

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

761075Orig1s000

PRODUCT QUALITY REVIEW(S)



Center for Drug Evaluation and Research
WO Building 22
10903 New Hampshire Ave.
Silver Spring, MD 20993

PRODUCT QUALITY MICROBIOLOGY REVIEW AND EVALUATION

Date: April 5, 2018
To: Administrative File, 761075/43
From: Scott Nichols, Ph.D., Reviewer, OPQ/OPF/DMA/BIV
Through: Reyes Candau-Chacon, Ph.D., Branch Chief, OPQ/OPF/DMA/BIV
Subject: Resubmission of 351(k) Biosimilar Biologics License Application (BLA)
US License: 2062
Sponsor: Mylan GmbH
Facilities: [REDACTED] (b) (4)
(FEI: [REDACTED] (b) (4))

Product: Fulphila (proposed), MYL-1401H
Dosage: sterile liquid for pre-filled syringe for subcutaneous injection (6 mg/0.6 mL)
Indication: decrease incidence of infection as manifested by febrile neutropenia, in patients with non-myeloid malignancies receiving myelosuppressive anti-cancer drugs associated with a clinically significant increase of febrile neutropenia
Action Date: June 4, 2018

Conclusion and Approvability Recommendation:

The drug substance portion of the original submission of BLA 761075 included a non-deficiency comment in the CR letter:

Only one batch of bulk drug substance (DE-B14-03-000018) was used to qualify the bioburden test. Conduct the qualification of the [REDACTED] (b) (4) bioburden test for the bulk drug substance using two additional batches of bulk drug substance.

The applicant submitted the additional qualification data, which were reviewed from a microbial control and microbiological product quality perspective. The application is recommended for approval.

Please refer to the review memo from Dr. Lindsey Brown for an assessment of microbial control and microbiological product quality for the drug product (DP) portions of the BLA resubmission.



Scott
Nichols

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**Center for Drug Evaluation and Research
Office of Pharmaceutical Quality
Office of Process and Facilities
Division of Microbiology Assessment**

PRODUCT QUALITY MICROBIOLOGY REVIEW AND EVALUATION

REVIEWER: Lindsey Brown, Ph.D.

Quality Assessment Lead (acting): Reyes Candau-Chacon, Ph.D.

Date: 3/28/2017

To: Administrative File

BLA: 761075/6

Applicant: Mylan Pharmaceuticals

US License Number: 1898

Manufacturing Site: [REDACTED] (b) (4)

FEI: [REDACTED] (b) (4)

Submission Reviewed: Biological License Application

Product: Proposed biosimilar to US approved Pegfilgrastim, MYL-1401H

Indication: To 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

Dosage Form: Pre-filled syringe (0.6 ml, 10 mg/ml)

FDA Receipt Date: 12/04/2017

Action Date: 6/4/2018

Recommendation for Approvability: BLA 761075 resubmission is recommended for approval from a sterility assurance and microbiology product quality perspective with the following post-marketing commitment:

To include (b) (4) as an in-process parameter in Section 3.2.P.3.4 of the BLA. (b) (4) acceptance criteria in (b) (4) should be within the parameters validated during the (b) (4) studies.

Review Summary

Drug Product Quality Microbiology Information Reviewed

Sequence	Date	Description
0042	12/04/2017	Resubmission
0048	5/02/2018	Response to Information Request
0049	5/03/2018	Response to Information Request

(b) (4)



Lindsey
Brown

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Reyes
Candau-Chacon

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Date: 5/04/2018 02:01:13PM
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QUALITY REVIEW



Recommendation: Approve, pending final assessment of facilities compliance

BLA 761075

Review 2

Review Date: May 15, 2018

Drug Name/Dosage Form	MYL-1401H/injection
Strength/Potency	6 mg/0.6
Route of Administration	Subcutaneous
Rx/OTC Dispensed	Rx
Indications	MYL-1401H is proposed for the following indication: Decrease the incidence of infection, as manifested by febrile a neutropenia, in patients with non-myeloid malignancies receiving myelosuppressive anti-cancer drugs associated with a clinically significant incidence of febrile neutropenia
Applicant/Sponsor	Mylan GmbH

Product Overview

MYL-1401H is a pegylated recombinant granulocyte colony stimulating factor (PEG-GCSF). GCSF is a growth factor that stimulates production of neutrophils and neutrophil precursors by binding the GCSF receptor on blood cells of the neutrophilic granulocyte. This binding initiates signal transduction that leads to the survival, proliferation, and differentiation of neutrophil progenitor cells into mature neutrophils.

