, while

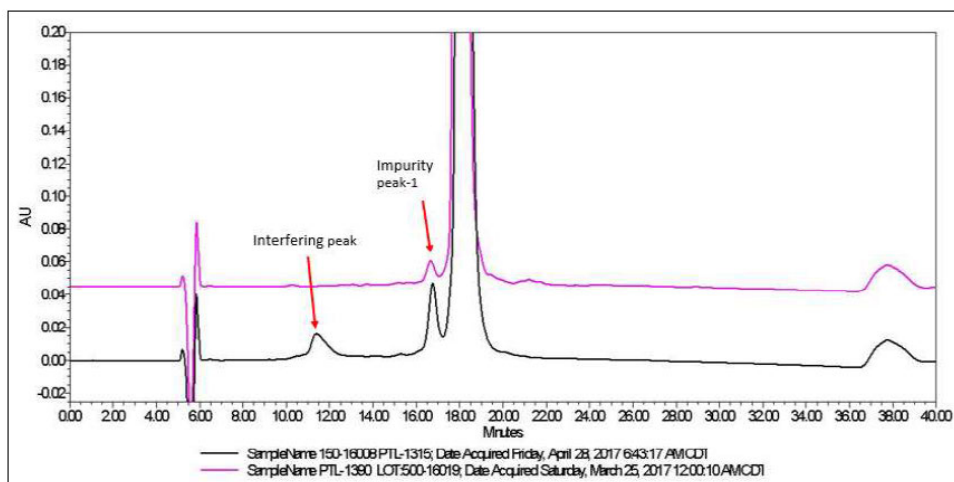
main peak is higher. These slight differences in values, which led to QR criteria not being met for main peak and acidic variants, are minor and do not preclude a demonstration of similarity as the levels of the impurities are very low and the potency of TPI-120 and US-licensed Neulasta is similar. In addition, the chromatograms support that both products contain the same types of charge variants aside from a low level basic variant uniquely present in TPI-120.

Hydrophobic Variants by RP-HPLC

Hydrophobic variants, such as oxidized methionine, di-PEGylated rhG-CSF, reduced, and other species were analyzed in TPI-120 and US-licensed Neulasta lots by RP-HPLC. Twelve lots of TPI-120 and 13 lots of US-licensed Neulasta were used in the initial analysis of hydrophobic variants by Tier 2 analysis (QR using $[\text{mean} \pm 3\sigma]$). Results for main peak, impurity peak 1 (oxidized variant at Met127), combined hydrophobic variants eluting before the main peak (pre-main peak), and combined hydrophobic variants eluting after the main peak (post-main peak) are reported in Table 32 (in the original CAA report; further discussed below).

Assessor comment: In the data provided originally in Table 32, a correction factor was applied to the assessment of impurity peak 1 and main peak, due to the presence of an interfering peak in the RP-HPLC analysis in some of the lots for both TPI-120 and US-licensed Neulasta. The use of a correction factor is not acceptable and suggests that the method was not executed or analyzed appropriately for the runs where a correction factor was applied. An IR was sent to the applicant (11/24/20) to clarify the root cause of the interfering peak, how this peak was identified, and to provide the reports for the investigation (i.e., TR-17-0032L, CAPA-17-038-A, and RPT-0975). Further, they were asked to provide chromatograms that clearly show the presence of the interfering peak, explain why the interfering peak is observed in some analyses and not others, and provide information to support the suitability of the method without the use of a correction factor. In response (12/8/20), the applicant stated that the root cause of the interfering peak was found to be due to an anomalous peak detected during the CAA analysis of samples, independent of which sample was being analyzed, and decreased with each injection, indicating that the cause was systemic in nature and not product-related. Additionally, the anomalous peak impacted the level of pre-peaks since it caused an upward shift in baseline (Figure 2 of IR response). It was found that the presence of the peak was due to inadequate and inappropriate column washing steps and storage after the sequence.

Figure 2. Overlaid chromatogram showing absence and presence of interfering peak in the different sample set of TPI-120.



Two CAPAs were implemented: (1) Revision of the SOP to include instructions for columns washing and storage; and (2) Calculation of a correction factor using a statistical approach to evaluate the data. While the implementation of the first CAPA is appropriate, the use of a correction factor to evaluate the data is inappropriate.

Since the use of the data from those analyses using a correction factor are not suitable to support the CAA, an IR was sent to the applicant (11/24/20) to remove the data from all lots of TPI-120 and US-licensed Neulasta lots that were analyzed using a correction factor to generate the data and to provide data from additional lots of TPI-120 and US-licensed Neulasta where the inferring peak was not observed, and a correction factor was not utilized. In response (1/8/21), the applicant removed all samples that were assessed using a correction factor from the CAA and analyzed an additional 5 lots of US-licensed Neulasta and 1 lot of TPI-120, along with 2 lots of TPI-120 that were previously analyzed without the use of a correction factor for data bridging purposes (Table 1 of the IR response), in a head-to-head assessment using RP-HPLC. The CAA report was updated to include the new lots; analysis of hydrophobic variants by RP-HPLC is included in the IR response as well as the CAA report and is reviewed below.

A total of 11 lots of TPI-120 and 8 lots of US-licensed Neulasta were used in the revised analysis of hydrophobic variants by RP-HPLC, after removal of lots reported to have an interfering peak and the additional of newly analyzed lots of TPI-120 and US-licensed Neulasta. Results for main peak (P6), impurity peak 1 (P4), combined hydrophobic variants eluting before the main peak (pre-main peak), combined hydrophobic variants eluting after the main peak (post-main peak), and total impurities are reported in Table 38 (further discussed below).

The mean main peak %area for US-licensed Neulasta lots was 96.17% (range: 95.71% – 96.85%), and for TPI-120 lots it was 97.10% (range: 96.55% – 97.72%), with a 0.5% CV for US-licensed Neulasta lots and 0.4% CV for TPI-120 lots (Table 39). The QR calculated from US-licensed Neulasta lots was 94.767% – 97.578%; 9 out of 11 TPI-120 lots were within the QR for main peak, and the 2 remaining lots were at borderline (Figure 21).

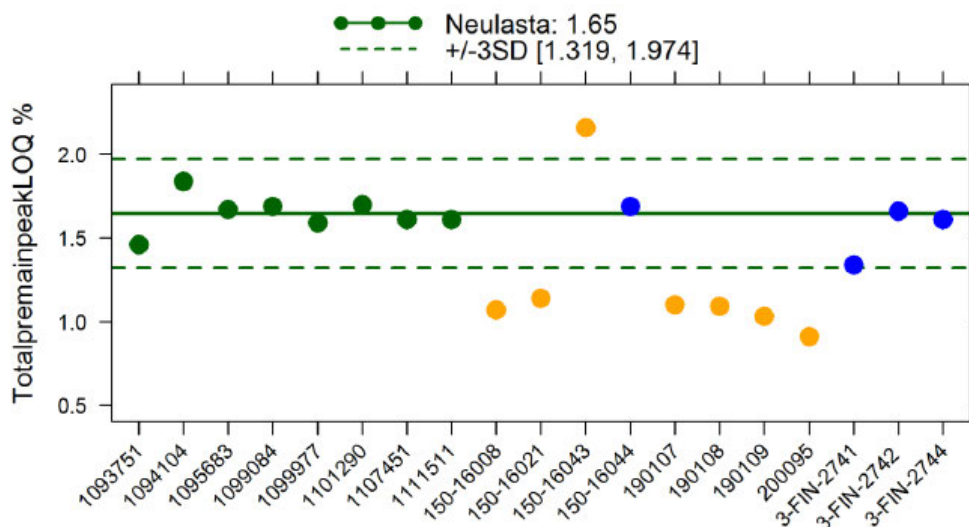
The mean impurity peak 1 %area for US-licensed Neulasta lots was 1.61% (range: 1.46% – 1.7%), and for TPI-120 lots it was 0.97% (range: 0.78% – 1.34%), with a standard deviation of 0.075 for US-licensed Neulasta lots and 0.152 for TPI-120 lots (Table 40). The QR calculated from US-licensed Neulasta lots was 1.382% – 1.830%; 11 out of 11 TPI-120 lots were below the lower quality limit of the QR for impurity peak 1 (i.e., lower level of impurity peak 1 in TPI-120 lots; Figure 22).

Assessor comment: *The lower level of impurity peak 1 is acceptable as the difference compared to US-licensed Neulasta is minimal (0.64%), with no difference in potency between the products. Therefore, this does not preclude a demonstrate of highly similar between TPI-120 and US-licensed Neulasta.*

For total pre-main peak hydrophobic variants, the mean %area for US-licensed Neulasta lots was 1.65% (range: 1.46% – 1.84%), and for TPI-120 lots it was 1.35% (range: 0.91% – 2.16%), with a standard deviation of 0.109 for US-licensed Neulasta lots and 0.385 for TPI-120 lots (Table 43). The QR calculated from US-licensed Neulasta lots was 1.319% – 1.974%; 4 out of 11 TPI-120 lots were within the QR while 6 lots were below the lower limit, and 1 lot was above the upper limit (Figure 25). This does not meet the criteria of at least 90% of TPI-120 lots within the QR.

Assessor comment: While only 4 lots of TPI-120 are within the QR, 6 lots are below the QR. These lots have a lower level of total pre-main peak impurities compared to US-licensed Neulasta lots, however the difference is minimal (0.30%). These TPI-120 lots also did not result in a difference in potency when compared to US-licensed Neulasta. Therefore, this does not preclude a demonstrate of highly similar between TPI-120 and US-licensed Neulasta. The one lot of TPI-120 that is above the upper limit of the QR (lot 150-16043) is further discussed below.

Figure 3. Graphical presentation of comparative results for Total pre-main peak impurities:



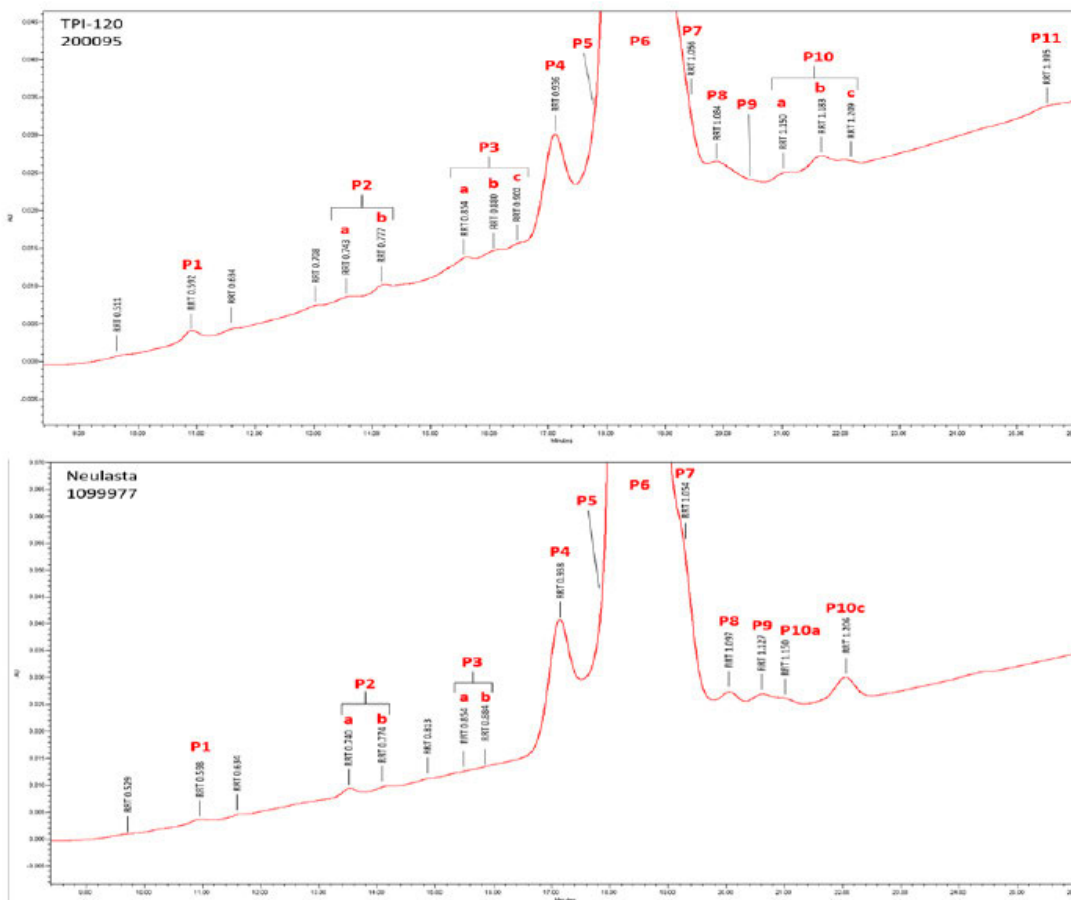
Before the re-analysis of the CAA using new lots, two lots of TPI-120 (i.e., 150-16043 and 3-FIN-2932) were above the upper QR limit for total pre-main peak. Subsequently lot 3-FIN-2932 was removed from the similarity assessment for hydrophobic variants due to the presence of the interfering peak/use of the correction factor for analysis, however lot 150-16043 was included and was observed to still be above the QR (Figure 3, see above). An IR was sent to the applicant (11/24/20) to provide characterization data for the lot above QR to determine the identity of any additional pre-main peak variants that were detected and their impact on potency, if possible. In response (1/8/21), the applicant stated that lot 150-16043 had a disturbed/elevated baseline in the pre-peak region but did not qualify for the definition of the interfering peak based on its usual RRT window and was therefore not analyzed using the correction factor and not excluded from analysis. The elevated baseline resulted in over-estimation of impurity peak 1, which contributed to the high level of total pre-main peak impurities in lot 150-16043, however, an assessment of the chromatogram did not show the presence of any additional pre-main peak impurities (further discussed below). While a root cause was not specified, this is acceptable as this appears to be related to the issue with interfering peaks that was later resolved.

For total post-main peak hydrophobic variants, the mean %area for US-licensed Neulasta lots was 1.93% (range: 0.94% – 2.49%), and for TPI-120 lots it was 1.11% (range: 0.80% – 1.52%), with a standard deviation of 0.682 for US-licensed Neulasta lots and 0.224 for TPI-120 lots (Table 44). The QR calculated from US-licensed Neulasta lots was -0.119% – 3.971%; 11 out of 11 TPI-120 lots were within the QR (Figure 26).

For total impurities the mean %area for US-licensed Neulasta lots was 3.56% (range: 2.61% – 4.19%), and for TPI-120 lots it was 2.44% (range: 1.88% – 3.14%), with a standard deviation of 0.704 for US-licensed Neulasta lots and 0.437 for TPI-120 lots (Table 45). The QR calculated from US-licensed Neulasta lots was 1.449% – 5.673%; 11 out of 11 TPI-120 lots were within the QR (Figure 27).

Representative chromatograms for US-licensed Neulasta and TPI-120 lots were provided in Figures 28, 29, and 30. A difference is observed in the peak profiles between materials (e.g., peaks P3 and P10 elute as three subpeaks in TPI-120, while only as two sub-peaks in US-Neulasta, and P11 is present only in TPI-120 material).

Figure 30: Representative RP-HPLC Expanded Chromatograms of TPI-120 and Neulasta Lots



Assessor comment: The applicant was asked (IR 11/24/20) to provide justification for each of the differences observed in the peak profiles between TPI-120 and US-licensed Neulasta. In response (2/19/21), the applicant provided data for the impurities (characterized by LC-MS/MS) present in each of the peaks. While the majority of the impurities are present in both products, some are found to be higher in TPI-120 (e.g., peak 2b, peak 3a and c, peak 10a and b, and Peak 11), while others higher in US-licensed Neulasta (e.g., peak 4, peak 7, peak 9, and peak 10c). Overall, since the levels of the impurity peaks uniquely present in TPI-120 are fairly low (up to 1.34% in TPI-120 and up to 1.7% in US-licensed

Neulasta), these minor differences do not preclude a demonstration of similarity between TPI-120 and US-licensed Neulasta.

Oxidized and Deamidated Species by RP-HPLC

The RP-HPLC method is capable of detecting oxidized and deamidated variant species; however, the CAA evaluation did not include a direct assessment of the identification and quantification of site-specific oxidized and deamidated residues.

Assessor comment: *The applicant was asked to provide data generated from the analyses of site-specific oxidation and deamidation at each of the applicable residues using a sufficient number of TPI-120 and US-licensed Neulasta lots. In response (1/8/21), the applicant stated that RP-HPLC peaks were identified by LC-MS/MS analysis, which resolves oxidized and deamidated variants of TPI-120. Oxidized variants include methionine residues Met-1 (pegylated), Met-122 (labeled P1, found in very low levels), Met-127 (major oxidized variant in both products, referred to as impurity peak 1, labeled as P4), and Met-128 (elutes very close to main peak, but found in both products). Deamidated variants were found to elute in two major peaks: peak 7 (P7) and peak 9 (P9). P7 was found to be mix of multiple deamidations, Q68,71,87,120,121,135, and 146, while peak P9 was found to be deamidation at Q33 residue (found in very low levels). P7 was also found to have acetylations at two lysine residues (Lys 24 and 35) in both products. A comparative assessment of these variants is provided in the IR response and the CAA and is discussed below.*

Statistical analysis was not performed for P1 (Met-122 oxidation) or P9 (Q33 deamidation) as levels are either very low (<LOQ), or not detected (ND), however, the numerical values are provided in Table 8 of the IR response and/or Table 38 of the revised CAA report. Levels of P1 ranges from ND – 0.21% in TPI-120 lots and 0.03 – 0.1% in US-licensed Neulasta lots, while levels of P9 ranged from ND – 0.44% in TPI-120 lots and ND – 0.27% in US-licensed Neulasta lots. The analysis of P6 (main peak) and P4 (impurity peak 1) is discussed above. For P7, the mean %area for US-licensed Neulasta lots was 0.98% (range: 0.04% – 1.58%), and for TPI-120 lots it was 0.33% (range: 0.04% – 0.74%), with a standard deviation of 0.782 for US-licensed Neulasta lots and 0.284 for TPI-120 lots (Table 40). The QR calculated from US-licensed Neulasta lots was -1.362% – 3.329%; 11 out of 11 TPI-120 lots were within the QR (Figure 23).

Assessor comment: *The data support that oxidation and deamidation levels are similar between TPI-120 and US-licensed Neulasta lots. However, oxidation may occur at tryptophan residues, in addition to methionine residues. An IR was sent to the applicant (2/4/21) to clarify whether oxidation at tryptophan residues was evaluated, and to provide data to support that the levels are similar between TPI-120 and US-licensed Neulasta. In response (2/19/21), the applicant clarified that oxidized variants at tryptophan residue 119 were identified in peaks 3a and 3b. The levels are very low in both products, though slightly higher in TPI-120 (ND – 0.23% in TPI-120 and ND – 0.14% in US-licensed Neulasta). Oxidation at W59 was not detected. The products can be considered similar in terms of levels of oxidized variants. The impact of these impurities on potency will be further discussed in the review of product related impurities in Section 3.2.S.3.2.*

Sequence Variants by RP-HPLC

In addition to hydrophobic, oxidized, and deamidated variants, sequence variants (non-terminal single amino acid misincorporations) were also detected by RP-HPLC. Each sequence variant, their location in

RP-HPLC peaks, their level, and at what level they are present in US-licensed Neulasta is provided in Table 3 below (modified from 3.2.S.3.2).

Table 3: Summary of impurities identified in TPI-120 and Reference Product

Peak Number	ID of TPI-120 DP variants	Min-Max Range TPI-120 DP (%)	Min-Max Range Reference Product (%)
RP-HPLC P2a	(b) (4)	ND - <LOQ	ND - 0.11
RP-HPLC P2b		<LOQ - 0.18	ND - <LOQ
RP-HPLC P3c		ND - 0.12	ND - <LOQ
RP-HPLC P8		ND - 0.54	0.25 - 0.44
RP-HPLC P10 ab		ND - 0.38	ND - 0.29

RP-HPLC: LOQ 0.10%, LOD 0.04%



Assessor co stability, dat not change on An IR was sent to the applicant (5/7/21) to provide characterization data for (i) each sequence variant that is present in TPI-120 but not in US-licensed Neulasta and (ii) each sequence variant that is common in TPI-120 and US-licensed but is present at higher levels in TPI-120 at release or at the end of shelf-life to determine an impact on potency, if feasible. In response (5/21/21), the applicant stated that because sequence variants in RP-HPLC peaks were found at very low levels, and could not be purified or enriched, potency evaluation could not be performed.

Overall, it appears that only one sequence variant is unique to TPI-120 that is not present in US-licensed Neulasta, though for sequence variants that are observed in both products, the levels of each variant differ

slightly between products.

(b) (4)

(b) (4) While there is a slight trend of higher sequence variants in TPI-120, the levels of the sequence variants are generally low in TPI-120, most variants are present in US-licensed Neulasta, and potency and other attributes between products were observed to be similar. Therefore, the presence of these sequence variants in TPI-120 does not preclude a demonstration of similarity.

Amino Acid Sequencing by Peptide Mapping LC-UV

The primary structure/identity of TPI-120 and US-licensed Neulasta was determined by peptide mapping of digested proteins using endoproteinase Glu-C after reduction and alkylation, followed by separation by UHPLC and detection by UV. Three lots of each product were evaluated by peptide mapping by Tier 3 analysis. Data is provided in Figures 37 – 39 and Tables 54 – 56, which show the stacked chromatograms of each product as well as %areas, retention times, and mass of the digested peptides. The peak profile in the stacked chromatograms and retention times of the different peptides are similar between products, while the %area of each peptide are different for G11 (17.7% – 18.7% for TPI-120 and 13.9% – 14.8% for US-licensed Neulasta) and PEG-G1 (7.8% – 11.4% for TPI-120 and 13.0% – 13.3% for US-licensed Neulasta). The measured mass of each of the peptides is also similar between lots of each product and between products and corresponds to the theoretical mass of each peptide.

Assessor comment: Peptide mapping analysis appears to be mostly similar between products, with similar peak profiles of digested peptides, retention times and calculated mass. However, due to the difference in the % area of some of the peptides, an IR was sent to the applicant (11/24/20) to provide justification for the differences observed between the PEG-G1 and G11 peptides in both products. In response (12/8/20), the applicant stated that the PEG-G1 peptide corresponds to the PEGylated N-terminal of the protein, which may not be accurately estimated on HPLC due possible differences in peptide recovery and/or shape due to the PEG moiety. Further, the G11 peptide is one of the longest peptides generated by Glu-C digestion and contains glutamine residues Q135 and Q146, which undergo deamidation. The RP-HPLC data shows that deamidation is slightly higher in US-licensed Neulasta lots compared to TPI-120 lots, which can result in a decrease in %area of the unmodified G11 peptide in US-licensed Neulasta lots compared to TPI-120 lots. The response is acceptable. Additional data provided in Section 3.2.S.3.1 shows that peptide mapping provides 100% sequence coverage for both products, which supports that the sequence of TPI-120 is identical to that of US-licensed Neulasta.

In terms of non-canonical peptides, data was provided from characterization of both theragrastim (representative of the IP) and TPI-120 (Section 3.2.S.3.1) and compared to US-licensed Neupogen and US-licensed Neulasta, respectively. Six peptides were generated by incomplete Glu-C digestion or fragmentation of peptides. Identification of these peptides was confirmed by MS and MS-MS signals. The measured mass of these peptides is comparable to the theoretical mass of each, and the measured mass of the IP/TPI-120 peptides is comparable to the respective reference product.

Table 5. Mass of Non-Canonical Peptides from Glu-C Digestion in Reduced and Alkylated Conditions

Peptide Designation	Theoretical Mass (Da)	Lot # 100-17015-RS (TPI-120 RS)		Lot # 1093751 (Neulasta)	
		RT (min)	Measured Mass (Da)	RT (min)	Measured Mass (Da)
A(100-110)	1184.6289	20.72	1184.6302	20.65	1184.6294
A(114-124)	1382.6177	21.94	1382.6165	21.85	1382.6184
A(111-123)	1538.7075	24.5	1538.7073	24.4	1538.7074
A(35-46)	1520.7003	14.9	1520.7014	14.82	1520.6978
A(106-124)	2238.0515	31.34	2238.0542	31.24	2238.0593
A(100-124)	2834.3684	36.53	2834.3718	36.43	2834.3650

Assessor comment: *The measured mass of the TPI-120 peptides is comparable to the theoretical mass and to the measured mass of the US-licensed Neulasta peptides. Therefore, the amino acid coverage is 100% between products and supports an identical primary structure between the products. The small difference in sequence variants (discuss above) between the products do not preclude a demonstration of identical amino acid sequence.*

Identity by Intact Mass

The intact mass of TPI-120 and US-licensed Neulasta lots was evaluated by Tier 3 analysis to confirm identity of the products. Three lots each were evaluated using LC-MS via electrospray ionization. The theoretical mass of TPI-120 and US-licensed Neulasta was calculated to be 39 kDa, while the experimental molecular mass was determined to be 40.2 kDa for TPI-120 and 40.0 for US-licensed Neulasta (standard deviation of 0 and RSD of 0% for each product). The deconvoluted intact mass spectra (Figure 50) are similar for each lot of each product.

Assessor comment: *The products are similar in terms of their theoretical and calculated intact mass, which is supported by the similarity of the representative intact mass spectra.*

Identity by Western Blot

The identity of TPI-120 and US-licensed Neulasta was evaluated by western blotting by Tier 3 analysis, in three lots of each product. A TPI-120 specific antibody was used to detect the products and confirm their immunological identity; a representative blot is provided in Figure 59. Heavy bands are observed in each product at ~50 kDa.

Assessor comment: *Both TPI-120 and US-licensed Neulasta products migrated to ~50 kDa and were similarly detected by the TPI-120 specific antibody. The products are similar in terms of their immunological identity.*

Disulfide Bond Integrity by LC-MS/MS

The number and location of disulfide bonds in three lots each of TPI-120 and US-licensed Neulasta was evaluated using LC-MS/MS by Tier 3 analysis. The products were digested with endoproteinase Glu-C in native (non-reduced) and non-alkylated conditions, then separated by UHPLC followed by MS/MS identification of the two unique peptides (i.e., G4 and G5) which contain one disulfide bond each. Both TPI-120 and US-licensed Neulasta lots contained both G4 and G5 peptides. The retention time of the G4 peptide was 12.7 minutes for all lots of both products, with a target and observed mass of 1531.7 Da for each, while the retention time of the G5 peptide was 24.3 minutes for all lots of both products, with a

target and observed mass of 4939.6 Da for each. Extracted compound chromatograms for G4 and G5 peptides for TPI-120 and US-licensed Neulasta are provided in Figures 51 – 53, while the MS spectra for the major ion are shown in Figure 54, each of which show a high level of similarity between products

Assessor comment: *The two peptides containing disulfide bond are found in both products, and the retention times and mass are similar for each peptide between TPI-120 lots and US-licensed Neulasta lots. Therefore, the number and location of disulfide bonds in TPI-120 and US-licensed Neulasta are similar.*

Free Thiol using Thiol Fluorometric Detector

The amount of free thiol in three lots each of TPI-120 and US-licensed Neulasta was determined using a proprietary fluorometric detector reagent that reacts with thiol groups to emit a fluorescent signal which is detected using excitation wavelengths between 380-390 nm and emission wavelengths between 510-520 nm. The free thiol concentration ranged from 431 – 504 nM in TPI-120 lots, and 405 – 433 nM in US-licensed Neulasta lots; free thiol per protein molecule was 0.7 – 0.8 in TPI-120 lots and 0.7 in US-licensed Neulasta lots (Tables 63 and 64).

Assessor comment: *The free thiol concentration in each product is similar, though a bit higher in TPI-120 lots, however, there is some overlap between one lot of each product. The free thiol per molecule for each product, however, is similar between TPI-120 and US-licensed Neulasta lots.*

Secondary Structure by Far-UV CD

The secondary structure of TPI-120 and US-licensed Neulasta was evaluated in terms of %helical content and peak position by Tier 3 analysis using Far-UV CD. Three lots of each product were evaluated; results are provided in Tables 47 and 48. The mean Peak 1 position for US-licensed Neulasta lots was 209.2 nm (range: 209.2 nm – 209.7 nm) and for TPI-120 lots it was 209.3 nm (range: 209.2 nm – 209.5 nm) with a standard deviation of 0.0 for US-licensed Neulasta lots and 0.2 for TPI-120 lots; the RSD was 0% for each. The mean Peak 2 position for US-licensed Neulasta lots was 222.0 nm (range: 222.0 nm – 222.0 nm) and for TPI-120 lots it was 221.9 nm (range: 221.9 nm – 222.0 nm) with a standard deviation of 0.0 for US-licensed Neulasta lots and 0.1 for TPI-120 lots; the RSD was 0% for each. The mean helical content for US-licensed Neulasta lots was 71.2% (range: 67.5% – 73.6%) and for TPI-120 lots it was 70.6% (range: 67.5% – 72.6%) with a standard deviation of 3.3 for US-licensed Neulasta lots and 2.7 for TPI-120 lots; the RSD was 5% and 4% for each, respectively. The CD spectra of TPI-120 and US-licensed Neulasta material is provided in Figure 32, which shows a high level of overlap between the products, with correlating peaks in each product.

Assessor comment: *The secondary structure of TPI-120 and US-licensed Neulasta product are observed to be similar in terms of helical content and peak positions, further supported by overlapping spectra, as evaluated by Far-UV CD.*

Amide Bonds by FTIR

The secondary structure of both products was also evaluated by FTIR spectroscopy by Tier 3 analysis. The average α -helix and β -sheet content was compared in four lots each of TPI-120 (including RS lot (b) (4)) and US-licensed Neulasta; data is provided in Table 49. The average α -helix content for US-licensed Neulasta lots ranged from 61.42% – 69.423% and for TPI-120 lots it ranged from 64.032% – 68.784%. The average β -sheet content for US-licensed Neulasta lots ranged from (-)9.332% – (-

)13.468% and for TPI-120 lots it ranged from (-)7.808% – (-)10.893%; the negative values represent negligible β -sheet content. The percentage of overlap in the spectra between each lot of TPI-120 and US-licensed Neulasta to all other lots evaluated by ATR-FTIR was 92.9% – 98.5%. The ATR-FTIR spectra for both products is provided in Figure 34 and shows a high level of overlap.

***Assessor comment:** The secondary structure of TPI-120 and US-licensed Neulasta, in terms of α -helix and β -sheet content, was evaluated orthogonally by FTIR. The data and spectra show comparability between materials in terms of their secondary structure, corroborating results from Far-UV CD analysis.*

Tertiary Structure by Intrinsic Fluorescence

The intrinsic fluorescence (IF) of TPI-120 and US-licensed Neulasta material was evaluated using an FP08200 spectrofluorometer to assess tertiary structure by Tier 3 analysis. Three lots of each product were evaluated for their maximum intensity, the wavelength at the maximum intensity ($\lambda_{\max}$), and the ratio of intensity at 310 nm and $\lambda_{\max}$ (Tables 50 and 51). The mean maximum intensity for US-licensed Neulasta lots was 8591 (range: 8508 – 8634) and for TPI-120 lots it was 8861 (range: 8510 – 9184) with a standard deviation of 71 for US-licensed Neulasta lots and 338 for TPI-120 lots; the RSD was 1% and 4% for each, respectively. The wavelength at the maximum intensity was ~341.5nm for each lot for both products. At 310nm, the mean intensity for US-licensed Neulasta lots was 5357 (range: 5282 – 5492) and for TPI-120 lots it was 5294 (range: 5211 – 5336) with a standard deviation of 117 for US-licensed Neulasta lots and 72 for TPI-120 lots; the RSD was 2% and 1% for each, respectively. The ratio of the intensity at 310 nm and $\lambda_{\max}$ was 0.6 for each lot for both products. Representative IF spectra are provided in Figure 35 for TPI-120 and US-licensed Neulasta materials; the spectra show a high level of overlap between products.

***Assessor comment:** The IF of TPI-120 and US-licensed Neulasta is similar for the lots evaluated, which supports that the tertiary structure of both products is similar.*

Tertiary Structure by Near-UV CD

The tertiary structure of TPI-120 and US-licensed Neulasta was also evaluated using near-UV CD, in the region of 260 nm to 350 nm, as an orthogonal method. Three lots of TPI-120 (including (b) (4)) and three lots of US-licensed Neulasta were analyzed; the overlay of spectra for both products is provided in Figure 36.

***Assessor comment:** The overlay of the spectra show similarity between TPI-120 material and US-licensed Neulasta material, which helps support comparability of the products in terms of tertiary structure.*

Thermal stability by DSC

The thermal stability of TPI-120 and US-licensed Neulasta lots was assessed through differential scanning calorimetry (DSC) by Tier 3 analysis. Three lots each of TPI-120 and US-licensed Neulasta were evaluated for their melting temperatures (T_m). The mean T_m for US-licensed Neulasta lots was 70.55°C (range: 70.49°C – 70.61°C) and for TPI-120 lots it was 70.62°C (range: 70.59°C – 70.65°C) with a standard deviation of 0.06 for US-licensed Neulasta lots and 0.03 for TPI-120 lots; the relative standard deviation (RSD) was 0% for each (Tables 45 and 46). A representative DSC thermogram for TPI-120 and US-licensed Neulasta is provided in Figure 31, which shows a high level of overlap between the products in terms of their T_m .

Assessor comment: *The melting point temperature between TPI-120 and US-licensed Neulasta lots is similar. Although only three lots were evaluated for each, since T_m was evaluated by Tier 3 analysis, and the data/thermogram are supportive of similarity, this is acceptable.*

Higher Order Structure by NMR

The primary and tertiary structure of TPI-120 and US-licensed Neulasta was evaluated using one- and two-dimensional (1D and 2D, respectively) nuclear magnetic resonance (NMR) analysis by Tier 3 analysis, in one lot of each product. Figures 60 – 63 show the overlay of 1D NMR spectra, and while the overlay of 2D NMR spectra are provided in Figure 64.

Assessor comment: *The overlays of the 1D and 2D NMR spectra show identical primary and tertiary structures between TPI-120 and US-licensed Neulasta and demonstrate high similarity and between products.*

Polydispersity using MALDI-TOF Mass Spectrometer

The polydispersity index (PDI) of three lots each of TPI-120 and US-licensed Neulasta was determined using matrix assisted laser desorption/ionization (MALDI), followed by time of flight (TOF) detection by Tier 3 analysis. The mass to charge ratio of singly charged ions was determined, from which the mass weighted molecular weight (M_w) and number weighted molecular weight (M_n) was calculated. The ratio of M_w/M_n (i.e., PDI) measures non-uniformity in the molecular weight of the PEG moiety in the PEG-rhG-CSF conjugate. Results are provided in Table 56 and Figure 40; while the M_w and M_n were similar between lots of each product, they were slightly different between products (M_w : 40182 – 40259 Da for TPI-120 and 39945 – 40033 Da for US-licensed Neulasta and M_n : 40167 – 40244 Da for TPI-120 and 39931 – 40018 Da for US-licensed Neulasta). However, the PDI of each lot of TPI-120 and US-licensed Neulasta was determined to be 1.0 (i.e., uniform).

Assessor comment: *The data show that the PDI of each lot of both products is 1.0, which means that the molecular weight of the PEG moiety in the PEG-rhG-CSF conjugate is uniform in TPI-120 and US-licensed Neulasta, and therefore similar. The mass spectra are also similar between products support similar PEG uniformity between the products.*

PEGylation Site using Enzymatic Digestion, MALDI-TOF, and LC-MS/MS

The PEGylation site of TPI-120 and US-licensed Neulasta was evaluated by Tier 3 analysis using enzymatic digestion followed by LC-MS/MS, in which the proteins are reduced and alkylated before enzymatic treatment by Glu-C or Lys-C, and then separated by UHPLC and detected by UV and MS. The theoretical sequence of digested peptides is provided in Tables 57 and 58 for digestion by Glu-C and Lys-C, respectively; PEGylated peptides have higher total ion chromatogram (TIC) responses compared to non-PEGylated peptides. The high intensity peak after Glu-C digestion, and separately, Lys-C digestion, of three lots each of TPI-120 and US-licensed Neulasta elutes ~23.5 minutes, suggesting a PEGylated peptide. The MS spectra for this peak were extracted from the TIC to confirm the identity of the PEGylated peptides. The presence of multiple peaks with m/z spacing of 44 with a Gaussian distribution suggest the peak corresponds to the PEGylated peptide, PEG-G1 from Glu-C digestion, or PEG-L1 from Lys-C digestion. N- and C- terminal sequencing was performed to further confirm the N-terminal to be the PEGylation site, using in-source decay (ISD) in MALDI-TOF. The ISD-MALDI-TOF MS peak was fit to the rhG-CSF sequence in which there was significant match at the C-terminal, but not the N-terminal (N-terminal is blocked due to PEGylation), suggesting attachment of the PEG at the N-terminal.

Assessor comment: Analysis of the PEGylation site of both TPI-120 and US-licensed Neulasta shows PEGylation of the same digested peptide, eluting ~23.5 minutes for each product. Additionally, the PEGylation was confirmed to be at the N-terminus in both products. Therefore, this supports that the PEGylation site is the same between the products.

However, while PEG characterization was evaluated for TPI-120 and US-licensed Neulasta lots, in terms of PEGylation site and the uniformity of PEG, data to supports that the PEG linker composition is similar between TPI-120 and US-licensed Neulasta was not provided. However, the evaluation of the pegylation site-determination by LC-MS/MS, together with the m-PEG heterogeneity determination based on polydispersity index determination by MS and SEC-MALS, and NMR analysis, along with the structural characterization data (infrared absorption spectroscopy, 1H-NMR spectroscopy and 13C-NMR spectroscopy) provided for m-PEG confirms the structure of the PEG moiety. (b) (4)

Therefore, the information provided is acceptable to support similarity of PEG structure and PEGylation site in both products. (b) (4)

Free PEG by Charged Aerosol Detector (CAD)

The amount of free PEG in TPI-120 and US-licensed Neulasta lots was evaluated using CAD by Tier 3 analysis. Three lots of each product were evaluated; results are provided in Table 68 while representative chromatograms are provided in Figure 66. US-licensed Neulasta lots had 0.03 mg/mL free PEG while TPI-120 lots were found to have 0.01 – 0.02 mg/mL free PEG.

Assessor comment: The amount of free PEG in both products is overall low, while TPI-120 is found to have slightly less free PEG compared to US-licensed Neulasta. This is acceptable; the products are similar in terms of free PEG content. However, it was unclear whether this analysis also included unreacted PEG-aldehyde. An IR was sent to the applicant (11/24/20) to report levels of unreacted PEG-aldehyde, if at quantifiable levels, for the lots evaluated. In response (12/8/20), the applicant stated that the CAD method detects total PEG-aldehyde and does not differentiate between reacted and unreacted PEG-aldehyde. Since the results are inclusive of both reacted and unreacted forms, and are overall very low, this is acceptable.

Free PEG by SDS-PAGE

The amount of free PEG in TPI-120 and US-licensed Neulasta lots was evaluated using SDS-PAGE with titrisol staining, by Tier 3 analysis. The gel images for three lots each of TPI-120 and US-licensed Neulasta are provided in Figures 55 – 58. A heavy band is observed at ~ 50 kDa, and a high molecular weight band is visible at ~125 kDa in each lot of TPI-120 and US-licensed Neulasta. There is not a band associated with free PEG visible in any lots of TPI-120 or US-licensed Neulasta evaluated for free PEG.

Assessor comment: The SDS-PAGE gel images for the different lots of TPI-120 and US-licensed Neulasta show bands that are consistent between lots of each product and between products. There does not appear to be free PEG in either TPI-120 or US-licensed Neulasta, therefore the products are similar in terms of free PEG content.

However, while LMWs in TPI-120 and US-licensed Neulasta lots were evaluated by SE-HPLC for rh-G-CSF content (no PEG moiety; discussed above) and by SDS-PAGE (and orthogonally by CAD) for free

PEG content, it is unclear whether additional low molecular weight variants are also present in the products. An IR was sent to the applicant (11/24/20 and 2/4/21), to provide additional information and/or data that characterizes the LMWs present in TPI-120 and US-licensed Neulasta lots to determine whether additional low molecular weight variants (e.g., N-terminal truncated species) are also present and assess their impact on potency, if possible. In response (1/8/21 and 2/19/21), the applicant stated that US-licensed Neulasta and TPI-120 lots were evaluated by the CE-SDS method, which was validated using free rhG-CSF since other LMWs are either not detected in the product (as demonstrated by forced degradation studies) or at very low level which are not adequate for validation, and no additional LMWs were observed in either product. A representative chromatogram for TPI-120 lot 3-FIN-2931 (6 replicates) and representative electropherograms for both products are provided, which do not show the presence of LMW peaks. The SDS-PAGE is a more sensitive method for detection of LMWs, which were detected in both TPI-120 and US-licensed Neulasta, however, the method is qualitative. SDS-PAGE data show that free filgrastim is the main LMW impurity detected in both products, while 1-2 additional LMW bands appear, which appear more prominently in US-licensed Neulasta lots compared to TPI-120 lots. This is corroborated by SE-HPLC data in which free-filgrastim is observed to be 0.08% – 0.14% in US-licensed Neulasta lots, and 0.01% – 0.06% in TPI-120 lots. Additionally, LC-MS/MS analysis, performed to evaluate truncated variants, shows the presence of very low levels of N-terminal truncation without the Met-1 residue, which is found in both products. The data provided show that additional LMWs, while detected in both products, are observed at very low levels in each. Therefore, the products can be considered similar in terms of LMWs.