Quality Review Team

DISCIPLINE	REVIEWER	DIVISION
Drug Substance	Merry Christie	DBRR III
Drug Product and Analytical Similarity	Paul Kirwan	DBRR III
Facilities	Laura Fontan/Michael Shanks/Peter Qiu	DIA
Microbiology	Scott Nichols/Lyndsey Brown/Lakshmi Narasimhan/Maria Reyes Candauchacon/Patricia Hughes	DMA
Immunogenicity	Zhenzhen Liu/ M.T. Gutierrez-Lugo/Susan Kirshner	DBRR III
Labeling	Vicky Borders-Hemphill	OBP
Application Technical Lead	Maria-Teresa Gutierrez-Lugo	DBRR III
Tertiary Reviewer	Susan Kirshner	DBRR III

Multidisciplinary Review Team

DISCIPLINE	REVIEWER	OFFICE/DIVISION
RPM	Beatrice Kallungal	OHOP/DHP
RBPM	Keith Olin	OHOP/DHP
CDTL/Medical Officer	Nicole Gormley	OHOP/DHP



QUALITY REVIEW



Clinical Reviewer	Rachel Ershler	OHOP/DHP
Pharm/Tox	Natalie E Simpson/Christopher Sheth	OHOP/DABT
Clinical Pharmacology	Saeho Chong /Sarah Schreiber	OCP/DCP V
CMC Statistics	Yaping Wang /Meiyu Shen	OB/DBVI

Submissions Reviewed

Submission:	Date Received:	Review Completed (yes or no)
STN 761075/0000	12/09/2016	Yes
STN 761075/0003	02/03/2017	Yes
STN 761075/0005	02/08/2017	Yes
STN 761075/0008	02/28/2017	Yes
STN 761075/0010	03/21/2017	Yes
STN 761075/0013	04/27/2017	Yes
STN 761075/0014	05/11/2017	Yes
STN 761075/0015	05/11/2017	Yes
STN 761075/0016	05/12/2017	Yes
STN 761075/0017	05/18/2017	Yes
STN 761075/0019	05/25/2017	Yes
STN 761075/0020	06/16/2017	Yes
STN 761075/0022	06/30/2017	Yes
STN 761075/0024	07/17/2017	Yes
STN 761075/0025	07/25/2017	Yes
STN 761075/0024	07/25/2017	Yes
STN 761075/0027	07/31/2017	Yes
STN 761075/0029	08/10/2017	Yes
STN 761075/0030	08/18/2017	Yes
STN 761075/0031	08/18/2017	Yes
STN 761075/0032	08/22/2017	Yes
STN 761075/0033	08/29/2017	Yes
STN 761075/0034	08/30/2017	Yes
STN 761075/0035	09/05/2017	Yes
STN 761075/0036	9/06/2017	Yes
STN 761075/0038	9/18/2017	Yes
STN 761075/0042 resubmission	12/4/2017	Yes
STN 761075/0045	2/27/2018	Yes
STN 761075/0048	5/2/2018	Yes
STN 761075/0049	5/3/2018	Yes

Quality Review Data Sheet

1. LEGAL BASIS FOR SUBMISSION: 351(k)

2. RELATED/SUPPORTING DOCUMENTS:

A. DMFs:

DMF#	DMF Holder	Item Referenced	Letter of Cross-Reference	Comments (status)
(b) (4)			Yes	Sufficient information in application
			Yes	Sufficient product quality information in application. For device functionality, refer CDRH/ODE review
			Yes	Sufficient product quality information in application. For device functionality, refer CDRH/ODE review
			Yes	Sufficient product quality information in application. For device functionality, refer CDRH/ODE review
			N/A	Sufficient product quality information in application. For device functionality, refer CDRH/ODE review
			N/A	Sufficient product quality information in application. For device functionality, refer CDRH/ODE review

3. CONSULTS:

Latoya Oliver-Powell (CDRH/OC) and Steven Basile (CDRH/ODE). Refer to reviews in DARRTS.

Executive Summary

I. Recommendations

A. Recommendation and Conclusion on Approvability

a. Recommendation

The Office of Pharmaceutical Quality, OPQ, CDER, completed review of BLA 761075 for MYL-1401H manufactured by (b) (4), a CMO for Mylan. MYL-1401H is a proposed biosimilar to US-licensed Neulasta. This review memo documents the review of a complete response (CR) action issued on October 6, 2017. The CR deficiencies were related to drug product manufacturing and cGMP deficiencies identified at (b) (4) (FEI # (b) (4)) the proposed drug product manufacturing facility. The CR deficiencies and additional comments were adequately addressed by the applicant in the December 4, 2017, resubmission.