Receptor Binding Kinetics by SPR

The receptor binding kinetics of three lots each of TPI-120 and US-licensed Neulasta was evaluated by surface plasmon resonance (SPR) by Tier 3 analysis, to evaluate the rate of association and dissociation between TPI-120 and the G-CSF receptor. Results are provided in Table 67 (average of duplicate runs); for TPI-120 the average rate of association (k_a) was 1.126E+06, rate of dissociation (k_d) was 1.766E-04, and equilibrium constant (K_D) was 1.574E-10, while for US-licensed Neulasta, the average k_a was 8.483E+05, k_d was 1.721E-04, and K_D was 2.056E-10. Representative sensograms for binding curves in the concentration range of 0.8, 1.6, 3.2, 6.4, and 12.8 nM, are provided in Figure 65.

Assessor comment: *The sensograms for binding curves are similar for all concentrations between TPI-120 and US-licensed Neulasta. While no quality ranges were used by Kashiv Biosciences in their evaluation, likely because of inherent method variability and the limited dataset, the Agency generated quality ranges (mean $\pm$ 3SD) to assess similarity with respect to receptor binding affinity and kinetics. All 3 TPI-120 lots reported values for K_D and k_a within the quality ranges; however, 1 TPI-120 lot reported a value for k_a that was slightly higher than the quality range upper limit. In an IR response received on 1/8/2021, additional data was provided by Kashiv Biosciences to show higher variability of k_a values for the US-licensed Neulasta lots (CV of 7.7%) compared to TPI-120 lots (CV of 3.5%). Qualification data for the method was also provided to show that there is higher variability for k_a values with respect to k_a values (8.9% vs. 3.8%, respectively). Changes in receptor binding affinity and kinetics could be an indication of structural changes that may impact receptor binding; however, data generated from the higher order structure assessments demonstrated that TPI-120 is similar to US-licensed Neulasta. Receptor binding by ELISA and the cell-based bioassay assessments, which report relative potency, also showed similar results for TPI-120 and US-licensed Neulasta. The difference in k_a values from the receptor binding by SPR assessment is likely the result of method variability. Therefore, the observed*

difference in k_a values does not preclude a demonstration that TPI-120 is highly similar to US-licensed Neulasta.

Overall Results of Comparative Analytical Assessment

The results from the comparative analytical assessment that support a demonstration that TPI-120 is highly similar to US-licensed Neulasta are summarized in Table 1 below. While not illustrated in the table below, the stability profile of TPI-120 was demonstrated to be similar to that of US-licensed Neulasta.

Table 1. Quality Attributes Analyzed in the Comparative Analytical Assessment

Physico-chemical/Functional Characteristics	Quality Attribute Assessed	Test Method		Supports a Demonstration of Highly Similar
<i>Primary Structure</i>	<i>Amino acid sequence</i>	<i>Peptide mapping LC-UV</i>		<i>Yes</i>
	<i>Intact Mass</i>	<i>LC-MS, electrospray ionization</i>		<i>Yes</i>
	<i>Disulfide mapping</i>	<i>LC-MS/MS</i>		<i>Yes</i>
	<i>Free Thiol</i>	<i>Thiol fluorometric detector</i>		<i>Yes</i>
<i>Pegylation Characteristics</i>	<i>Pegylation site</i>	<i>MALDI-TOF, LC-MS/MS</i>		<i>Yes</i>
	<i>Polydispersity</i>	<i>MALDI-TOF MS</i>		<i>Yes</i>
	<i>Free PEG</i>	<i>CAD</i>		<i>Yes</i>
		<i>SDS-PAGE (Titrisol staining)</i>		<i>Yes</i>
<i>Higher Order Structure</i>	<i>Secondary structure</i>	<i>Far-UV CD</i>		<i>Yes</i>
		<i>FTIR spectroscopy</i>	<i>α-helix content</i>	<i>Yes</i>
			<i>β-sheet content</i>	<i>Yes</i>
	<i>Tertiary structure</i>	<i>Intrinsic fluorescence</i>		<i>Yes</i>
		<i>Near-UV CD</i>		<i>Yes</i>
		<i>DSC</i>		<i>Yes</i>
		<i>NMR</i>		<i>Yes</i>
<i>Purity and product-related variants</i>	<i>Size variants</i>	<i>SE-HPLC</i>	<i>Monomer</i>	<i>Yes</i>
			<i>Total size variants</i>	<i>Yes</i>
			<i>Di-pegylated variants</i>	<i>Yes</i>
			<i>Aggregates</i>	<i>Yes</i>
			<i>Free rhG-CSF</i>	<i>Yes</i>
	<i>Charge variants</i>	<i>CEX-HPLC</i>	<i>Main species</i>	<i>Yes</i>
			<i>Acidic variants</i>	<i>Yes</i>
			<i>Basic variants</i>	<i>Yes</i>
	<i>Hydrophobic variants</i>	<i>RP-HPLC</i>	<i>Main species</i>	<i>Yes</i>
			<i>Total variants</i>	<i>Yes</i>
			<i>Post-main variants</i>	<i>Yes</i>

Physico-chemical/Functional Characteristics	Quality Attribute Assessed	Test Method		Supports a Demonstration of Highly Similar	
		<i>Pre-main variants</i>		Yes	
		<i>Oxidized variants</i>		Yes	
		<i>Deamidated variants</i>		Yes	
		<i>Truncated variants</i>	<i>CE-SDS (non-reduced)</i>		Yes
		<i>Aggregate characterization</i>	<i>AUC</i>		Yes
			<i>DLS</i>		Yes
<i>Bioactivity</i>	<i>Cell proliferation</i>	<i>In vitro cell-based bioassay</i>		Yes	
	<i>Receptor binding</i>	<i>ELISA (relative potency)</i>		Yes	
		<i>SPR (binding affinity and kinetics)</i>	<i>K_D (M)</i>	Yes	
			<i>k_a (1/Ms)</i>	Yes	
			<i>k_d (1/s)</i>	Yes	
<i>Drug Product Attributes</i>	<i>Protein concentration</i>	<i>UV Absorbance Spectroscopy</i>		Yes	

Overall Conclusion: *The CAA was consistent with the FDA Guidance for Industry, Development of Therapeutic Protein Biosimilar: Comparative Analytical Assessment and Other Quality-Related Considerations, May 2019. Extensive analytical characterization of TPI-120 and US-licensed Neulasta was performed, which included comparisons of primary structure, pegylation characteristics, higher order structure, purity and product-related variants, size-variant characterization, bioactivity (potency and receptor binding), and protein concentration. Attributes were selected based on the established quality target product profile for US-licensed Neulasta and the defined critical quality attributes (CQAs) for TPI-120. Overall, the CAA assessment that evaluated TPI-120 and US-licensed Neulasta is meaningful, extensive, and robust, using multiple orthogonal methods to allow for adequate characterization of both products, appropriate selection of lots, and an acceptable data analysis plan. The results from the CAA studies, including the comparative stability and forced degradation studies, which are described below, support a demonstration that TPI-120 is highly similar to US-licensed Neulasta, notwithstanding minor differences in clinically inactive components.*

Comparative Assessment of Stability Profiles

Studies were performed to evaluate the degradation pathways and rates of TPI-120 compared to US-licensed Neulasta. These include stability studies at varying storage conditions as well as forced degradation studies, both of which are further reviewed below.

Comparative Stability Studies (Document PTL-1791-R)

TPI-120 DP and US-licensed Neulasta lots were evaluated in a comparative stability study to assess whether the products have comparable degradation profiles in accelerated and stressed temperature conditions, in order to support the determination of highly similar. Three lots of each product (i.e., TPI-120 lots 150-16008 (clinical), 3-FIN-2742 (PPQ) and 3-FIN-2744 (PPQ) and US-licensed Neulasta lots 1068889, 1066654 and 1073267) were evaluated at the accelerated condition of 25 ± 2°C/60 ± 5% RH

and the stressed condition $40 \pm 2^\circ\text{C}/75 \pm 5\% \text{ RH}$ for up to 6 months. The lots were analyzed for their physiochemical properties (e.g., purity, product-related impurities, PEGylation characterization, etc.), and biological activity (e.g., cell proliferation assay).

Assessor comment: *The number of lots and time points evaluated as part of the comparative stability studies is acceptable. While only one storage condition (i.e., heat stress) was evaluated, the forced degradation studies, described below, included multiple relevant pathways of degradation. The accelerated and stressed temperature conditions are adequate to allow for incremental degradation of DP over time in storage.*

Results from Comparative Stability Studies

Appearance

The appearance of three lots each of TPI-120 and US-licensed Neulasta was evaluated at both accelerated and stressed conditions for up to 6 months. The acceptance criteria (AC) of “clear, colorless to slightly yellow liquid” was met for each lot at each time point (i.e., 0, 3, and 6 months) for both products at both 25°C and 40°C . Results are provided in Table 4.1a and 4.1b.

SDS-PAGE with Silver Staining (non-reduced)

Non-reduced SDS-PAGE with silver staining was used to qualitatively evaluate degradation of TPI-120 and US-licensed Neulasta lots (e.g., HMW and LMWs) at the accelerated and stressed conditions. The AC were based on system suitability criteria: (1) The high molecular weight (HMW) impurity band, if present in the sample(s), should not be more intense than the TPI 120 band in system suitability high (0.1 μg) solution, (2) the low molecular weight (LMW) impurity band, if present in the sample, should not be more intense than the filgrastim band in system suitability high (0.1 μg) solution, and (3) the sample should not have any more bands in addition to the ones observed in the RS solution; minor bands may be present in the sample provided the intensity of each is not more than the principal band in system suitability low (0.02 μg) solution. At the 25°C accelerated condition, both TPI-120 and US-licensed Neulasta lots had results of “Pass” for each AC at each time point. At 40°C , both TPI-120 and US-licensed Neulasta lots had results of “Fail” at the 3-month and 6-month time point for AC 3, while only TPI-120 lots had results of “Fail” at the 3-month and 6-month time point for AC 2. Results are provided in Table 4.2a – 4.2d while representative gel images for each product at 0- and 6-month timepoints for each condition are provided in Figures 4.2a – 4.2f. The HMW and LMW bands visible in the gels are more intense after 6 months in storage at both 25°C and 40°C for both products. Additional HMW and LMW bands are also visible after 6 months of storage, compared to 0 months and the RS.

Assessor comment: *An increase in both HMW and LMWs is visible (in terms of number and intensity of bands) in the gel images for both TPI-120 and US-licensed Neulasta lots after storage at 25°C and 40°C for up to 6 months. The results of “Fail” are expected due to the accelerated/stressed temperature conditions, therefore this is acceptable. Though the number and intensity of the bands appears to be similar between products, quantitative data was not provided for confirmation.*

Free rhG-CSF by SDS-PAGE with silver staining (non-reduced)

The level of free rhG-CSF in TPI-120 and US-licensed Neulasta lots was also qualitatively evaluated using SDS-PAGE (non-reduced) with silver staining, for both accelerated and stressed storage conditions up to 6 months. The AC was based on system suitability criteria: Intensity for the free rhG-CSF band in the sample should not be more intense than the free rhG-CSF band in the system suitability low (0.02 μg)

solution. While TPI-120 lots had results of “Pass” through 6 months of storage at 25°C, US-licensed Neulasta lots “Failed” at 3 and 6 months at 25°C. Both TPI-120 and US-licensed Neulasta lots “failed” at the 3- and 6-month time point at 40°C. Results are provided in Table 4.3a and 4.3b, and representative gel images are provided in Figures 4.2a – 4.2f. The free rhG-CSF band was more intense after storage in both conditions after 6 months.

***Assessor comment:** The results of “Fail” are expected due to the accelerated/stressed temperature conditions, therefore this is acceptable. The intensity of the free rhG-CSF band appears to be similar between TPI-120 and US-licensed Neulasta lots. However, quantitative data was not provided for confirmation (see below for quantitative analysis of rhG-CSF levels). An IR was sent to the applicant (11/24/20) to provide a quantitative assessment of the data for “free filgrastim”, if available, in order to assess the stability of PEG linkage in TPI-120 and US-licensed Neulasta lots, or a justification as to why this data is not necessary. In response (1/8/21), the applicant stated that free filgrastim was also evaluated by SE-HPLC and provided graphical data to show that at both 25°C and 40°C, TPI-120 and US-licensed Neulasta lots show a similar increase in free filgrastim over time in storage up to 6 months. Results are similar between products (discussed further below under SE-HPLC).*

Free PEG by SDS-PAGE with Titrisol Staining (non-reduced)

The level of free PEG in TPI-120 and US-licensed Neulasta lots was qualitatively evaluated using SDS-PAGE (non-reduced) with titrisol staining, for both accelerated and stressed storage conditions up to 6 months. The AC was based on system suitability criteria: Intensity for the free PEG band in the sample should not be more intense than the free PEG band in the system suitability low (0.1 µg) solution. Both TPI-120 lots had results of “Pass” through 6 months of storage at 25°C, and both TPI-120 and US-licensed Neulasta lots “Fail” at the 3- and 6-month time point at 40°C. Results are provided in Table 4.4a and 4.4b, and representative gel images are provided in Figures 4.4a – 4.4c. The free PEG band was more intense in both products after storage in both temperature conditions after 6 months.

***Assessor comment:** The results of “Fail” are expected due to the accelerated/stressed temperature conditions, therefore this is acceptable. The free PEG band appears to be more intense for both TPI-120 and US-licensed Neulasta lots at the 6-month time point compared to the 0-month time point after storage at both 25°C and 40°C, however whether the intensity of the bands is similar between products is unclear as quantitative data was not provided. An IR was sent to the applicant (11/24/20) to provide a quantitative assessment of the data for free PEG, if available, to assess the stability of PEG linkage in TPI-120 and US-licensed Neulasta lots, or a justification as to why this data is not necessary. In response (1/8/21), the applicant stated that free PEG was evaluated only by SDS-PAGE with titrisol staining. However, since the level of free PEG corresponds to the level of free filgrastim observed over time in storage at 25C and 40C, which is found to increase similarly in both products as evaluated by SE-HPLC evaluation, the level of free PEG is likely to correspond to the level of free filgrastim. Since the gel analysis also shows a similar increase in free PEG over time in storage in both products, the data provided is sufficient to support similarity.*

Size Exclusion by HPLC

Size variants in TPI-120 and US-licensed Neulasta lots after up to 6 month of storage in accelerated and stressed conditions were evaluated by SE-HPLC. The levels of monomer, di-PEGylated species, free filgrastim, aggregates, any other single impurity peak, and sum of total impurities in both products at both storage conditions is provided in Tables 4.5a – 4.5d. At 25°C, a marginal decrease was observed in

monomer content for all 3 lots of each product (i.e., decrease of 0.7% – 0.8% in TPI-120 lots and 1.0% – 1.2% in US-licensed Neulasta lots), while marginal increases were observed for di-PEG species (i.e., increase of 0.2% for TPI-120 lots and 0.2% – 0.3% for US-licensed Neulasta lots), free filgrastim (i.e., increase of 0.3% for each lot of each product), aggregates (i.e., increase of 0.4% in TPI-120 lots and 0.6% – 0.7% in US-licensed Neulasta lots), as well as the sum of all impurities (i.e., increase of 0.7% – 0.9% in TPI-120 lots and 1.0% – 1.2% in US-licensed Neulasta lots). There was no increase observed overall in any other single impurity peak in either product (i.e., not detected in TPI-120 and no increase in US-licensed Neulasta). At 40°C, a greater decrease was observed in monomer content for all 3 lots of each product (i.e., decrease of 13.7% – 20.2% in TPI-120 lots and 19.6% – 25.2% in US-licensed Neulasta lots), while increases were observed for di-PEG species (i.e., increase of 0.4% – 0.5% for TPI-120 lots and 0.3% – 0.5% for US-licensed Neulasta lots), free filgrastim (i.e., increase of 1.5% – 1.7% for TPI-120 lots and 1.4% – 1.5% for US-licensed Neulasta lots), aggregates (i.e., increase of 11.7% – 18.5% in TPI-120 lots and 17.9% – 23.3% in US-licensed Neulasta lots), as well as the sum of all impurities (i.e., increase of 13.8% – 20.4% in TPI-120 lots and 19.6% – 25.2% in US-licensed Neulasta lots). There was no increase in any other single impurity peak in TPI-120 lots (i.e., not detected), while one lot of US-licensed Neulasta had an increase of 0.6% (remained two lots had results of “not detected”). Trend plots are provided in Figures 4.5a – 4.5j. Representative chromatograms are provided in Figures 4.5.1a – 4.5.1f.

Assessor comment: *The rates of degradation of TPI-120 and US-licensed Neulasta lots in terms of size variants was similar at the accelerated storage condition of 25°C up to 6 months of storage; however, the changes are minor. All lots of both products met the AC for monomer content (AC: ≥ 96.5%), any other single impurity peak (AC: no more than (NMT) 2.0%), and sum of all impurities (AC: NMT 3.5%) after time in storage (AC of report results for other variants). At 40°C, the rates of degradation were similar, though the US-licensed Neulasta product was observed to have a slightly higher level of degradation by 6 months. The lots of both products met the AC for any other single impurity peak, but not for monomer content and the sum of all impurities after time in storage. This is acceptable, as degradation of the product is expected due to the stressed storage conditions. The chromatograms for both products are similar at each storage condition, in terms of number and position of peaks. Overall, the degradation of TPI-120 and US-licensed Neulasta lots is similar in terms of size variants, with potentially less overall degradation in TPI-120 material, and therefore a slightly more stable product. This is acceptable.*

Purity by RP-HPLC

Purity of TPI-120 and US-licensed Neulasta lots was assessed by RP-HPLC, after up to 6 month of storage in accelerated and stressed conditions. The levels of main peak, any other single impurity peak, and sum of total impurities for both products at both storage conditions is provided in Tables 4.6a – 4.6d. At 25°C, a decrease was observed in main peak for all 3 lots of each product (i.e., decrease of 3.0% – 4.0% in TPI-120 lots and 3.3% – 4.2% in US-licensed Neulasta lots), while an increase was observed in a single impurity peak (i.e., increase of 0.8% – 1.4% for TPI-120 lots and 1.6% – 2.2% for US-licensed Neulasta lots). Overall, the sum of all impurities increased (i.e., increase of 2.9% – 3.9% in TPI-120 lots and 2.7% – 3.8% in US-licensed Neulasta lots). At 40°C, a greater decrease was observed in main peak for all 3 lots of each product (i.e., decrease of 15.5% – 16.8% in TPI-120 lots and 17.5% – 24.5% in US-licensed Neulasta lots), while a greater increase was observed in a single impurity peak (i.e., increase of 7.6% – 8.9% for TPI-120 lots and 9.0% – 9.4% for US-licensed Neulasta lots). Overall, the sum of all impurities increased (i.e., increase of 15.6% – 16.8% in TPI-120 lots and 17.4% – 24.6% in US-licensed Neulasta lots). Trend plots are provided in Figures 4.6a – 4.6f and representative chromatograms are provided in Figures 4.6.1a – 4.6.1f.

Assessor comment: *The degradation profile of TPI-120 and US-licensed Neulasta lots is similar at the accelerated storage condition of 25°C up to 6 months of storage. The lots of both products did not meet the AC for main peak (AC: $\geq 96.5\%$), any other single impurity peak (AC: NMT 2.0%), and sum of all impurities (AC: NMT 3.5%) after time in storage. This is expected due to the accelerated storage conditions at the higher temperature. At 40°C, the rates of degradation were similar, though the US-licensed Neulasta lots were observed to have a slightly higher level of degradation by 6 months. The lots of both products did not meet the AC for main peak, any other single impurity peak, and the sum of all impurities after time in storage. This is expected due to the stressed storage conditions. The chromatograms for both products are similar at each storage condition, in terms of number and position of peaks. Overall, the degradation of TPI-120 and US-licensed Neulasta lots is similar in terms of purity, with potentially less overall degradation in TPI-120 material, and therefore a slightly more stable product. This is acceptable.*

Charge Heterogeneity by CEX

The charge heterogeneity of TPI-120 and US-licensed Neulasta lots was assessed by CEX, after up to 6 month of storage in accelerated and stressed conditions. The levels of total acidic impurities, total basic impurities and total charge variants for both products at both storage conditions is provided in Tables 4.7a – 4.7d. At 25°C, total acidic impurities were below the limit of quantitation (LOQ) in each lot of TPI-120 up to 6 months, while a marginal increase was observed in US-licensed Neulasta lots (i.e., increase of 0.2% – 0.3% at 3 months, but only 0% – 0.1% at 6 months). A marginal decrease was observed for total basic impurities in TPI-120 lots (i.e., decrease of 0% – 0.1%), while in US-licensed Neulasta, one lot had a marginal decrease in total basic impurities and two lots had a marginal increase (i.e., 0.2% decrease in lot 1066654, and 0.1% increase in lots 1068889 and 1073267). Total charge variants marginally decreased in TPI-120 lots (i.e., 0% – 0.1%), while one lot of US-licensed Neulasta had a marginal decrease and two lots had a marginal increase (i.e., 0.1% decrease in lot 1066654, 0.2% increase in lot 1068889, and 0.1% increase in lot 1073267). At 40°C, total acidic impurities slightly increased by 6 months in TPI-120 lots (i.e., <LOQ to 0.2%), while a larger increase was observed in US-licensed Neulasta lots (i.e., increase of 0.2% – 1.1%). A greater increase was observed for total basic impurities in TPI-120 and US-licensed Neulasta lots (i.e., increase of 12.6% – 18.5% in TPI-120 lots and 19.0% – 26.5% in US-licensed Neulasta lots). Total charge variants also had a larger increase in both products (i.e., 12.9% – 18.5% in TPI-120 lots and 19.5% – 27.5% in US-licensed Neulasta lots). Trend plots are provided in Figures 4.7a – 4.7f, and representative chromatograms are provided in Figures 4.7.1a – 4.7.1f.

Assessor comment: *There was not much change in the level of charge variants in either product after storage at 25°C for 6 months; the charge profile is stable and similar between TPI-120 and US-licensed Neulasta at this storage condition. At 40°C, the increase in charge variants was similar between products, though the US-licensed Neulasta lots were observed to have a slightly higher level of acidic and basic variants by 6 months. This is acceptable, as the TPI-120 product degrades slower than and/or to a slightly lesser extent than US-licensed Neulasta in the accelerated condition. The chromatograms for both products appear mostly similar, with the exception of an additional minor acidic peak (labeled “Acidic 11”) observed in TPI-120 lots in each condition, at both 0 and 6 months. This was also observed in the comparison of charge variants in the CAA and is discussed further in that section.*

Potency by Cell Proliferation Assay

The potency of TPI-120 and US-licensed Neulasta lots was evaluated using a cell proliferation assay with rhG-CSF adapted M-NFS-60 cells, after up to 6 month of storage in accelerated and stressed conditions.

The biological activity is provided in terms of relative potency in comparison to RS (US-licensed Neulasta lot 1053071). The results are provided in Tables 4.8a and 4.8b for both products. At 25°C, a marginal decrease in potency is observed in each lot of each product after 6 months of storage (e.g., 98% to 95% in TPI-120 and 101% to 99% in US-licensed Neulasta). At 40°C, a greater decrease in potency is observed in each lot of each product after 6 months of storage (e.g., 102% to 80% in TPI-120 and 97% to 79% in US-licensed Neulasta). Representative dose response curves are provided in Figures 4.8a – 4.8c with no significant differences noted between TPI-120 and US-licensed Neulasta.

Assessor comment: *The change in potency is similar between TPI-120 and US-licensed Neulasta in stressed storage conditions.*

Overall, the comparative stability study, using accelerated and stressed storage conditions of 25°C and 40°C, respectively, showed that TPI-120 lots and US-licensed Neulasta lots are similar in terms of their degradation profiles after storage for up to 6 months.

Forced Degradation Studies (Document PTL-2249-R)

TPI-120 DP and US-licensed Neulasta lots were evaluated in a forced degradation study to assess whether the products have comparable degradation profiles and pathways, in order to support the determination of highly similar. Three lots of each product (i.e., commercial TPI-120 lots 190107, 190108, and 190109 and US-licensed Neulasta lots 1094104, 1095683, and 1093751) were evaluated using the conditions listed in Table 2. The lots were analyzed for their physiochemical properties (e.g., purity, product-related impurities, and concentration), and biological activity (e.g., cell proliferation assay).

Table 2: Stress Conditions for Forced Degradation

Degradation Stress	Conditions	Time Points
Thermal	45 ± 2 °C	0, 1, 8, 14 days
Light exposure (ICH Q1B Option II)	White light (kLux/hr), UV (watts/m ²)	0, 0.5, 1 ICH described cycles
Oxidation (Peroxide)	RT; 0.012% H ₂ O ₂	0, 2, 7, 24 hours
Low pH, 2.4±0.3	~6 µL of 0.5 N HCl treatment for 300 µL sample	0, 1, 7, 14 days
High pH, 9.5±0.3	~10.5 µL of 0.5 N NaOH treatment for 300 µL sample	0, 1, 4, 11 days

Assessor comment: *The number of lots, time points, and conditions evaluated as part of the forced degradation studies is acceptable. The comparative stability studies included a longer storage duration for the thermal stress condition, i.e., samples were stored at 40°C for up to 6 months; therefore, changes under thermal stress in this evaluation are not as robust as the changes observed during the comparative stability study. While the purity and potency of the samples was assessed, the stability of the PEG linkage was not directly assessed in the forced degradation studies. An IR was sent to the applicant (11/24/20) to provide data to support that the stability of the PEG linker is similar between TPI-120 and US-licensed Neulasta lots under the forced degradation conditions. In response (1/8/21), the applicant stated that*

while the stability of the PEG linkage was not directly assessed during forced degradation studies, the instability of the linkage will lead to de-pegylation, which can be monitored through the evaluation of free-filgrastim. Since free-filgrastim was evaluated by SE-HPLC and RP-HPLC in each stress condition, information was indirectly provided for the stability of the PEG linker. The data provided for free filgrastim is reviewed within each section below.

Results from Forced Degradation Studies

Thermal Stress

Each lot of TPI-120 and US-licensed Neulasta was stored at 45°C for up to 14 days; aliquots were removed for analysis at each time point. Samples were analyzed using RP-HPLC, SE-HPLC, CEX, and a cell proliferation assay using M-NFS-60 cells.

Purity by RP-HPLC

An overlay of chromatograms is provided in Figure 1A and 1B for Day 0 and Day 14, respectively, while trend plots are provided in Figure 2. At Day 0, the chromatograms appear similar in peak profiles, though with more prominent peaks after the main peak in US-licensed Neulasta lots. At Day 14, the peak profiles appear similar in terms of number and position of peaks between TPI-120 and US-licensed Neulasta lots. The trend plots show a similar decrease in main peak in both products, while a similar increase is observed for the peak at RRT 1.084 – 1.102 and RRT 1.056 – 1.059. The peak observed at RRT 0.931 – 0.943 remains stable (though at different levels) in both products.

Size Variants by SE-HPLC

An overlay of chromatograms is provided in Figure 3A and 3B for Day 0 and Day 14, respectively, while trend plots are provided in Figure 4. At Day 0, the chromatograms appear similar in peak profiles in terms of peak position, though more prominent dimer peaks are observed in US-licensed Neulasta lots, while more prominent aggregate peaks are observed in TPI-120 lots. Additionally, a larger free filgrastim peak is observed in US-licensed Neulasta lots. At Day 14, the peak profiles appear similar in terms of number and position of peaks between TPI-120 and US-licensed Neulasta lots. The trend plots show a similar decrease in main peak in both products, while a similar increase is observed in aggregates and total size variants. The di-PEG (i.e., dimer) variant remains stable in both products over time (though at different levels).

Assessor comment: *The overlay of the chromatograms in Figure 3B show that an additional large peak after the main peak is observed for TPI-120 lot 190107. An IR was sent to the applicant (11/24/20) to provide an explanation for this additional large peak, including information and data to characterize this peak. In response (1/8/21), the applicant stated that the additional peak was observed only in one lot of TPI-120 and elutes very close to the formulation buffer peak which elutes as a doublet peak in the end, at ~22-24 minutes. Since the method requires a mixture of water and mobile phase to be run as a blank diluent, in water elutes in the first part of the doublet, it is likely that the blank diluent was used as the diluent instead of water, which lead to a larger first part of the doublet peak, corresponding to formulation buffer. Since additional LMW variants are not observed in this sample, the peak is only observed in one sample, and the presence of this peak is attributed to matrix components, the peak does not impact the comparability of the products overall.*

Charge Variants by CEX

An overlay of chromatograms is provided in Figure 5A and 5B for Day 0 and Day 14, respectively, while trend plots are provided in Figure 6. At Day 0, the chromatograms appear similar in peak profiles in terms of peak position, though the RRT 0.91 – 0.97 peak is larger in US-licensed Neulasta lots, and an additional acidic peak is observed in TPI-120 lots (further discussed under CAA review). At Day 14, the peak profiles remain largely unchanged. The trend plots show that the main peak and peak at RRT 0.92 – 0.97 remain stable over 14 days in both products.

Potency by Cell Proliferation Assay

Results for potency are provided in Table 4 for each lot of TPI-120 and US-licensed Neulasta at each time point, while a trend plot is provided in Figure 7. A decrease in potency is observed for both products over time (e.g., 99% to 87% for TPI-120 and 96% to 76% for US-licensed Neulasta), though one lot of TPI-120 did not show any decrease in potency over 14 days. The trend plot shows an overall decrease in potency in both products, though to a greater extent in US-licensed Neulasta lots compared to TPI-120 lots.

Summary of Results

Data is provided for analysis of main peak (%area) by RP-HPLC, SE-HPLC, and CEX at each time point, for each lot of TPI-120 and US-licensed Neulasta in Table 5; the rate of degradation of main peak by each method is provided in Table 6. The rates of degradation are similar between TPI-120 and US-licensed Neulasta (i.e., -1.5702 for TPI-120 and -1.5855 for US-licensed Neulasta for SE-HPLC, and 0.0176 for TPI-120 and 0.0119 for US-licensed Neulasta for CEX), though slightly larger in US-licensed Neulasta lots in the analysis of main peak by RP-HPLC (i.e., -0.3705 for TPI-120 and -0.4267 for US-licensed Neulasta).

Assessor comment: *While tabular data was provided for main peak content by RP-HPLC, SE-HPLC, and CEX under thermal stress, from which rates of degradation for main peak were determined, the data for impurities was not provided. An IR was sent to the applicant (11/24/20) to provide individual data points (i.e., all time points) for each impurity (individual and total) that was evaluated under thermal stress in tabular format, and for each of the included datasets, provide an assessment of the rates of formation for the individual/total impurity in the TPI-120 and US-licensed Neulasta lots. In response (1/8/21), the applicant provided the requested data, which is reviewed below.*

Under thermal stress, impurities at RRt ~0.74-0.75, RRt ~1.06, RRt 1.08-1.10, and RRt 1.19-1.22 are observed to increase (though very low) over 14 days for each of the 3 lots of TPI-120 and US-licensed Neulasta as evaluated by RP-HPLC. The rates of formation for each individual impurity peak are somewhat similar between TPI-120 and US-licensed Neulasta (i.e., 0.01 for TPI-120 and 0.01 for US-licensed Neulasta for RRt ~0.74-0.75, 0.11 for TPI-120 and 0.17 for US-licensed Neulasta for RRt ~1.07, 0.1 for TPI-120 and 0.01 for US-licensed Neulasta for RRt 1.08-1.10, and 0.01 for TPI-120 and 0.01 for US-licensed Neulasta for RRt 1.19-1.22). The rate of degradation of main peak is equivalent to the rate of formation of total impurities (i.e., -0.37 for TPI-120 and -0.43 for US-licensed Neulasta).

Evaluation by SE-HPLC shows that aggregates increase largely over time, while levels of dimers and free rhG-CSF remain stable or slightly increase over 14 days for each of the 3 lots of TPI-120 and US-licensed Neulasta. The rates of formation for aggregates is similar between TPI-120 and US-licensed Neulasta (i.e., 1.55 for TPI-120 and 1.59 for US-licensed Neulasta), while the rates of formation are essentially stable

for dimers and free rhG-CSF (i.e., 0.01 for each for TPI-120 and 0.00 for each for US-licensed Neulasta). The rate of degradation of main peak is similar to the rate of formation of total impurities (i.e., -1.57 for TPI-120 and -1.59 for US-licensed Neulasta).

Degradation was not observed in the evaluation impurities by CEX-HPLC, therefore rates of degradation/formation for this evaluation were not provided.

Assessor comment: The data provided show that both products are similar in terms of their degradation profiles and resulting impurities arising from thermal stress.

Overall, thermal stress at 45°C for up to 14 days results in degradation of TPI-120 and US-licensed Neulasta products in a similar manner; purity and potency decreases slightly while size variants increase. This corresponds to the results in the comparative stability study (discussed above), in which lots were stored at 40°C for up to 6 months, confirming temperature as a pathway for degradation in both products.

Light Stress

Each lot of TPI-120 and US-licensed Neulasta was subjected to a half and one ICH-described light exposure cycle (i.e., 0.6 million lux hours of cool white fluorescent light and 100-watt hours/square meter of near-UV fluorescent light at $25 \pm 2^\circ\text{C}$ and 1.2 million lux hours of cool white fluorescent light and 200-watt hours/square meter of near-UV fluorescent light at $25 \pm 2^\circ\text{C}$, respectively). Samples were analyzed using RP-HPLC, SE-HPLC, CEX, and a cell proliferation assay using M-NFS-60 cells.

Purity by RP-HPLC

An overlay of chromatograms is provided in Figure 8A and 8B for $t = 0$ and $t = 1$ cycle, respectively, while trend plots are provided in Figure 9. The $t = 0$ chromatograms are the same as for thermal stress. At $t = 1$ cycle, the peak profiles appear similar in terms of number and position of peaks between TPI-120 and US-licensed Neulasta lots. The trend plots show a similar decrease in main peak in both products, while a similar increase is observed for the peaks at RRT 0.91 – 0.96 and RRT 0.97 – 0.98.

Size Variants by SE-HPLC

An overlay of chromatograms is provided in Figure 10A and 10B for $t = 0$ and $t = 1$ cycle, respectively, while trend plots are provided in Figures 11 and 12. The $t = 0$ chromatograms are the same as for thermal stress. At $t = 1$ cycle, the peak profiles appear similar in terms of number and position of peaks between TPI-120 and US-licensed Neulasta lots. The trend plots show a similar decrease in main peak in both products. A similar increase is observed in aggregates (though to a marginally higher extent in US-licensed Neulasta lots), free filgrastim, di-PEG variants, and total size variants in both products.

Charge Variants by CEX

An overlay of chromatograms is provided in Figure 13A and 13B for $t = 0$ and $t = 1$ cycle, respectively, while trend plots are provided in Figures 14 and 15. The $t = 0$ chromatograms are the same as for thermal stress. At $t = 1$ cycle, the chromatograms appear similar in peak profiles in terms of number and position of peaks, except for US-licensed Neulasta lot 1094104, which is observed to have a very large peak at RRT 0.63 – 0.69 (aligns with the additional acidic peaks observed in TPI-120 lots) and RRT 0.85 – 0.91, in addition to a peak eluting after the main peak. The trend plots show a similar decrease in main peak for both products (except for one lot of US-licensed Neulasta, which decreased to a larger extent by $t = 1$ cycle). Similar increases are observed for peaks eluting at RRT 0.87 – 0.91, RRT 0.92 – 0.97, and RRT

1.33 – 1.45. A minor peak is observed for TPI-120 lots at RRT 1.31 – 1.32 at the end of $t = 1$ cycle (total area $\sim 0.4\% - 0.5\%$).

Potency by Cell Proliferation Assay

Results for potency are provided in Table 7 for each lot of TPI-120 and US-licensed Neulasta for $t = 0, 0.5,$ and 1 cycle, while a trend plot is provided in Figure 16. A decrease in potency is observed for both products over time (e.g., 99% to 68% for TPI-120 and 96% to 63% for US-licensed Neulasta). The trend plot shows an overall decrease in potency in both products, though to a greater extent in US-licensed Neulasta lots compared to TPI-120 lots.

Summary of Results

Data is provided for analysis of main peak (%area) by RP-HPLC, SE-HPLC, and CEX at each time point, for each lot of TPI-120 and US-licensed Neulasta in Table 8; the rates of degradation were not provided. A significant decrease in main peak is observed by RP-HPLC for both products, with only minor decreases in main peak by SE-HPLC and CEX analyses for both products (i.e., 97.72% to 52.77% for TPI-120 and 96.52% to 47.21% for US-licensed Neulasta for RP-HPLC; 99.38% to 97.00% for TPI-120 and 98.27% to 95.96% for US-licensed Neulasta for SE-HPLC; and 99.28% to 94.48% for TPI-120 and 98.77% to 93.75% for US-licensed Neulasta for CEX). An independent analysis of the numbers in Table 8 finds that the rates of degradation are similar between TPI-120 and US-licensed Neulasta lots, though marginally higher in US-licensed Neulasta lots, particularly in purity by RP-HPLC.

Assessor comment: *While tabular data was provided for main peak content by RP-HPLC, SE-HPLC, and CEX under light stress, from which rates of degradation for main peak were determined, the data for impurities was not provided. An IR was sent to the applicant (11/24/20) to provide individual data points (i.e., all time points) for each impurity (individual and total) that was evaluated under light stress in tabular format, and for each of the included datasets, provide an assessment of the rates of formation for the individual/total impurity in the TPI-120 and US-licensed Neulasta lots. In response (1/8/21), the applicant provided the requested data, which is reviewed below.*

Under light stress, impurities at RRt $\sim 0.54,$ RRt $\sim 0.87,$ RRt $\sim 0.94,$ and RRt ~ 0.97 are observed to increase over 1 cycle for each of the 3 lots of TPI-120 and US-licensed Neulasta as evaluated by RP-HPLC. The rates of formation for each individual impurity peak are somewhat similar between TPI-120 and US-licensed Neulasta (i.e., 7.38 for TPI-120 and 8.83 for US-licensed Neulasta for RRt $\sim 0.54,$ 3.81 for TPI-120 and 6.69 for US-licensed Neulasta for RRt $\sim 0.87,$ 11.85 for TPI-120 and 12.83 for US-licensed Neulasta for RRt $\sim 0.94,$ and 10.68 for TPI-120 and 14.23 for US-licensed Neulasta for RRt ~ 0.97). The rate of degradation of main peak is equivalent to the rate of formation of total impurities, though higher in US-licensed Neulasta compared to TPI-120 (i.e., -42.44 for TPI-120 and -55.73 for US-licensed Neulasta).

Evaluation by SE-HPLC shows that impurities at RRt ~ 0.80 and RRt ~ 0.843 (aggregates and HMWs), RRt ~ 0.89 (di-PEG/dimer), and RRt ~ 1.49 (free rhG-CSF) are observed to increase over 1 cycle for each of the 3 lots of TPI-120 and US-licensed Neulasta. The rates of formation for the individual impurity peaks are similar between TPI-120 and US-licensed Neulasta (i.e., 0.85 for TPI-120 and 1.12 for US-licensed Neulasta for RRt $\sim 0.80,$ 1.12 for TPI-120 and 1.33 for US-licensed Neulasta for RRt $\sim 0.89,$ and 0.13 for TPI-120 and 0.15 for US-licensed Neulasta for RRt ~ 1.49). The rate of degradation of main peak

is similar to the rate of formation of total impurities (i.e., -2.24 for TPI-120 and -2.72 for US-licensed Neulasta).

Evaluation by CEX shows that impurities at $RR_t \sim 0.92-0.97$ and $RR_t \sim 1.31-1.45$ are observed to increase over 1 cycle for each of the 3 lots of TPI-120 and US-licensed Neulasta. The rates of formation for the individual impurity peaks are somewhat similar between TPI-120 and US-licensed Neulasta for $RR_t \sim 0.92-0.97$, though not for $RR_t \sim 1.31-1.45$ (i.e., 1.99 for TPI-120 and 2.69 for US-licensed Neulasta for $RR_t \sim 0.92-0.97$, and 0.90 for TPI-120 and 0.22 for US-licensed Neulasta for $RR_t \sim 1.31-1.45$). The rate of degradation of main peak is higher for US-licensed Neulasta compared to TPI-120 (i.e., -4.66 for TPI-120 and -7.70 for US-licensed Neulasta).

Assessor comment: *The data provided show that both products are similar in terms of their degradation and resulting impurities, though not always to the same extent, arising from light stress. The rates of degradation are at times observed to be slightly greater in US-licensed Neulasta lots compared to TPI-120 lots, however, this can be interpreted as TPI-120 being a slightly more stable product and does not preclude a demonstration of similarity between the products.*

Overall, light stress of up to 1 ICH cycle results in degradation of TPI-120 and US-licensed Neulasta products in a similar manner; purity and potency decreases slightly while impurities, size and charge variants increase. Light stress is observed to be a pathway of degradation for both products.

Oxidative Stress

Samples from each lot of TPI-120 and US-licensed Neulasta were treated with hydrogen peroxide and then quenched with a saturated methionine solution at 2, 7, and 24 hours. Samples were analyzed using RP-HPLC, SE-HPLC, CEX, and a cell proliferation assay using M-NFS-60 cells.

Purity by RP-HPLC

An overlay of chromatograms is provided in Figure 17A and 17B for $t = 0$ and $t = 24$ hours, respectively, while trend plots are provided in Figure 18. The $t = 0$ chromatograms are the same as for thermal stress. At $t = 24$ hours, the peak profiles appear similar in terms of number and position of peaks between TPI-120 and US-licensed Neulasta lots. The trend plots show a similar decrease in main peak in both products, while a similar increase is observed for the peaks at $RR_t 0.884 - 0.903$, $RR_t 0.928 - 0.956$, and $RR_t 0.968 - 0.974$.