The analytical similarity data submitted by the applicant support a determination that MYL-1401H is highly similar to US-licensed Neulasta notwithstanding minor differences in clinically inactive components. The data also support that the proposed MYL-1401H drug product has the same strength as US-licensed Neulasta. The manufacturing data and information provided in the submission are sufficient to support a determination that the manufacturing process of MYL-1401H is well controlled and leads to a product that is safe, pure, and potent for the duration of the product shelf life. Therefore, OPQ recommends MYL-1401H be approved for human use under the conditions specified in the package insert. This recommendation is pending final assessment of facilities compliance.

b. Draft approval action letter language

- Manufacturing locations:
- Drug Substance: (b) (4))
- Drug product: (b) (4))
- Presentation, fill size, strength, dosage form: Single-dose, pre-filled syringe, 6 mg/0.6 ml, injection.
- Dating period
 - Drug Substance: (b) (4) months at (b) (4)°C
 - Drug Product: 24 months at 5 ± 3°C.
- Except from lot release per Docket No 95-29960 because MYL-1401H is a recombinant protein

b. Benefit/Risk Considerations

MYL-1401H is a proposed biosimilar to US-licensed Neulasta. The applicant requested the neutropenia indication for which US-licensed Neulasta is approved (see page 1 of this review).

The manufacturing process and control strategy of MYL-1401H are sufficient and lead to a drug product of acceptable quality to ensure drug safety and effectiveness for patients. The drug substance (DS) and drug product (DP) manufacturing processes are well controlled and should consistently deliver DS and DP of desired quality. Adequate (b) (4) controls are established throughout the DP process. However, enhancements in the DP process can be made by including (b) (4). This enhancement can be conducted post licensure as a PMC because information provided in the license application show that the applicant can consistently produce DP that is safe, pure, and potent.

As part of demonstration of biosimilarity, Mylan conducted analytical similarity studies. Up to 24 lots of Neulasta, 12 of MYL-1401H drug product (DP), and 31 of EU-approved Neulasta were evaluated, including MYL-1401H lots used in the PK/PD similarity and additional clinical studies that were manufactured by the proposed commercial manufacturing process. This assessment was supported by comparative statistical analysis. To determine the comparative analyses that would be used to support a demonstration of highly similar through analytical testing, quality attributes were ranked into categories of high, medium and low criticality using information about the importance of that attribute to product safety, potency, PK and immunogenicity. The sponsor then selected the two high criticality attributes one of which is most important for the mechanism of action of the product. Analytical similarity of these attributes, referred as Tier 1 attributes, was evaluated using statistical equivalence testing. Per FDA advice, if a Tier 1 attribute was measured using more than one analytical method, results from only one of the methods need to be evaluated using statistical equivalence testing. Results from other attributes were assessed using “quality ranges” and comparison of graphical data, referred as Tier 2 and Tier 3 attributes, respectively. The methods and results of the analytical similarity assessment are summarized in Table A below and are discussed in detail in the OBP primary review.

Table A. Analytical Similarity Summary

Quality Attribute	Method/Test	Supports a Conclusion of Highly Similar	Supports an Analytical Bridge	Residual Differences	Notes
Protein Content	UV Abs 280 nm	Yes	Yes		Met equivalence testing; Theoretical Extinction Coefficient
Potency	Mouse NFS-60 cell-based proliferation assay	Yes	Yes		Met equivalence testing Ref Stand Lots bridged
G-CSF Receptor Binding	SPR	Yes	Yes		
Primary Sequence	Peptide Mass Fingerprinting Glu-C and Trypsin	Yes	Yes		
	MALDI-TOF MS	Yes	Yes		
Secondary and Higher Order Structure	Non-reduced Peptide Mass Fingerprint, Glu-C	Yes	Not Tested		Far UV-CD & 1D-NMR supports analytical bridge
	Far-UV-CD	Yes	Yes		$\theta_{208}/\theta_{222}$ ratio supports similarity & analytical bridge
Free Cysteine	FT-IR	Yes	Yes		
	Ellman's Assay	Yes	Yes	Minor differences due to method variability	peptide mapping supports Cys similarity
HMWP - Aggregates	Extrinsic Fluorescence	Yes	Yes		
	Near-UV-CD	---	---	Spectrum deconvolution inconsistent with literature	1D-NMR supports similar tertiary structure & analytical bridge
	DSC	Yes	Yes		
	1D-NMR (Fingerprint)	Yes	Yes		
HMWP - Aggregates	Intrinsic Fluorescence	Yes	Yes		
	SEC-MALS	Yes	Yes	MYL-1401H lots outside quality ranges 0.55% vs 0.33%	AUC (aggregates) & MFI (particles) support similarity & analytical bridge; HMWP levels are low; Immunogenicity risk historically low
Molecular Weight	AUC	Yes	Yes		
	SEC-MALS	Yes	Yes		
PEG Polydispersity	SEC-MALS	Yes	Yes		
Hydrodynamic radius	SEC-DLS	Yes	Yes		
Dimer	RP-HPLC	Yes	Yes		
N-term PEG confirmation	Peptide Mass Fingerprinting Glu-C and Trypsin	Yes	Yes		
	CNBr, MALDI, MS/MS	Yes	Not Tested		Peptide Mapping Glu-C and Trypsin supports analytical bridge
Di-PEG-G-CSF	CIEX-HPLC	Yes	Yes		
Deamidation Q108	RP-HPLC	Yes	Yes		
LMWP	SEC-MALS	Yes	Yes		
DES-PEG-G-CSF	RP-HPLC	Yes	Yes		
Methionine Oxidation	RP-HPLC	Yes	Yes		Met122, 127, 138
Free PEG	RP-HPLC-CAD	Yes	Yes		
pI	cIEF	Yes	Yes		
Sub-Visible Particles	MFI	Yes	Yes		
Size Variants	CE-SDS	Yes	Yes		
Stability Profile	Multiple Methods	Yes	Yes		