Size Variants by SE-HPLC

An overlay of chromatograms is provided in Figure 19A and 19B for $t = 0$ and $t = 24$ hours, respectively, while trend plots are provided in Figures 20 and 21. The $t = 0$ chromatograms are the same as for thermal stress. At $t = 24$ hours, the peak profiles appear similar in terms of number and position of peaks between TPI-120 and US-licensed Neulasta lots, and largely unchanged from $t = 0$. The trend plots show that the main peak remains stable over time in both products. Similarly, aggregates, free filgrastim, di-PEG variants, and total size variants also remain stable over time up to 24 hours in both products.

Charge Variants by CEX

An overlay of chromatograms is provided in Figure 22A and 22B for $t = 0$ and $t = 24$ hours, respectively, while trend plots are provided in Figures 14 and 15. The $t = 0$ chromatograms are the same as for thermal stress. At $t = 24$ hours, the chromatograms appear similar in peak profiles in terms of number and position

of peaks between TPI-120 and US-licensed Neulasta lots, and largely unchanged from $t = 0$. The trend plots show that the main peak, peak eluting at RRT 0.93 – 0.96 and RRT 1.33 – 1.44 remain stable over time.

Potency by Cell Proliferation Assay

Results for potency are provided in Table 9 for each lot of TPI-120 and US-licensed Neulasta for $t = 0, 2, 7,$ and 24 hours, while a trend plot is provided in Figure 24. A decrease in potency is observed for both products over time (e.g., 97% to 80% for TPI-120 and 96% to 79% for US-licensed Neulasta). The trend plot shows an overall decrease in potency in both products to the same extent.

Summary of Results

Data is provided for analysis of main peak (%area) by RP-HPLC, SE-HPLC, and CEX at each time point, for each lot of TPI-120 and US-licensed Neulasta in Table 10; the rate of degradation of main peak by each method is provided in Table 11. The rates of degradation are similar between TPI-120 and US-licensed Neulasta (i.e., -1.8178 for TPI-120 and -1.7294 for US-licensed Neulasta for RP-HPLC, -0.0022 for TPI-120 and 0.0010 for US-licensed Neulasta for SE-HPLC, and 0.0067 for TPI-120 and 0.0025 for US-licensed Neulasta for CEX).

Assessor comment: *While tabular data was provided for main peak content by RP-HPLC, SE-HPLC, and CEX under oxidative stress, from which rates of degradation for main peak were determined, the data for impurities was not provided. An IR was sent to the applicant (11/24/20) to provide individual data points (i.e., all time points) for each impurity (individual and total) that was evaluated under oxidative stress in tabular format, and for each of the included datasets, provide an assessment of the rates of formation for the individual/total impurity in the TPI-120 and US-licensed Neulasta lots. In response (1/8/21), the applicant provided the requested data, which is reviewed below.*

Under oxidative stress, impurities at RRt ~0.89, RRt ~0.94 and RRt ~0.97 are observed to increase over 24 hours for each of the 3 lots of TPI-120 and US-licensed Neulasta as evaluated by RP-HPLC. The rates of formation for each individual impurity peak are similar between TPI-120 and US-licensed Neulasta (i.e., 0.29 for TPI-120 and 0.27 for US-licensed Neulasta for RRt ~0.89, 0.41 for TPI-120 and 0.38 for US-licensed Neulasta for RRt ~0.94, and 1.10 for TPI-120 and 1.08 for US-licensed Neulasta for RRt ~0.97). The rate of degradation of main peak is equivalent to the rate of formation of total impurities, and similar between products (i.e., -1.82 for TPI-120 and -1.73 for US-licensed Neulasta).

Degradation was not observed in the evaluation impurities by SEC and CEX-HPLC, therefore rates of degradation/formation for these evaluations were not provided.

Assessor comment: *The data provided show that both products are similar in terms of their degradation profile, resulting from oxidative stress, as evaluated by RP-HPLC.*

Overall, exposure to oxidative stress for up to 24 hours does not result in significant degradation of TPI-120 and US-licensed Neulasta products in terms of size and charge variants. A similar decrease in purity with a concomitant increase in impurities is observed for both products after 24 hours (RP-HPLC analysis). Oxidative stress is observed to be a pathway of degradation for both products, in a similar manner.

Low pH Stress

Each lot of TPI-120 and US-licensed Neulasta was exposed to a pH of 2.4 at 25°C for up to 14 days. Samples were analyzed using RP-HPLC, SE-HPLC, CEX, and a cell proliferation assay using M-NFS-60 cells.

Purity by RP-HPLC

An overlay of chromatograms is provided in Figure 25A and 25B for Day 0 and Day 14, respectively, while trend plots are provided in Figures 26 and 27. The Day 0 chromatograms are the same as for thermal stress. At Day 14, the peak profiles appear similar in terms of number and position of peaks between TPI-120 and US-licensed Neulasta lots. The trend plots show a similar marginal decrease in main peak in both products. An increase is observed for the peak at RRT 1.051 – 1.059, RRT 1.078 – 1.106, and RRT 1.194 – 1.214 in both products. The peak observed at RRT 0.931 – 0.956 remains stable (though at different levels) in both products.

Size Variants by SE-HPLC

An overlay of chromatograms is provided in Figure 28A and 28B for Day 0 and Day 14, respectively, while trend plots are provided in Figure 29. The Day 0 chromatograms are the same as for thermal stress. At Day 14, the peak profiles appear similar in terms of number and position of peaks between TPI-120 and US-licensed Neulasta lots, though a large peak is observed in one lot of US-licensed Neulasta (lot 1093751) after the free filgrastim peak. The trend plots show that the main peak remains stable over up to 14 days of exposure, similarly in both products. Marginal increases are observed in aggregates, di-PEG variants, and total size variants over time, similarly in both products (though at different levels).

Charge Variants by CEX

An overlay of chromatograms is provided in Figure 30A and 30B for Day 0 and Day 14, respectively, while trend plots are provided in Figure 31. The Day 0 chromatograms are the same as for thermal stress. At Day 14, the peak profiles remain largely unchanged from Day 0, with the exception of a peak at RRT 1.58 – 1.69, which is similar in both products. The trend plots show that the main peak and peak at RRT 0.93 – 0.96 remain stable over 14 days in both products. The peak at RRT 1.33 – 1.45 slightly increases in both products (though at different levels).

Potency by Cell Proliferation Assay

Results for potency are provided in Table 12 for each lot of TPI-120 and US-licensed Neulasta at each time point, while a trend plot is provided in Figure 32. On average, a marginal decrease in potency is observed for both products over time (e.g., 99% to 95% for TPI-120 and 96% to 94% for US-licensed Neulasta), though one lot of each product did not show a decrease in potency over 14 days. The trend plot shows comparable results for potency in both products.

Summary of Results

Data is provided for analysis of main peak (%area) by RP-HPLC, SE-HPLC, and CEX at each time point, for each lot of TPI-120 and US-licensed Neulasta in Table 13; the rate of degradation is not provided. No significant decrease is observed for main peak by any evaluation method for either product (i.e., 97.72% to 93.79% for TPI-120 and 96.52% to 91.63% for US-licensed Neulasta for RP-HPLC; 99.38% to 97.91% for TPI-120 and 98.27% to 96.73% for US-licensed Neulasta for SE-HPLC; and 99.28% to 99.25% for TPI-120 and 98.77% to 98.64% for US-licensed Neulasta for CEX). An independent analysis of the

numbers in Table 13, however, finds that the rates of degradation are similar between TPI-120 and US-licensed Neulasta lots, though marginally higher in US-licensed Neulasta lots, but overall low.

Assessor comment: *While tabular data was provided for main peak content by RP-HPLC, SE-HPLC, and CEX under low pH stress, from which rates of degradation for main peak were determined, the data for impurities was not provided. An IR was sent to the applicant (11/24/20) to provide individual data points (i.e., all time points) for each impurity (individual and total) that was evaluated under low pH stress in tabular format, and for each of the included datasets, provide an assessment of the rates of formation for the individual/total impurity in the TPI-120 and US-licensed Neulasta lots. In response (1/8/21), the applicant provided the requested data, which is reviewed below.*

Under low pH stress, impurities were detected at low levels at $RR_t \sim 1.05-1.06$, and $RR_t \sim 1.19-1.21$ over 14 days for each of the 3 lots of TPI-120 and US-licensed Neulasta as evaluated by RP-HPLC. The rates of formation for each individual impurity peak are similar between TPI-120 and US-licensed Neulasta (i.e., 0.17 for TPI-120 and 0.23 for US-licensed Neulasta for $RR_t \sim 1.05-1.06$, and 0.03 for both TPI-120 and US-licensed Neulasta for $RR_t \sim 1.19-1.21$). The rate of degradation of main peak is equivalent to the rate of formation of total impurities, (i.e., -0.28 for TPI-120 and -0.35 for US-licensed Neulasta).

Evaluation by SE-HPLC shows that the products remained mostly stable over time, with very low levels of impurities at $RR_t \sim 0.80$ and ~ 0.84 (aggregates), as well as low levels of di-PEG/dimer, and free rhG-CSF. The rates of formation for aggregates were similar between products (i.e., 0.05 and 0.06 for $RR_t \sim 0.80$ for TPI-120 and US-Neulasta, respectively, and 0.01 for both products for $RR_t \sim 0.84$). The rate of formation for di-PEG/dimer impurities was 0.2 and 0.03 for TPI-120 and US-Neulasta, respectively, and for free rhG-CSF it was 0.01 and 0.00 for TPI-120 and US-Neulasta, respectively. The rate of degradation of main peak was also similar between products (i.e., -0.09 for TPI-120 and -0.11 for US-licensed Neulasta).

Degradation was not observed in the evaluation impurities by CEX-HPLC, therefore the rates of degradation/formation for this evaluation were not provided.

Assessor comment: *The data provided show that both products are similar in terms of their degradation and resulting impurities, though not always to the same extent, arising from light stress. The rates of degradation are a times observed to be slightly greater in US-licensed Neulasta lots compared to TPI-120 lots, however, this can be interpreted as TPI-120 being a slightly more stable product and does not preclude a demonstration of similarity between the products.*

Overall, low pH stress at pH 2.4 at 25°C for up to 14 days does not results in significant degradation of either TPI-120 or US-licensed Neulasta products; results are overall similar. Low pH stress does not appear to a major pathway of degradation for either product.

High pH Stress

Each lot of TPI-120 and US-licensed Neulasta was exposed to a pH of 9.4 at 25°C for up to 11 days. Samples were analyzed using RP-HPLC, SE-HPLC, CEX, and a cell proliferation assay using M-NFS-60 cells.

Purity by RP-HPLC

An overlay of chromatograms is provided in Figure 33A and 33B for Day 0 and Day 11, respectively, while trend plots are provided in Figures 34 and 35. The Day 0 chromatograms are the same as for thermal stress. At Day 11, the peak profiles appear similar in terms of number and position of peaks between TPI-120 and US-licensed Neulasta lots, though two lots of US-licensed Neulasta were similar to one lot of TPI-120 in terms of peak profile (e.g., prominent peaks at RRT 0.88, and RRT 1.11 – 1.14), while the third lot of US-licensed Neulasta was similar to the remaining two lots of TPI-120 in terms of peak profile. The trend plots show a similar decrease in main peak in both products. An increase is observed for other impurity peaks in a similar manner between TPI-120 and US-licensed Neulasta (peaks at RRT 0.881 – 0.93, RRT 0.931 – 0.956, RRT 1.051 – 1.059, RRT 1.078 – 1.098, RRT 1.114 – 1.132, and RRT 1.337 – 1.367).

Size Variants by SE-HPLC

An overlay of chromatograms is provided in Figure 36A and 36B for Day 0 and Day 11, respectively, while trend plots are provided in Figure 37. The Day 0 chromatograms are the same as for thermal stress. At Day 11, the peak profiles appear similar in terms of number and position of peaks between TPI-120 and US-licensed Neulasta lots. The trend plots show that the main peak remains decreases over up to 11 days of exposure, similarly in both products. Increases are observed in aggregates, di-PEG variants, and total size variants over time, similarly in both products.

Charge Variants by CEX

An overlay of chromatograms is provided in Figure 38A and 38B for Day 0 and Day 11, respectively, while trend plots are provided in Figures 39 and 40. The Day 0 chromatograms are the same as for thermal stress. At Day 11, the peak profiles remain largely unchanged from Day 0 in terms of acidic variants. Basic variant peaks are similar in terms of number and position of peaks. The trend plots show that the main peak decreases similarly in both products. The peak at RRT 0.91 – 0.96 (i.e., acidic) remains stable over 11 days in both products, while the basic peaks at RRT 1.41 – 1.49 and RRT 1.51 – 1.72 increase in a similar manner in both products.

Potency by Cell Proliferation Assay

Results for potency are provided in Table 14 for each lot of TPI-120 and US-licensed Neulasta at each time point, while a trend plot is provided in Figure 41. A similar decrease in potency is observed for both products over time (e.g., 97% to 62% for TPI-120 and 96% to 62% for US-licensed Neulasta). The trend plot shows comparable decreases for potency in both products.

Summary of Results

Data is provided for analysis of main peak (%area) by RP-HPLC, SE-HPLC, and CEX at each time point, for each lot of TPI-120 and US-licensed Neulasta in Table 15; the rate of degradation of main peak by each method is provided in Table 16. The rates of degradation of main peak are similar between TPI-120 and US-licensed Neulasta lots (i.e., -3.7497 for TPI-120 and -3.8218 for US-licensed Neulasta for RP-HPLC, -4.4945 for TPI-120 and -4.4688 for US-licensed Neulasta for SE-HPLC, and -1.6439 for TPI-120 and -1.5245 for US-licensed Neulasta for CEX).

Assessor comment: *While tabular data was provided for main peak content by RP-HPLC, SE-HPLC, and CEX under high pH stress, from which rates of degradation for main peak were determined, the data for impurities was not provided. An IR was sent to the applicant (11/24/20) to provide individual data points*

(i.e., all time points) for each impurity (individual and total) that was evaluated under high pH stress in tabular format, and for each of the included datasets, provide an assessment of the rates of formation for the individual/total impurity in the TPI-120 and US-licensed Neulasta lots. In response (1/8/21), the applicant provided the requested data, which is reviewed below.

Under high pH stress, post-main peak impurities (i.e., RRt ~0.88-0.91, RRt ~1.05-1.06, RRt ~1.08-1.10, RRt ~1.11-1.13, RRt ~1.21-1.26, RRt ~1.34-1.37, and RRt ~1.43-1.50) were observed to slightly increase, corresponding to glutamine deamidation, over 11 days for each of the 3 lots of TPI-120 and US-licensed Neulasta as evaluated by RP-HPLC. The rates of formation for each individual impurity peak were similar, while the rate of degradation of main peak was equivalent to the rate of formation of total impurities, and similar between products (i.e., -3.75 for TPI-120 and -3.84 for US-licensed Neulasta).

Evaluation by SE-HPLC shows that aggregates and di-PEG/dimer variants increased over 11 days, while free rhG-CSF remained stable over time for each of the 3 lots of TPI-120 and US-licensed Neulasta. The rates of formation for aggregates and dimers were similar between products (i.e., 3.19 for TPI-120 and 3.31 for US-licensed Neulasta for aggregates, and 11.31 for TPI-120 and 1.18 for US-licensed Neulasta for di-PEG/dimer variants). The rate of degradation of main peak is similar to the rate of formation of total impurities, and similar between products (i.e., -4.49 for TPI-120 and -4.47 for US-licensed Neulasta).

Evaluation by CEX shows that basic variants at RRt ~1.41-1.49 and RRt ~1.51-1.72 slightly increased over time for each of the 3 lots of TPI-120 and US-licensed Neulasta. The rates of formation for the individual impurity peaks are similar between TPI-120 and US-licensed Neulasta (i.e., 0.07 for TPI-120 and 0.5 for US-licensed Neulasta for RRt ~1.41-1.49, and 1.57 for TPI-120 and 1.48 for US-licensed Neulasta for RRt ~1.51-1.72). The rate of degradation of main peak is similar between US-licensed Neulasta and TPI-120 (i.e., -1.64 for TPI-120 and -1.52 for US-licensed Neulasta), and equivalent to the rate of formation of total impurities.

Assessor comment: *The data provided show that both products are similar in terms of their degradation resulting from high pH stress.*

Overall, high pH stress at pH 9.4 at 25°C for up to 11 days results in significant degradation of both TPI-120 or US-licensed Neulasta products, terms of impurities, size, and charge variants; in results are overall similar. High pH stress is a pathway of degradation for both products.

Overall Conclusion: *The comparative stability and forced degradation studies demonstrate that the stability profile of TPI-120 is similar to that of US-licensed Neulasta.*

3.2.R.1 Executed Batch Records

IP, DS, and DP are manufactured per master batch records (MBR) to ensure control of the manufacturing process. The proposed commercial MBRs and executed batch records (EBRs) for PPQ/PV material were provided for IP, DS, and DP.

Assessor comment: *The adequacy of the MBRs and EBRs will be assessed on inspection during the resubmission review cycle.*

3.2.R.2 Method Validation Package

Information pertaining to the QC methods, including validation of these methods, is provided in Sections 3.2.S.2.4, 3.2.S.4.2, 3.2.S.4.3, 3.2.P.5.2, and 3.2.P.5.3.

3.2.R.3 Comparability Protocols

No comparability protocols were included in the BLA.

5.3.1.4 Reports of Bioanalytical and Analytical Methods for Human Studies

Immunogenicity of TPI-120 in comparison to that of US-licensed Neulasta was evaluated in healthy adult subjects in a single-dose PK/PD clinical study (TPI-CL-109-A) and a multi-dose confirmatory clinical study (Study ADL-CL-112). Syneos Health performed the immunogenicity analysis and developed and validated the methods that assessed for immunogenicity. A multi-tiered approach was implemented using a binding antibody assay to screen, confirm, and characterize (i.e., domain specificity and titer) samples for ADAs, followed by a neutralizing antibody assay to characterize confirmed positive samples for neutralizing antibody activity. The applicant utilized a single-assay approach for both the binding and neutralizing antibody assays using TPI-120 as labeled-reagents for capture and detection of binding and neutralizing antibodies.

Assessor comment: The tiered approach for ADA assessment, which is described in greater detail below, is acceptable and consistent with FDA Guidance for Industry: Immunogenicity Testing of Therapeutic Protein Products – Developing and Validating Assays for Anti-Drug Antibody Detection (2019). In addition, the use of a single-assay with the proposed biosimilar as labeled reagents for capture and detection of antibodies is consistent with FDA Guidance for Industry: Scientific Considerations in Demonstrating Biosimilarity to a Reference Product (2015). An assessment of the antigenic equivalence of the assay is described below as part of the drug tolerance discussion. As discussed below, sufficient data are provided to support that the binding and neutralizing antibody assays are suitable for their intended purpose in support of the BLA.

Binding Antibody Assay Validation Report (Report 11508.053017)

Anti-drug antibodies (ADAs) against TPI-120 and US-licensed Neulasta were detected using an electrochemiluminescence (ECL) binding assay using Meso-Scale Discovery (MSD) technology, which was developed and validated for the detection, confirmation, and titration of binding ADAs against TPI-120 or US-licensed Neulasta in the serum of healthy adult subjects. Samples were initially evaluated in a screening assay, and positive samples from the screening assay were then evaluated in a confirmatory assay (Tier 1 and Tier 2, respectively) to demonstrate that the ADA response is specific to TPI-120 or US-licensed Neulasta. As part of the confirmatory assay, samples were further evaluated to determine whether ADAs to TPI-120 or US-licensed Neulasta bind to the PEG portion or the G-CSF portion of the product. The confirmed positive ADA samples were then titrated.

Assessor comment: The ECL bridging format allows for the detection of all antibody isotypes, thus, the assay format is acceptable.

Assay Principle

Under the screening assay format, samples were co-incubated with biotinylated-TPI-120 and Ru-TPI-120 to allow ADAs specific to TPI-120, if present, to bind to the components. The antibody complex was then added to a streptavidin-coated MSD plate and incubated. The ruthenium in the immune complexes

produces an ECL signal when voltage is applied to the plate; only samples containing Ru-TPI-120/ADA/B-TPI-120 complexes generate a signal. The signal is directly proportional to the level of binding ADAs present in the sample. Samples with mean replicate response < the screening cut-point are reported as “negative”, while samples with mean replicate response $\geq$ the screening cut-point are considered “potential positive” (Tier 1) and are evaluated by the confirmatory assay (Tier 2). Under the confirmatory assay format, samples are left untreated or pre-treated with excess drug (TPI-120 or US-licensed Neulasta) as a competitor to determine whether samples that screened positive for ADAs are confirmed positive to the drug. Samples that are evaluated in the Tier 2 confirmatory assay are also incubated with excess drug components (PEG or G-CSF domains) to determine the specificity of binding to the region of the product. Theragrastim or US-licensed Neupogen were used as the competitor for the G-CSF domain and PEG was used as well in this assessment. Samples with %inhibition < confirmatory cut-point are reported as “negative”, while samples with inhibition $\geq$ confirmatory cut-point are considered “confirmed positive” to the product or domain, depending on the competitor used. Samples that are positive in the Tier 2 confirmatory assay are evaluated for ADA titer. Under the titer assay format, confirmed positive ADA samples are titered prior to analysis using the screening assay format. The reciprocal of the last dilution (inclusive of assay MRD) resulting in the last positive response at or above the titration cut-point is reported as the sample titer.

Assessor comment: *To demonstrate that the assay is acceptable to detect antibody responses to all domains of the product, PCs to the whole product (TPI-120) and each domain (PEG and G-CSF) were generated and adequately validated under the screening and confirmatory assay formats. Each PC was detected at adequate levels to demonstrate that the assay is capable of detecting antibody responses to each domain. The responses to each domain were able to be competed adequately by its respective domain that was unlabeled and used at an excess amount to support that the responses against the PCs are specific to each domain.*

Negative Control

Healthy adult human serum samples were pre-screened and pooled. Six samples were combined, aliquoted, and stored at -80°C to serve as a negative control (NC).

Assessor comment: *The use of healthy adult human serum as the NC is acceptable as it resembles the clinical population and is derived from treatment-naïve subjects. The values generated from the NC are below but close to the cut-point.*

Positive Control

Four positive controls (PCs) were said to have been used in the ADA binding assay: an anti-TPI-120 antibody supplied by (b) (4) as well as a TPI-120 purified anti-drug antibody from (b) (4) along with an anti-PEG monoclonal antibody (b) (4) and a rabbit polyclonal anti-G-CSF antibody (b) (4). TPI-120 PCs were prepared by spiking the TPI-120 purified anti-drug antibody stock into the NC pool at 500 ng/mL (high positive control; PCH), 40 ng/mL (low positive control-1; PCL-1), and 20 ng/mL (low positive control-2; PCL-2). PEG PCs were prepared by spiking the anti-PEG antibody stock into the NC pool at 2000 ng/mL (PCH-PEG), 175 ng/mL (PCL1-PEG) and 125 ng/mL (PCL2-PEG). G-CSF PCs were prepared by spiking the anti-G-CSF antibody stock into the NC pool at 2000 ng/mL (PCH-G-CSF) and 60.0 ng/mL (PCL-GCSF).

Assessor comment: Information concerning the PCs was insufficient. An IR was sent to the applicant (2/19/21) to clarify whether the anti-TPI-120 antibody is monoclonal or polyclonal and which anti-TPI-120 antibody was used during the validation exercises. It was also requested that the applicant clarify whether a polyclonal antibody against PEG was also evaluated during development. Polyclonal antibodies as PCs are typically recommended for validation of the binding assay as this allows for the assessment of heterogenous antibody responses. In response (3/9/21), the applicant stated that the TPI-120 antibody PC used during validation was the PC prepared from (b) (4) and that the PC is polyclonal, affinity purified rabbit antisera. However, polyclonal antibodies against PEG were not evaluated during development, because they were not commercially available. Considering that the sensitivity level of the assay is acceptable, this is not an issue. In a follow-up IR (4/22/21), it was also requested that the applicant state whether the same type of PEG molecule that was used to generate the PC is the same type of PEG that is used in TPI-120, and if there are any differences, provide a justification to support the suitability of the anti-PEG monoclonal antibody PC.

(b) (4)
(D) (4)

Overall, sufficient information was provided to support the suitability of the PCs.

Screening Assay Cut Point

The screening assay cut-point was derived from 50 normal human serum samples, which were analyzed six times by two analysts over nine days (12 sets of data; Table 3). The distribution of the data was determined not to be normal (JMP evaluation, Shapiro-Wilk test) after identification and removal of outliers (outliers defined as values less than $Q1 - 3 * IQR$ or greater than $Q3 + 3 * IQR$). A non-parametric approach was applied, and the cut point was calculated using the 95th percentile in Excel. The screening cut point factor (SCPF) was determined to be 1.115. The variance of the data sets was analyzed using an Anova F-test in JMP. A floating cut-point was determined because the p-value was < 0.05 . The assay cut point for sample analysis was calculated as the SCPF multiplied by the geometric mean of NCs from the run, which is shown below:

Screening Assay Floating Cut-Point = Mean responses of the NCs * SCPF

Additionally, the correlation of the NCs and samples was examined by comparing the mean response of the NC replicates from each run to the mean response of the cut point samples from each run. The correlation coefficient (R^2 ; Pearson correlation function in Excel) was above 0.8, indicating a correlation between the response of NC and response of the samples. The false positive rate was evaluated to be 5.008%

Assessor Comment: The cut-points were determined after removal of outliers using an IQR factor of 3, however, the standard approach involves using an IQR factor of 1.5. This could lead to exclusion of outliers, resulting in inflated cut-points and misidentification of low positive ADA samples. An IR was sent to the applicant (2/19/21) to re-determine the screening, confirmatory, and titer cut-points using the standard approach (e.g., $1.5 * IQR$), and if these cut-points are significantly different from the originally validated cut-points, to consider using the more conservative assay cut-points in the analysis of clinical samples for detection of ADAs. In response (3/9/21), the applicant re-determined the cut-points using an IQR factor of 1.5, which resulted in lower screening, confirmatory (i.e., TPI-120, US-licensed Neulasta,

PEG, Theragrastim, and US-Neupogen) and titer cut-points. An additional 61 samples screened ADA positive using the new screening cut-point of 1.096 (282 samples originally, 343 with new cut-point). The new confirmatory cut-points were used to analyze the originally screened ADA positive samples (282) in the Tier 1 confirmatory assay, among which, an additional 4 samples were confirmed positive against TPI-120, 9 samples against US-licensed Neulasta. In the Tier 2 confirmatory assay (originally 52 samples evaluated), 1 additional sample was confirmed positive against Neupogen; no additional samples were confirmed positive against PEG and Theragrastim. Of these 52 confirmed samples, 15 samples resulted in a higher titer using the re-determined titer cut-point. The applicant stated that the more conservative re-determined cut-point will be used to reanalyze the clinical samples, and relevant sections of the BLA and reports will be revised and submitted in a subsequent amendment. This is acceptable.

*Additionally, it was unclear whether the samples used to determine the cut-points were obtained from a commercial source or were the pre-dose clinical samples from study ADL-CL- 112. An IR was sent to the applicant (2/19/21) to clarify the source of samples that were used to determine the cut-points, and if commercial samples were used, then to verify the suitability of the validated cut-points to analyze the ADL-CL-112 clinical study samples (i.e., generate appropriate false positive rates of ~5%) and/or redetermine the cut-points using the pre-dose clinical samples from study ADL-CL-112. In response (3/9/21), the applicant clarified that the normal human serum samples were obtained from a commercial source. The original and re-determined screening cut-points both resulted in a false positive rate of ~5% (5.008% vs. 4.811%, respectively). The pre-dose clinical samples were then used to determine the cut-point (1.173 vs. 1.115 (3*IQR) or 1.096 (1.5*IQR) using commercial samples), which resulted in a false positive rate of 11.283%. As described above, the applicant plans on using the re-determined cut-point from commercial samples (1.5*IQR approach) to evaluate the clinical samples. This is acceptable as it results in an appropriate false positive rate. The applicant indicated that the data from the reanalysis will be provided by June 30, 2021 via an amendment.*

Confirmatory Assay Cut-Point

The confirmatory assay was run in a competitive binding format. The same 50 normal human serum samples that were analyzed to determine the screening assay cut-point were left untreated or spiked with an excess drug. Samples were then processed in the same manner as in the screening assay and data was collected from three assay runs by two analysts; the mean signal inhibition (%) was calculated for each sample. Samples at or above the confirmatory cut point (defined as percent of inhibition) were determined to be positive for antibodies specific to a compound, and below the confirmatory cut point were defined to be negative. A confirmatory cut point was established for TPI-120, US-licensed Neulasta, PEG, Theragrastim and US-licensed Neupogen.

TPI-120 Confirmatory Cut Point

Each normal human serum sample was spiked with TPI-120 at 10.0 µg/mL (the concentration determined to reduce the signal response of the PCH by at least 50.0%, or to below the screening assay cut point). The log ratio (signal inhibition ratio, SIR) for each sample was calculated by:

$$\text{Signal Inhibition Ratio (SIR)} = \text{Log}_{10} (\text{Inhibited Sample} / \text{Uninhibited Sample})$$

Outliers were determined as values less than $Q1 - 3*IQR$ or greater than $Q3 + 3*IQR$; no outliers were identified. The data had a normal distribution when evaluated with JMP software (Shapiro-Wilk test), and

a parametric approach was applied to determine the confirmatory cut point (CCP). The CCP was calculated as 16.913%, such that a 1% false positive rate was allowed, using the following equation:

$$CCP = (100 * (1 - \text{Anti Log} (\text{Mean of SIR} - t_{0.01,df} * SD)))$$

US-licensed Neulasta Confirmatory Cut Point

Each normal human serum sample was spiked with US-licensed Neulasta at 10.0 µg/mL (the concentration determined to reduce the signal response of the PCH by at least 50.0%, or to below the screening assay cut point). The SIR was calculated for each sample. The distribution was not normal after removal of outliers (values less than Q1 – 3*IQR or greater than Q3 + 3*IQR). Therefore the % Inhibition was calculated on each sample using the following equation:

$$\% \text{ Inhibition} = (1 - (\text{Inhibited Sample} / \text{Uninhibited Sample})) * 100$$

A non-parametric approach was applied; the 99th percentile of the compiled %inhibition data in Excel was used to determine the CCP. The CCP of US-licensed Neulasta was calculated as 22.972%, allowing for a 1% false positive rate.

PEG Confirmatory Cut Point

Each normal human serum sample was spiked with PEG at 5.0 µg/mL (the concentration determined to reduce the signal response of the PCH by at least 50.0%, or to below the screening assay cut point). The SIR was calculated for each sample. Outliers were determined as values less than Q1 – 3*IQR or greater than Q3 + 3*IQR; no outliers were identified, and the data had a normal distribution. A parametric approach was applied to determine the CCP, allowing for a 1% false positive rate, calculated by:

$$CCP = (100 * (1 - \text{Anti Log} (\text{Mean of SIR} - t_{0.01,df} * SD)))$$

The CCP for PEG was calculated to be 17.006%.

Theragrastim Confirmatory Cut Point

Each normal human serum sample was spiked with Theragrastim at 10.0 µg/mL (the concentration determined to reduce the signal response of the PCH by at least 50.0%, or to below the screening assay cut point). The SIR was calculated for each sample. The data had a normal distribution after identification and removal of outliers (values less than Q1 – 3*IQR or greater than Q3 + 3*IQR). A parametric approach was applied to determine the CCP, allowing for a 1% false positive rate, calculated by:

$$CCP = (100 * (1 - \text{Anti Log} (\text{Mean of SIR} - t_{0.01,df} * SD)))$$

The CCP for Theragrastim was calculated to be 17.223%.

US-licensed Neupogen Confirmatory Cut Point

Each normal human serum sample was spiked with US-licensed Neupogen at 10.0 µg/mL (the concentration determined to reduce the signal response of the PCH by at least 50.0%, or to below the screening assay cut point). The SIR was calculated for each sample. The data had a normal distribution after identification and removal of outliers (values less than Q1 – 3*IQR or greater than Q3 + 3*IQR). A

parametric approach was applied to determine the CCP, allowing for a 1% false positive rate, calculated by:

$$\text{CCP} = (100 * (1 - \text{Anti Log} (\text{Mean of SIR} - t_{0.01,df} * \text{SD})))$$

The CCP for US-licensed Neupogen was calculated to be 16.945%.

Assessor Comment: The confirmatory cut-points calculated for TPI-120, US-licensed Neulasta, PEG, Theragrastim, and US-licensed Neupogen will be redetermined using the conservative approach in identifying and removing outliers as described above.

Titer Assay Cut-Point

The titer cut point factor was calculated using the same data as the screening cut point factor. A non-parametric approach was applied, and the cut point was calculated using the 99th percentile in Excel. The titer cut point factor (TCPF) was determined to be 1.226. A floating assay cut point for sample analysis was calculated as the TCPF multiplied by the geometric mean of NCs from the run, which is shown below:

$$\text{Titer Assay Floating Cut-Point} = \text{Mean responses of the NCs} * \text{TCPF}$$

The reciprocal of the highest dilution of the PC or sample that yields a positive result which is equal to or greater than the titer cut point multiplied by the MRD (10) was reported as the titer value.

Assessor comment: The titer assay cut-point was calculated using the 99th percentile, which should give a false positive rate of 1%, which is acceptable.

Screening Assay Sensitivity and PCL Calculations

The sensitivity of the assay is the lowest concentration level at which the PC antibody can be detected and was evaluated in the screening assay by serially diluting a high concentration of positive control 1:2 to span the screening assay cut-point such that at least 5 concentrations were above the assay cut point and at least 1 concentration was below the assay cut point. Assay sensitivity was calculated using the equation:

$$\text{Assay Sensitivity} = \text{Anti Log} (\text{mean of log}_{10} \text{ values} + (t_{0.05,df} * \text{SD of log}_{10} \text{ values}))$$

The calculated assay sensitivity was then used to calculate the theoretical concentration of the PCL using the equation:

$$\text{Theoretical PCL Concentration} = \text{Anti Log} (\text{mean of log}_{10} \text{ values} + (t_{0.01,df} * \text{SD of log}_{10} \text{ values}))$$

The anti-TPI-120 antibody PC was diluted 1:2 starting at 1000 ng/mL to 3.91 ng/mL. The sensitivity was calculated as 11.105 ng/mL and the theoretical LPC was calculated as 14.249 ng/mL (Table 9). The PCL-1 and PCL-2 levels were evaluated for suitability in the screening and confirmatory assays. Both levels were positive in the screening and confirmatory assays, had concentrations above the assay sensitivity, and were above the assay cut point in the selectivity evaluations (further reviewed below). Therefore, the lower of the two (PCL-2 20.0 ng/mL) was selected as the PCL for the screening and confirmatory assays.

For the anti-PEG antibody, the PC was diluted 1:2 starting at 4000 ng/mL to 15.6 ng/mL. The sensitivity was calculated as 99.747 ng/mL and the theoretical LPC was calculated as 123.371 ng/mL (Table 10). The PCL1-PEG and PCL2-PEG levels were evaluated for suitability in the Tier 2 confirmatory assay. Only PCL2-PEG was positive in the screening and confirmatory assays, had a concentration above the assay sensitivity, and was above the assay cut point in the selectivity evaluations (further reviewed below). Therefore, PCL2-PEG was selected as the PCL for the PEG assay.

The anti-G-CSF antibody PC was diluted 1:2 starting at 2000 ng/mL to 0.977 ng/mL. The sensitivity was calculated as 31.974 ng/mL and the theoretical LPC was calculated as 53.142 ng/mL (Table 11). The anti-G-CSF PCL level was evaluated for suitability in the Tier 2 confirmatory assay and was confirmed as the PCL for the G-CSF assay.

***Assessor Comment:** The sensitivity of the screening assay is acceptable as the assay is able to detect an adequate level of ADAs against TPI-120. Furthermore, the sensitivity levels of the assay to detect antibodies to specific domains of the product are acceptable. In addition, the chosen concentrations for the PCLs provide adequate monitoring of assay performance near the cut-point level.*

Confirmatory Assay Sensitivity

The sensitivity of the confirmatory assay was defined as the lowest concentration level at which the PC antibody could be inhibited was evaluated similarly by serially diluting a high concentration of PC 1:2; samples were assays with and without excess drug such that at least one concentration was above the confirmatory cut point and at least 1 below the confirmatory cut point. The lowest nominal concentration which yielded a consistently positive result was considered the confirmatory assay sensitivity.

The anti-TPI-120 antibody PC (starting at 500 ng/mL) was diluted and treated with 10.0 µg/mL of TPI-120. The level at which %inhibition remained positive (above the confirmatory cut point) was 15.6 ng/mL, which was determined to be the sensitivity of the TPI-120 confirmatory assay (Table 12). The anti-PEG antibody PCs were diluted and evaluated with 5.00 µg/mL excess PEG. The level at which %inhibition remained positive (above the confirmatory cut point) was 125 ng/mL, which was determined to be the sensitivity for the PEG confirmatory assay (Table 13). The anti-G-CSF antibody PCs were diluted and treated with 10.00 µg/mL excess Theragrastim. The level at which %inhibition remained positive (above the confirmatory cut point) was 62.5 ng/mL, which was determined to be the sensitivity for the G-CSF confirmatory assay (Table 14).

***Assessor Comment:** The sensitivity of the confirmatory assay is acceptable as the assay is able to detect an adequate level of ADAs against TPI-120 and each of the product domains.*

Precision

The intra- and inter-assay precision of the screening assay was determined by evaluating multiple lots of the PCs of each antibody by two analysts on different days. The intra- and inter-assay precision of the confirmatory assay was also determined by evaluating PCs of each antibody in the presence of excess drug and calculating %inhibition using the following equation:

$$\% \text{ Inhibition} = (1 - (\text{Inhibited Sample} / \text{Mean of Uninhibited Samples})) * 100$$

The acceptance criteria were met if $CV \leq 20.0\%$ for intra-assay precision and $CV \leq 25.0\%$ for inter-assay precision for all PC levels (for both assays).

TPI-120 Precision

Three PC levels were evaluated in 18 lots on 5 different days to determine inter-assay and intra-assay precision (Tables 15-17). The screening assay intra-assay precision for the PCH, PCL-1, and PCL-2 had $CV \leq 8.382\%$, $CV \leq 4.019\%$, and $CV \leq 7.530\%$, respectively. For the TPI-120 confirmatory assay, the intra-assay precision of the PCH, PCL-1, and PCL-2 had $CV \leq 0.742\%$, $CV \leq 9.054\%$, and $CV \leq 12.288\%$, respectively, when treated with TPI-120, and $CV \leq 0.547\%$, $CV \leq 6.255\%$, and $CV \leq 10.161\%$, respectively, when treated with US-licensed Neulasta.

For the screening assay inter-assay precision, the PCH, PCL-1, and PCL-2 had 8.899% CV, 6.447% CV, and 6.367% CV. For the confirmatory assay inter-assay precision, the PCH, PCL-1, and PCL-2 had 0.708% CV, 5.162% CV, and 9.056% CV, respectively, when treated with TPI-120, and 0.688% CV, 4.879% CV, and 9.740% CV, respectively, when treated with US-licensed Neulasta. The inter-assay %inhibition of the PCH, PCL-1, and PCL-2 was 93.92%, 55.87%, and 37.95% when treated with TPI-120 and 94.18%, 57.2%, and 39.77% when treated with US-licensed Neulasta.

Assessor comment: *The data met the acceptance criteria for intra- and inter-assay precision for the TPI-120 PC in the screening and confirmatory assays.*

PEG Precision

Three PC levels were evaluated in 6 lots on 5 different days to determine inter-assay and intra-assay precision (Table 18 and 19). The screening assay intra-assay precision for PCH-PEG, PCL1-PEG and PCL2-PEG had $CV \leq 5.251\%$, $CV \leq 5.839\%$, and $CV \leq 6.629\%$, respectively. For the PEG confirmatory assay, the intra-assay precision of the PCH-PEG, PCL1-PEG and PCL2-PEG had $CV \leq 2.822\%$, $CV \leq 13.364\%$, and $CV \leq 12.704\%$ (4 out of 6 runs), respectively, when treated with PEG.

For screening assay inter-assay precision, the PCH-PEG, PCL1-PEG and PCL2-PEG had 21.515% CV, 8.669% CV, and 7.433% CV, respectively. For confirmatory assay inter-assay precision, the PCH-PEG, PCL1-PEG and PCL2-PEG had 9.096% CV, 29.909% CV, and 36.220% CV, respectively, when treated with PEG. The inter-assay %inhibition of the PCH-PEG, PCL1-PEG and PCL2-PEG was 75.001%, 35.197%, and 27.253% when treated with PEG.

Assessor comment: *The results for inter-assay precision for the confirmatory assay did not meet the acceptance criteria of $CV \leq 20\%$ for PCL1-PEG and PCL2-PEG. The data were accepted with a deviation to the validation protocol and determined to not have an impact of data integrity as the replicate %inhibition values were $\geq CCP$. Although the inter-assay precision for the confirmatory assay was outside the acceptance criteria for the PCL1-PEG and PCL2-PEG samples, the lower levels of precision are reasonable considering the lower level of antibodies in the preparations. Overall, this is acceptable and does not impact the conclusions of the validation study.*

G-CSF Precision

Two PC levels were evaluated in 6 lots on 3 different days (Table 20-22). The screening assay intra-assay precision for the PCH and PCL had $CV \leq 9.796\%$ and $CV \leq 9.901\%$, respectively. For the G-CSF confirmatory assay, the intra-assay precision of the PCH and PCL had $CV \leq 0.682\%$ and $CV \leq 12.826\%$,

respectively, when treated with Theragrastim, and $CV \leq 0.594\%$ and $CV \leq 13.153\%$, respectively, when treated with US-licensed Neupogen.

For the screening assay inter-assay precision, the PCH and PCL had 17.555% CV and 28.197% CV, respectively. For confirmatory assay inter-assay precision, the PCH and PCL had 1.169% CV, and 15.032% CV, respectively, when treated with Theragrastim, and 0.772% CV, and 14.632% CV, respectively, when treated with US-licensed Neupogen. The inter-assay %inhibition of the PCH and PCL was 94.651% and 38.544%, respectively, when treated with Theragrastim, and 94.021% and 36.612%, respectively, when treated with US-licensed Neupogen.

***Assessor comment:** The results for inter-assay precision for the PCL for screening assay did not meet acceptance criteria of $CV \leq 20\%$. The data were accepted with a deviation to the validation protocol and determined to not have an impact of data integrity as the replicate %inhibition values were $\geq CCP$ and the CV value is reasonable considering the level of antibodies in the preparation. Overall, this is acceptable and does not impact the conclusions of the validation study.*

Overall, intra- and inter-assay precision data were provided for the screening and confirmatory assays, which were found to be generally acceptable. However, precision data was not provided for the titer assay. An IR was sent to the applicant (2/19/21), to provide data to support that the intra- and inter-assay precision of the titer assay is adequate. In response (3/9/21), the applicant clarified that titer precision data was not generated during the validation exercise; however, titer assay controls were included in the evaluation of clinical samples. Data from this assessment support that the titer assay controls were run with acceptable precision (i.e., NMT 6.3% CV for PCL and NMT 10.7% for PCH; Table 16, report ADL-CL-112 report 11509.043017.1). This is acceptable. If the reanalysis of clinical data includes new evaluations of titer, the precision of the controls in the titer assay should be confirmed.

Selectivity: Matrix Interference

Selectivity was evaluated to assess the possible interference from serum matrix in assay performance. Pooled NC samples were used to prepare blank, low, and high matrix interference controls using the PC antibodies. These controls (i.e., low and high) were prepared at same levels as prepared for the PCL and PCH, respectively. Samples were evaluated in the screening and confirmatory assays.

TPI-120 and US-licensed Neulasta Selectivity

Ten NC samples were prepared by spiking anti-TPI-120 antibody PC at the PCH, PCL-1, and PCL-2 levels. For the Tier 1 confirmatory assay, spiked and unspiked samples were evaluated with and without excess TPI-120 and US-licensed Neulasta. All spiked samples tested positive for each PC level in the screening and confirmatory assays. The % inhibition was 93.6 – 95.7%, 56.2 – 67.8%, and 36.1 – 52.4% for the PCH, PCL-1, and PCL-2, respectively, when treated with TPI-120, and 92.1 – 93.7%, 48.4 – 55.0%, and 29.8 – 38.6% for the PCH, PCL-1, and PCL-2, respectively, when treated with US-licensed Neulasta (Tables 23 and 24).

PEG Selectivity

Ten NC samples were prepared by spiking anti-PEG antibody PC at the PCH-PEG, PCL1-PEG, and PCL2-PEG levels, and ten samples were prepared by spiking anti-TPI-120 antibody PC at the PCH, PCL-1, and PCL-2 levels. For the Tier 2 confirmatory assay, spiked and unspiked samples were evaluated with and without excess PEG. All spiked samples screened positive for the PCH-PEG and PCL1-PEG levels,

and 9 of 10 samples screened positive at the PCL2-PEG level. In the confirmatory assay, all spiked samples tested positive for the PCH-PEG and PCL1-PEG levels, and 8 of 10 samples screened positive at the PCL2-PEG level. For the TPI-120 PC, all samples screened positive at each level, but only 1 of 10 samples were confirmed positive at the PCH and PCL-1 levels when treated with PEG; 0 samples were confirmed positive at the PCL-2 level when treated with PEG. The % inhibition was 65 – 82.7%, 17-3 – 31.7%, and 14.4 – 25.6% for the PCH-PEG, PCL1-PEG, and PCL2-PEG, respectively, when treated with PEG and -15.3 – 25.4%, -20.6 – 22.6%, and -5.9 – 15.8% for the PCH, PCL-1, and PCL-2, respectively, when treated with PEG (Table 25).

Theragrastim and US-licensed Neupogen Selectivity

Ten NC samples were prepared by spiking anti-G-CSF antibody PC at the PCH-G-CSF and PCL-G-CSF levels. For the Tier 2 confirmatory assay, spiked and unspiked samples were evaluated with and without excess Theragrastim and US-licensed Neupogen. All spiked samples tested positive for each PC level in the screening and confirmatory assays. The % inhibition was 92.8 – 94.8% and 27.4 – 43.7% for the PCH and PCL, respectively, when treated with Theragrastim, and 92.1 – 93.9% and 26.5 – 42.5% for the PCH and PCL, respectively, when treated with US-licensed Neupogen (Tables 26 and 27).

Assessor comment: The data show that matrix present in serum samples does not negatively interfere in ADA detection for each PC in both screening and confirmatory assays.

Effect of Hemolysis

The effect of hemolysis on ADA detection was evaluated in the screening and confirmatory assays by evaluating blank, PCL, and PCH samples prepared in hemolyzed serum.

TPI-120 and US-licensed Neulasta

Five samples were prepared by spiking anti-TPI-120 antibody PC at the PCH, PCL-1, and PCL-2 levels in hemolyzed serum. For the Tier 1 confirmatory assay, spiked and unspiked samples were evaluated with and without excess TPI-120 and US-licensed Neulasta. All spiked samples tested positive for each PC level in the screening and confirmatory assays. The % inhibition was 93.0 – 93.6%, 49.1 – 53.7%, and 29.4 – 35.6% for the PCH, PCL-1, and PCL-2, respectively, when treated with TPI-120, and 91.9 – 93.6%, 46.2 – 51.9%, and 32.2 – 37.2% for the PCH, PCL-1, and PCL-2, respectively, when treated with US-licensed Neulasta (Tables 28 and 29).

PEG

Five samples were prepared by spiking anti-PEG antibody PC at the PCH-PEG, PCL1-PEG, and PCL2-PEG levels in hemolyzed serum, and five samples were prepared by spiking anti-TPI-120 antibody PC at the PCH, PCL-1, and PCL-2 levels in hemolyzed serum. For the Tier 2 confirmatory assay, spiked and unspiked samples were evaluated with and without excess PEG. All spiked samples screened positive at the PCH-PEG level, but no samples screened positive at the PCL1-PEG and PCL2-PEG levels. In the confirmatory assay, all spiked samples tested positive for the PCH-PEG, but no samples tested positive at the PCL1-PEG and PCL2-PEG levels. For the TPI-120 PC, all samples screened positive at each PC level, but no samples were confirmed positive at any PC level when treated with PEG. The % inhibition was 19.4 – 34.5%, -9.3 – 8.9%, and -27.5 – 8.0% for the PCH-PEG, PCL1-PEG, and PCL2-PEG, respectively, when treated with PEG and -4.3 – 2.9%, -3.8 – 3.9%, and -13.0 – 6.6% for the PCH, PCL-1, and PCL-2, respectively, when treated with PEG (Table 30).

Theragrastim and US-licensed Neupogen

Five samples were prepared by spiking anti-G-CSF antibody PC at the PCH-G-CSF and PCL-G-CSF levels in hemolyzed serum. For the Tier 2 confirmatory assay, spiked and unspiked samples were evaluated with and without excess Theragrastim and US-licensed Neupogen. All spiked samples tested positive for each PC level in the screening and confirmatory assays. The % inhibition was 90.8 – 92.7% and 22.6 – 40.2% for the PCH and PCL, respectively, when treated with Theragrastim, and 91.5 – 92.3% and 22.0 – 45.6% for the PCH and PCL, respectively, when treated with US-licensed Neupogen (Tables 31 and 32).

***Assessor comment:** The data show that the hemolyzed matrix did not affect the PCL and PCH samples in terms of detection of anti-TPI-120 and anti-G-CSF antibodies in the screening and confirmatory assays. However, the detection of anti-PEG antibodies was impacted in hemolyzed serum. An IR was sent to the applicant (2/19/21) to provide justification for the levels chosen for hemolyzed serum (i.e., how the levels correlate to clinical samples). In response (3/9/21), the applicant stated that commercially available human serum samples with hemolysis levels at approximately 500 mg/dL were used, which is on the high end of the index for hemolysis. Since this would represent worse-case for hemolyzed serum, the assay is able to detect the PEG PCL and PCH samples adequately, and hemolysis at 50 mg/dL did not impact the detection of ADAs to the whole product, the assay appears to be suitable for its intended purpose.*

Effect of Lipemia

The effect of lipemia on ADA detection was evaluated in the screening and confirmatory assays by evaluating blank, PCL, and PCH samples prepared in lipemic serum.

TPI-120 and US-licensed Neulasta

Five samples were prepared by spiking anti-TPI-120 antibody PC at the PCH, PCL-1, and PCL-2 levels in lipemic serum. For the Tier 1 confirmatory assay, spiked and unspiked samples were evaluated with and without excess TPI-120 and US-licensed Neulasta. All spiked samples tested positive for each PC level in the screening and confirmatory assays. The % inhibition was 93.0 – 94.1%, 49.1 – 54.5%, and 34.3 – 38.7% for the PCH, PCL-1, and PCL-2, respectively, when treated with TPI-120, and 92.7 – 94.1%, 47.1 – 55.6%, and 27.4 – 38.0% for the PCH, PCL-1, and PCL-2, respectively, when treated with US-licensed Neulasta (Tables 33 and 34).

PEG

Five samples were prepared by spiking anti-PEG antibody PC at the PCH-PEG, PCL1-PEG, and PCL2-PEG levels in lipemic serum, and five samples were prepared by spiking anti-TPI-120 antibody PC at the PCH, PCL-1, and PCL-2 levels in lipemic serum. For the Tier 2 confirmatory assay, spiked and unspiked samples were evaluated with and without excess PEG. All spiked samples screened positive at each PC level. In the confirmatory assay, all spiked samples tested positive for the PCH-PEG and PCL1-PEG, and 4 of 5 samples tested positive at the and PCL2-PEG levels. For the TPI-120 PC, all samples screened positive at each PC level, but no samples were confirmed positive at any PC level when treated with PEG. The % inhibition was 78.2 – 84.6%, 25.3 – 38.5%, and 14.6 – 27.1% for the PCH-PEG, PCL1-PEG, and PCL2-PEG, respectively, when treated with PEG and -6.5 – -2.1%, -4.5 – 6.2%, and -16.1 – 5.8% for the PCH, PCL-1, and PCL-2, respectively, when treated with PEG (Table 35).

Theragrastim and US-licensed Neupogen

Five samples were prepared by spiking anti-G-CSF antibody PC at the PCH-G-CSF and PCL-G-CSF levels in lipemic serum. For the Tier 2 confirmatory assay, spiked and unspiked samples were evaluated with and without excess Theragrastim and US-licensed Neupogen. All spiked samples tested positive for each PC level in the screening and confirmatory assays. The % inhibition was 93.8 – 94.2% and 37.2 – 42.4% for the PCH and PCL, respectively, when treated with Theragrastim, and 92.9 – 94.7% and 34.2 – 45.8% for the PCH and PCL, respectively, when treated with US-licensed Neupogen (Tables 36 and 37).

Assessor comment: The data show that lipemia did not interfere with the detection of ADAs in the LPC and HPC samples in both the screening and confirmatory assays. An IR was sent to the applicant (2/19/21) to provide justification for the levels chosen for lipemic serum (i.e., how the levels correlate to clinical samples). In response (3/9/21), the applicant stated that commercially available human serum samples that were “visually lipemic” were purchased and used. This is acceptable.

Hook Effect

Assessor comment: An IR was sent to the applicant (2/19/21) to provide data to demonstrate that very high concentrations of ADA above the HPC level for each positive control do not interfere with assay performance, since hook effects for ADA binding assays result in a reduction in signal and may ultimately lead to false-negative results. In response (3/9/21), the applicant stated that hook effects were not evaluated as part of the validation of the binding ADA assay, but were later evaluated and submitted in an updated validation report (Section 5.3.1.4, Sequence 0018, 5/17/21).

An ultra-high concentration of PC stock (4.23 mg/mL) was prepared in human serum and diluted 3-fold with negative control pool (423000ng/mL – 193 ng/mL). Three samples were analyzed, and the data was graphed as the nominal PC concentration vs. the S/N ratio. For the results to be acceptable, all PC concentrations above the PCL must be determined positive and no false negative results should be observed. The data passed the acceptance criteria (Table G2).

Table G2. Prozone (Hook Effect)

Run ID	Mean NC PC Concentration	Theoretical	Normalized	Normalized	Normalized
		Concentration	Response (S/N) Replicate 1	Response (S/N) Replicate 2	Response (S/N) Replicate 3
21329rza10z	67.500	193.000	4.333	4.030	4.156
		580.000	10.956	10.437	10.659
		1741.000	32.052	31.304	31.474
		5222.000	100.541	98.244	91.615
		15667.000	315.311	308.570	308.074
		47000.000	1026.170	982.822	941.607
		141000.000	2720.874	2752.726	2617.985
		423000.000	1113.963	1172.163	1035.874

S/N: Sample Response / Mean Run NCs

Note: Mean ECL responses were normalized with the NC mean of the assay

Note: No values fall below the screening cut point factor (1.115)

Assessor comment: Increasing concentrations of ADAs did not have a significant impact on assay signal. Although there is a slight impact that caused a reduction in assay signal, the concentration of antibody in this sample is really high (4.23 mg/ml) and did not result in an assay signal value below or near the cut-

point. Overall, the data support an adequate correlation with assay signal and increasing concentration of ADAs.

Drug Tolerance

The drug tolerance of the assay was evaluated to determine the level of drug that would interfere with ADA detection in the assay. Four PC antibody levels (20 ng/mL, 40 ng/mL, 100 ng/mL, and 500 ng/mL) were spiked into six levels of TPI-120 or US-licensed Neulasta (0 – 1.5 µg/ml). The lowest PC antibody level (20 ng/mL) could be detected in 0.25 µg/mL TPI-120. The assay could detect 100 ng/mL of antibody in the presence of up to 1 µg/mL of TPI-120, or 1.5 µg/mL of US-licensed Neulasta (Tables 38 and 39).

Table 38. Drug Tolerance (TPI-120)

Run ID	Assay Cut	TPI-120	20.0 ng/mL	40.0 ng/mL	100 ng/mL	500 ng/mL
	Point	(µg/mL)				
17607bta10z	75.123	1.50	65.000	65.500	74.000	106.000
		1.00	66.000	67.500	79.500	117.500
		0.500	68.000	71.000	89.000	131.000
		0.250	78.500	76.000	89.000	135.000
		0.00	109.000	148.500	246.500	1114.000

Note: Bold values are below the assay cut point

Table 39. Drug Tolerance (Neulasta®)

Run ID	Assay	Neulasta	20.0 ng/mL	40.0 ng/mL	100 ng/mL	500 ng/mL
	Cut Point	(µg/mL)				
17607bta10z	75.123	1.50	65.000	69.500	75.500	112.500
		1.00	68.500	74.500	78.000	126.000
		0.500	70.000	77.500	81.000	133.500
		0.250	72.000	75.000	85.000	142.500

Note: Bold values are below the assay cut point

Assessor comment: An adequate level of ADAs (100 ng/mL) can be detected in the presence of 1 µg/mL of TPI-120 and 1.5 µg/mL of US-licensed Neulasta. However, it is unclear what are the levels of onboard drug in patient samples. An IR was sent to the applicant (2/19/21), to clarify what the onboard levels of drug are at the time of immunogenicity testing of patients during clinical study ADL-CL-112. In response (3/9/21), the applicant stated that at the time of sampling (i.e., pre-dose, Day 1, and Day 8), the onboard PK concentration levels were below 1 ng/mL on the days that immunogenicity samples were taken. (Table 2, Report 16203, Section 5.3.1.4), which is much lower than the drug tolerance of the assay. While the assessment of drug levels in patient samples is deferred to the clinical pharmacology reviewer, this appears to support that the assay has acceptable drug tolerance to TPI-120 and US-licensed Neulasta in patient samples to allow for detection of low levels of ADA that may be clinically relevant.

In the IR response, the applicant clarified that they plan to provide an assessment to support antigenic equivalence (i.e., the ability of both products to bind in a similar manner to the PCs) at the time of reanalysis of the clinical samples. This assessment will be submitted on June 30, 2021. This is not an issue as the data provided in the validation study from the drug tolerance and precision assessments generally support that the products are binding in a similar manner to the PC, which would support similar assay

performance when analyzing clinical samples treated with either TPI-120 or US-licensed Neulasta, and ADAs were confirmed to both TPI-120 (7%) and US-licensed Neulasta (14.8%).

Stability

The stability of the PCH and PCL levels for each PC were evaluated at ambient conditions, after freeze/thaw, and at long-term conditions. Three samples were evaluated for each PC at each level, in each condition.

Short-Term Stability (Ambient)

PC samples were removed from frozen storage (-80°C) and placed at ambient, room temperature (RT) conditions for 24 to 25 hours before testing. The CV of each PC ranged from 3.3 – 5% for anti-TPI-120, 0.7 – 3.6% for anti-PEG, and 4.8 – 12.9% for anti-G-CSF, which are within the %CV ≤ 20.0% AC. Data are provided in Tables 48 – 50.

Freeze/Thaw Stability (-80°C, Nominal)

Samples were subject to 5 to 6 freeze/thaw (F/T) cycles for TPI-120 PC levels, 5 F/T cycles for PEG PC levels, and 12 F/T cycles for G-CSF PC levels prior to testing. The CV of each PC ranged from 1 – 4.4% for anti-TPI-120, 3.1 – 4.5% for anti-PEG, and 2.9 – 3.3% for anti-G-CSF, which are within the %CV ≤ 20.0% AC. Data are provided in Tables 51 – 53.

Long-Term Stability (-80°C, Nominal)

TPI-120 PC levels were stored at -80 for up to 8 and 118 days, PEG PC levels were stored at -80C for 8 days, and G-CSF PC levels were stored at -80C for 21 days prior to testing. The CV of each PC ranged from 1.2 – 3.2% for anti-TPI-120, 0.5 – 3.3% for anti-PEG, and 5.3 – 6.3% for anti-G-CSF, which are within the %CV ≤ 20.0% AC. Data are provided in Tables 54 – 56.

Long-Term Stability of Working Solutions (-80°C, Nominal)

Very high levels of PC were prepared for anti-TPI-120 (50,000 ng/mL) and of anti-PEG (100,000 ng/mL) and stored at -80C for up to 30 days, after which they were used to prepare the PCH and PCL levels for each control. The CV of each PC ranged from 1.1 – 2.5% for anti-TPI-120 and 0.7 – 1.5% for anti-PEG, which are within the %CV ≤ 20.0% AC. Data are provided in Tables 57 and 58.

***Assessor Comment:** The provided data suggest that the PC preparations are stable without much deviation in assay responses after freeze/thaw cycles from -80°C storage to RT, as well as stored at RT for 24-25 hours, which cover the working conditions for the assay. Data was also provided for the controls stored under long-term storage conditions, which show that the PC preparations and working solutions are stable over time when stored at -80°C. Sufficient information and data are provided to support the stability of the PCs during assay in-use conditions.*

System Suitability/Data Acceptance Criteria

For a run to be considered acceptable, at least 4 replicates of NC and 2 replicates of each PC level must be included in the screening, confirmatory, and titer assays. In the screening, confirmatory and titer assays, the CV of the NC and PC response must be ≤ 20%; 3/4 of the NC and at least 1 of each PC level replicate responses must meet the acceptance criteria. In the screening and titer assays, the PC must meet the paradigm of PCH > PCL ≥ CPF. In the confirmatory assay, the % inhibition of PCs must be ≥ confirmatory cut point (CCP) established by pre-study validation.

Assessor Comment: System suitability criteria are supported and were met during the assay validation exercise.

Deviations

There were a few deviations that occurred during the validation of the binding assay, including improper preparation and processing of PC samples, sample mix up, improper documentation, and improper instructions for preparation. These resulted in experimental failures after which an investigation was conducted to find the root cause. The samples were re-prepared, and runs repeated, as necessary; the test method was also updated with the correct instructions for sample preparation. Due to the incorrect preparation of PC (8.57 µg/mL TPI-120 instead of 10 µg/mL), the data integrity of the assay was impacted, though the addition of this PC still resulted in excess TPI-120 in the samples as intended. Planned deviations were also implemented for stability studies in order to reflect actual working conditions of the assay. Additionally, the stock drug concentration for Theragrastim was documented to be 10 mg/mL, but the correct concentration (as per CoA) was 0.62 mg/mL, which was used during the evaluation of the confirmatory cut-point and sensitivity runs. Investigative experiments showed that a CCP established with a 10 µg/mL confirmatory working solution was comparable to the CCP established with the 0.62 µg/mL confirmatory working solution in the validation, as the "investigative" CCP value was within the range between the minimum and maximum CCPs calculated across the 6 cut point datasets from the validation.

Assessor comment: The deviations do not impact the validation exercises for the binding assay. Overall, sufficient information and data are provided to support that the binding antibody assay is adequately validated for its intended purpose in support of the BLA.

ADA Bioanalytical Report (Report 11509.043017.1)

Serum samples from clinical study ADL-CL-112 were evaluated using the screening, confirmatory (Tier 1 and Tier 2), and titer assays. Samples were obtained from subjects receiving TPI-120 or US-licensed Neulasta for immunogenicity testing at baseline (pre-dose), and on Days 8, 22 (prior to dose 2), 29, and 58 (end of treatment) of the study (n = 1126 samples for analysis; 1123 duplicate samples).

Treatment-emergent anti-Peg-G-CSF antibodies were observed in 8 subjects (7%). Of these, 7 subjects showed specificity for TPI-120 only while 1 subject showed specificity for US-licensed Neulasta only. None of the subjects showed specificity for the active Theragrastim component of TPI-120, the active Neupogen component of US-licensed Neulasta, or the inactive PEG component. None of the subjects showed neutralizing activity of the antibody. Titer levels were also reported for samples confirmed positive for ADAs.

For subjects receiving US-licensed Neulasta, treatment-emergent anti-Peg-G-CSF antibodies were observed in 16 subjects (14.8%). Of these, 11 subjects showed specificity for both TPI-120 and US-licensed Neulasta, while 5 subjects showed specificity for only TPI-120. Nine subjects (8.3%) showed specificity to PEG, 1 subject (0.9%) showed specificity to Theragrastim, and 1 subject (0.9%) showed specificity to Neupogen. One subject (0.9%) showed neutralizing activity of the antibody.

The comparison of the incidence rates between TPI-120 and US-licensed Neulasta show that the post-dose incidence of ADAs varied between 1% to 7% and incidence of nAbs was less than 2%. Since the

difference was less than 10%, TPI-120 was not considered different from US-licensed Neulasta in terms of treatment emergent immune response.

***Assessor comment:** It was unclear whether the titers reported in the analysis report considered all possible dilutions in the titer assay. An IR was sent to the applicant (2/19/21) to clarify, and if necessary, update the report to include titer values that consider all possible dilutions. In response (9/2/20), the applicant clarified that all possible dilutions were considered for reporting antibody titers for the clinical samples. Overall, the information provided in the clinical report demonstrates that the binding antibody assay was performed in the validated state as intended without impactful changes to the method and met system suitability criteria during clinical sample analysis, which supports that the clinical immunogenicity data generated from the assessment is reliable. The reanalysis report using the new cut-points should be reviewed when submitted to the BLA.*

Neutralizing Antibody Assay Validation Report (Report 11866.122617)

Neutralizing antibodies (nAbs) against TPI-120 and US-licensed Neulasta were detected using a proliferation assay in which the product binds to the G-CSF receptor on the surface of target cells and induces dimerization of the receptor and initiates a cascade of events resulting in hematopoietic stem cell proliferation and differentiation into neutrophils. nAbs prevent binding of PEG-G-CSF to the receptor, and therefore prevent proliferation. The assay uses the M-NFS-60 (murine leukemia) cell line as target cells, which express the G-CSF receptor and proliferate in response to human G-CSF and murine IL-3 (mIL-3); the cells were adapted with Theragrastim to respond to stimulation from PEG-G-CSF. mIL-3 was used to develop a specificity assay to evaluate the specificity of the inhibition detected in the assay. Viability was measured as the assay end point, using MTS dye; the formazan product produced is directly proportional to the number of living cells, which can be measured by absorbance.

***Assessor comment:** The M-NSF-60 mouse cell line was used in the validation of the nAb assay, however, no control over the use of these cells is indicated in the validation report. Since the number of cell passages can impact assay performance, an IR was sent to the applicant (2/19/21) to clearly state how the number of cell passages is controlled at the time of cryopreservation and to show that the limits placed on the passage number are appropriate, as well as to clarify whether the cells used in the validation of the nAb assay were within that criteria. In response (3/9/21), the applicant stated that while the cell line was purchased from (b) (4) a passage number was not provided, therefore they were designated as passage 0 at the time of purchase, and the cells were cryopreserved at passage 6. The cell passage number that was used for method validation was between passages 10-23 and for sample analysis was between passages 10-24. Data was provided to show that cells at passage 10 performed comparably to cells at passage 23 (i.e., NC, and PC/DC readings). Furthermore, the results from the PCs used in the clinical sample assessment were acceptable. Overall, this is acceptable for the intended purpose of supporting the BLA.*

Assay principle

Samples are first analyzed in a Tier 1 assay in which PCs and samples are evaluated using TPI-120 (or US-licensed Neulasta) as a proliferating stimulator to distinguish nAb positive from nAb negative samples, and then nAb positive samples are evaluated in a Tier 2 is a specificity assay using mIL-3 as a proliferating stimulator to evaluate if the nAb positive samples are specific to TPI-120.

In the Tier 1 assay, TPI-120 (or US-licensed Neulasta) was incubated with samples and controls 60 ± 15 minutes, and then added, along with target cells, to cell culture plates and incubated for 72 ± 2 hours at

37°C. The MTS reagent was then added, and the plates were incubated for 4 hours $\pm$ 15 minutes. At the end of incubation, the OD at 490 nm was measured; if neutralizing antibodies were present in the serum samples, TPI-120 would be unable to induce cell proliferation. The ratio of bioactivity was calculated as: (Mean response of the sample or PC)/(Mean response of drug control). If the ratio of a sample was above the assay cut-point, the samples were considered “negative” for nAbs, while samples with a ratio at or below the assay cut-point were “positive” and further evaluated in the Tier 2 assay. In the Tier 2 assay, mIL-3 was mixed with samples instead of TPI-120, and the assay continued as above. Samples above the cut-point were reported as “negative” (nAbs specific to TPI-120), while samples at or below the assay cut point were reported as “positive” (non-specific nAbs).

Assessor comment: *An IR was sent to the applicant (2/129/21) to clarify the concentration of drug (i.e., TPI-120 and US-licensed Neulasta) that was used in the nAb assay and to provide activity dose response curves for TPI-120 and US-licensed Neulasta to demonstrate that the concentration of the drug chosen for each product is on the linear range of the curve to support that the assay response is sensitive to inhibition by nAbs. In response (3/9/21), the applicant stated that a concentration of 0.25 ng/ mL was selected for TPI-120 and US-licensed Neulasta because it provided 70% activity; assay curves were provided to show that the concentration is on the linear range of the curve. The response is acceptable and supports that an adequate concentration of TPI-120 and US-licensed is used in the assay to ensure proper response. In addition, the assay curves are generally comparable.*

Negative Control

Six pre-screened human serum lots were pooled, aliquoted, and stored -80°C to generate a NC pool.

Assessor comment: *The use of healthy adult human serum as the NC is acceptable as it resembles the clinical population and is derived from treatment-naïve subjects. The values generated from the NC are above but close to the cut-point (negative result).*

Positive Control

A rabbit anti-G-CSF polyclonal antibody was used as a PC. PC samples were prepared by spiking the NC with the rabbit pAb at PCH (1000 ng/mL), PCL-A (300 ng/mL) and PCL-B (250 ng/mL) levels, which were aliquoted and stored -80°C.

Assessor comment: *A TPI-120 purified anti-drug antibody is also listed as a PC in Section 2.2; while the description of the assay does not include the use of this antibody as PC, the results refer to the PC as anti-TPI-120 antibody. Therefore, it is unclear whether both antibodies were used for validation (anti-G-CSF and anti-TPI-120). IRs were sent to the applicant (2/19/21, 4/22/21) to clarify which antibody was used as PC for the validation of the nAb assay and provide justification to support the selection of the PC. In response (3/9/21, 5/17/21), the applicant clarified that only the anti-TPI-120 polyclonal antibody was used during validation; the report will be amended in a subsequent amendment. Additionally, the applicant was asked whether an anti-PEG positive control antibody was used during development because ADAs specific to PEG may lead to false positives results to PEG in the nAb assay. The applicant stated that the response of an anti-PEG PC antibody was not evaluated in the nAb assay since specificity was confirmed using mIL-3 in the Tier 2 confirmatory assay.*

Drug Control

TPI-120, US-licensed Neulasta, or mIL-3 were used as drug controls (DC); these were diluted in assay medium and added to the NC or PC during the assay.

Tier 1 Assay Cut-Point

The Tier 1 (screening) assay cut-point was derived from 30 samples of normal human serum. Data was collected over two days by three analysts. The distribution of the data was determined to be normal (JMP evaluation, Shapiro-Wilk test) after identification and removal of outliers (outliers defined as values less than $Q1 - 1.5 * IQR$ or greater than $Q3 + 1.5 * IQR$). A parametric approach was applied, and the cut point was calculated, allowing a 1% false positive rate, using the following equation:

$$\text{Cut Point Factor (CPF)} = \text{Mean of responses} - t_{0.01,df} * \text{SD}$$

The CPF was calculated to be 0.672 (Table 2). Additionally, the correlation of the DC and samples was examined by comparing the mean response of the DC replicates from each run to the mean response of the samples from each run. The correlation coefficient (R^2 ; Pearson correlation function in Excel) was 0.9754, indicating a correlation between the response of DC and response of the samples.

Assessor comment: The Tier 1 screening assay cut-point allows for a 1% false positive rate; therefore, the cut-point is appropriate. An IR was sent to the applicant (2/19/21) to correct the equation used for deriving the cut-point factor, which was amended as part of the response (3/9/21; as written above).

Tier 2 Assay Cut-Point

The Tier 2 (confirmatory) assay was performed in order to evaluate whether the reduction in signal is observed due to the presence of nAbs specific to TPI-120 or due to non-specific matrix components. The Tier 2 assay cut-point was derived from 30 samples of normal human serum. Data was collected over two days by three analysts. The distribution of the data was determined to be normal (JMP evaluation, Shapiro-Wilk test) after identification and removal of outliers (outliers defined as values less than $Q1 - 1.5 * IQR$ or greater than $Q3 + 1.5 * IQR$). A parametric approach was applied, and the cut point was calculated, allowing a 1% false positive rate, using the following equation:

$$\text{Specificity Cut Point Factor (SCPF)} = \text{Mean of responses} - t_{0.01,df} * \text{SD}$$

The SCPF was calculated to be 0.689 (Table 3). Additionally, the correlation of the DC and samples was examined by comparing the mean response of the DC replicates from each run to the mean response of the samples from each run. The correlation coefficient (R^2 ; Pearson correlation function in Excel) was 0.975, indicating a correlation between the response of DC and response of the samples.

Assessor comment: The Tier 2 confirmatory assay cut-point allows for a 1% false positive rate; therefore, the cut-point is appropriate. An IR was sent to the applicant (2/19/21) to correct the equation used for deriving the cut-point factor, which was amended as part of the response (3/9/21; as written above).

Sensitivity and PCL Calculations

The assay sensitivity was evaluated by serially spiking the PCH antibody into the NC pool to create an antibody curve from 1000 ng/mL to 15.6 ng/mL (1:2 dilutions). Ten antibody curves were generated for each run type. The assay was run using TPI-120 and US-licensed Neulasta, and assay sensitivity was

calculated using each DC. The assay sensitivity is 202 ng/mL for the anti-TPI-120/TPI-120 DC assay and 247 ng/mL for the anti-TPI-120/US-licensed Neulasta DC assay. From this, the theoretical PCL concentration was calculated to be of 215 ng/mL for anti-TPI-120/TPI-120 DC and 276 ng/mL for anti-TPI-120/US-licensed Neulasta DC. Data are provided in Tables 4 and 5.

The PCL-A (300 ng/mL) and PCL-B (250 ng/mL) levels were evaluated for suitability. Both levels had positive results in the screening and confirmatory precision assays, and data for each PC level were above the assay cut-point in the Tier 2 selectivity assay. Since both PCL levels were above assay sensitivity, the PCL-B level was established as the assay PCL since it was the lower concentration.

Assessor comment: The sensitivity of the nAb assay is acceptable to detect low levels of Nabs and the level of the PCL is acceptable to monitor assay performance near the cut-point level.

Matrix Interference

The ability to detect anti-TPI-120 nAbs in the presence of matrix components was evaluated. The PCH, PCL-A, and PCL-B were spiked into ten lots of human serum and analyzed, in addition to unspiked serum, with TPI-120 and US-licensed Neulasta. Ten of ten unspiked samples were above the cut-point (i.e., negative), while all of the spiked samples were below the assay cut-point (i.e., positive). The PC samples met the criteria of $CPF > PCL > PCH$. Data are provided in Table 9 and 10.

Assessor comment: The data show that the detection of nAbs is not affected by the presence of serum matrix in normal human samples.

Precision

The intra- and inter-assay precision of the assay was evaluated using PCH, PCL-A, and PCL-B levels with TPI-120 and US-licensed Neulasta, by 2 analysts over 3 days. Five replicates of each of the PC levels were evaluated 4 times (Tables 11 and 12).

Intra-assay precision

For TPI-120 at the PCH, PCL-A, and PCL-B levels, the intra-assay precision had 2.1 – 3.4% CV, 2.9 – 15.9% CV, and 2.3 – 5.3% CV, respectively. For US-licensed Neulasta at the PCH, PCL-A, and PCL-B levels, the intra-assay precision had 1.8 – 3.7% CV, 2.2 – 16.5% CV, and 1.8 – 4.2% CV, respectively. The standard deviation for intra-assay precision for each PC level evaluated using both TPI-120 and US-licensed Neulasta was 0 – 0.1.

Inter-assay precision

For inter-assay precision using TPI-120, each PC level had 4.8% CV for PCH, 7.9% CV for PCL-A, and 5.7% CV for PCL-B. For inter-assay precision using US-licensed Neulasta, each PC level had 7.5% CV for PCH, 11.6% CV for PCL-A, and 6.2% CV for PCL-B. The standard deviation for inter-assay precision for each PC level evaluated using both TPI-120 and US-licensed Neulasta was 0 – 0.1.

Assessor comment: The data met the acceptance criteria of $\%CV \leq 25.0\%$ for each PC level evaluated using TPI-120 and US-licensed Neulasta. The precision of the nAb assay is acceptable to ensure that the results are reliable.

Drug Tolerance

The drug tolerance of the assay was evaluated to determine the potential of TPI-120 and US-licensed Neulasta to interfere with the detection of nAbs. Three antibody levels (PCH 1000 ng/mL, PCL-A 300 ng/mL, and PCL-B 250 ng/mL), were spiked with 8 levels of drug (1:2 dilutions ranging from 0.0313 - 2 ng/mL, along with a blank control) to evaluate tolerance. For both TPI-120 and US-licensed Neulasta, the PCL-A and PCH results indicate that the assay was tolerant up to 2 ng/mL, while the PLC-B was tolerant up to 1 ng/mL. Data are provided in Tables 6 - 8.

Table 6. Drug Tolerance (TPI-120)

TPI-120 (ng/mL)	250 ng/mL	300 ng/mL	1000 ng/mL
2.00	0.702	0.308	0.340
1.00	0.569	0.311	0.351
0.500	0.491	0.300	0.348
0.250	0.442	0.296	0.352
0.125	0.447	0.301	0.337
0.0625	0.429	0.302	0.333
0.0313	0.419	0.297	0.337
0.00	0.402	0.306	0.350

Note: Bold value above the cut point (0.672)

Note: Results compiled as ((Response of sample-Mean of NC)/Mean response of DCC)

Table 7. Drug Tolerance (Neulasta)

Neulasta (ng/mL)	250 ng/mL	300 ng/mL	1000 ng/mL
2.00	0.727	0.659	0.727
1.00	0.585	0.529	0.500
0.500	0.516	0.459	0.499
0.250	0.470	0.420	0.466
0.125	0.452	0.401	0.432
0.0625	0.445	0.396	0.431
0.0313	0.442	0.407	0.433
0.00	0.449	0.412	0.428

Note: Bold values above the cut point (0.672)

Note: Results compiled as ((Response of sample-Mean of NC)/Mean response of DCC)

Table 8. Drug Tolerance (Neulasta; Repeat)

Neulasta (ng/mL)	1000 ng/mL
2.00	0.351
1.00	0.314
0.500	0.309
0.250	0.303
0.125	0.329
0.0625	0.316
0.0313	0.308
0.00	0.306

Note: Results compiled as ((Response of sample-Mean of NC)/Mean response of DCC)

Assessor comment: *The data demonstrate that the nAb assay can tolerate drug concentrations up to 2.0 ng/ml for TPI-120 and US-licensed Neulasta for the detection of an adequate level (i.e., 300 - 1000 ng/ml) of nAbs. As described above, the drug levels in patient samples at the time of immunogenicity sampling did not exceed 1 ng/ml. Therefore, the drug tolerance is acceptable for the evaluation of clinical samples with TPI-120 and US-licensed Neulasta. Furthermore, the applicant plans to submit an assessment to support antigenic equivalence in the nAb assay for TPI-120 and US-licensed Neulasta. This is not an issue as the sensitivity and drug tolerance data do not show any concerns regarding antigenic equivalence.*

Stability Experiments

The stability of the PC was evaluated by subjecting 3 replicates of each level to short-term ambient conditions, as well as freeze thaw cycles in a separate experiment, prior to analysis. For the short-term stability experiments, samples removed from frozen storage and were left at room temperature (bench top) for 22 hours prior to analysis. All three replicates for PCH, PCL-A, and PCL-B were below the assay cut-points when evaluated with TPI-120 (Table 20), and US-licensed Neulasta (Table 21). For freeze-thaw experiments, samples were removed from frozen storage and subjected to 5 freeze-thaw cycles prior to analysis. All three replicates for PCH, PCL-A, and PCL-B were below the assay cut-points when evaluated with TPI-120 (Table 22), and US-licensed Neulasta (Table 23). Additionally, the data met the criteria of CPF > PCL > PCH

Assessor Comment: *The data provided is acceptable and support the stability of PC in the validation matrix and the stability of ADAs in clinical samples during execution of the assay.*

Assay Robustness

Assessor comment: *Data was not provided for assay robustness in the validation report for the nAb assay. Since evaluation of the robustness of the assay provides assurance that subtle deviations in the method (e.g., changes in incubation time or temperature) would not affect method performance, an IR was sent to the applicant (2/19/21) to provide development data, if available, assessing the robustness of the nAb assay using deliberate variations during testing. In response (3/9/21), the applicant provided the incubation times for the various runs performed during validation, in which the 72 ± 2 hour incubation of cells and samples varied from 69.17 hours to 71.33 hours (2 hour 10 minute difference), and the 4 hours ± 30 minutes incubation of the MTS reagent varied from 3.42 hours to 4.25 hours (50 minute difference) and stated that the QC data was comparable between runs, which supports robustness to small changes in incubation steps.*

Assay Acceptance Criteria

All controls (NC, PC, and DC) and samples must have responses with CV $\leq 25.0\%$. For the Tier 1 and Tier 2 assays, 50% of the NC and PCH must meet %CV criteria, and 2/3 of DC must meet %CV criteria. For the drug tolerance and selectivity assays, 50% of NC and each PC level must meet %CV criteria, 2/3 of DC must meet %CV criteria, and the relationship of PCH, PCL and CPF for Tier 1 and Tier 2 assays must follow the paradigm: PCH < PCL $\leq$ CPF. For the precision assay, 2/3 of the NC must meet %CV criteria.

Assessor Comment: *System suitability was met during assay validation exercise (for acceptable runs that were used in evaluations). Overall, sufficient information and data are provided to support that the nAb assay is adequately validated for its intended purpose in support of the BLA.*

Deviations

One deviation was noted that resulted in a slightly shorter incubation step for the addition of cell titer reagent to cells step. This run met the acceptance criteria and had comparable results with the other runs. Therefore, this deviation did not impact the validity of the study. Two other deviations were noted but were very minor.

Assessor comment: The deviations that occurred did not impact the conclusions of the validation exercise.

nAb Bioanalytical Report (Report 11563.061618)

Patient serum samples from clinical study ADL-CL-112 were first evaluated using the screening, confirmatory, and titer assays for detection of ADAs. Samples that were confirmed positive (n = 54) were analyzed using the Tier 1 cell-based nAb assay using both TPI-120 and US-licensed Neulasta. If either assay had positive results, the samples were evaluated using the Tier 2 nAb assay using mIL-3, to assess whether the nAbs were specific to TPI-120/US-licensed Neulasta. The validated methods were not modified during patient sample analysis.

Of the 54 samples evaluated, 52 samples were negative for nAbs, while the 2 that were positive were further evaluated in the Tier 2 assay and were found to be specific to TPI-120.

Assessor comment: The information provided in the clinical report demonstrates that the nAb assay was performed in the validated state as intended without impactful changes to the method and met system suitability criteria during clinical sample analysis, which supports that the clinical immunogenicity data generated from the assessment is reliable. As noted before, reanalysis of clinical samples for binding antibodies is planned using the conservative cut-point approach, which may result in additional samples being evaluated in the nAb assay. Any additional clinical samples analysis reports for the nAb assay that were generated from the clinical sample reanalysis should be review when submitted to the BLA.