Note: Gray boxes are used to facilitate readability of the information contained in this table.

Briefly, the analytical similarity assessment of MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta is extensive and robust and included multiple orthogonal methods that

adequately evaluated the necessary quality attributes of the products. The characterization of the products is consistent with FDA Guidance: Quality Considerations in Demonstrating Biosimilarity of a Therapeutic Protein Product to a Reference Product, April 2015. The observed differences between MYL-1401H and U.S.-licensed Neulasta were adequately addressed as follows, and would not preclude a demonstration of highly similar:

- Data submitted for near-UV-CD analyses to compare the tertiary structure of MYL-1401H and U.S.-licensed Neulasta were inadequate to establish similarity because the experiments were not conducted appropriately. However, the sponsor provided 1D-NMR, intrinsic and extrinsic fluorescence, and differential scanning calorimetry data to support a similar tertiary structure between the products.
- Levels of high molecular weight proteins (HMWP) in MYL-1401H detected by SE-HPLC and identified as aggregates by the sponsor were slightly higher than the quality range for both U.S.-licensed Neulasta and E.U.-approved Neulasta. The difference in HMWP species between MYL-1401H and the comparator products is ~0.19%. On the other hand, AUC and MFI results show similar low levels of aggregates and particles, respectively. The difference in HMWP species identified by SEC is justified by their low levels of aggregates and a risk assessment of potential impact on product quality.
- A photostability study showed higher levels of oxidized species, corresponding to methionine oxidation, by RP-HPLC in MYL-1401H as compared to U.S.-licensed Neulasta and E.U.-approved Neulasta. This has a low risk of impacting product quality because the photoexposed samples showed similar relative potency values for MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta.

The totality of the analytical similarity exercise supports a determination that MYL-1401H is highly similar to US-licensed Neulasta notwithstanding minor differences in clinically inactive components. The pair-wise analytical comparisons of MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta, support an analytical bridge based on the extensive pair-wise analytical characterization. The analytical similarity data do not raise residual uncertainties about the conclusion that MYL-1401H is highly similar to U.S.-licensed Neulasta.

The assessment of other review disciplines is as follows:

The totality of the nonclinical data indicates that MYL-1401H is similar to US-licensed Neulasta. This assessment was based on two studies in rats. One Sprague-Dawley rat study assessed the pharmacodynamics (PD), toxicity, and toxicokinetics of MYL-1401H and EU-approved Neulasta. The second study, intended to support a bridge between MYL-1401H, EU- approved and US-licensed Neulasta, compared PD of the three products in neutropenic rats. The animal model, dose, route of administration and dosing regimen used in these studies were found adequate from non-clinical perspective.

Clinical studies MYL-1401H-1001, MYL-1401H-1002, and MYL-1401H-3001 were submitted by the applicant to support a determination of no clinically meaningful differences between

MYL-1401H and US-licensed Neulasta. The following is clinical pharmacology's assessment of these studies.

Study MYL-1401H-1001 was a single-dose, randomized, double-blind, 3-period, 3-treatment, 3-way crossover study in 216 healthy subjects designed to determine the PK and PD (absolute neutrophil count (ANC)) similarity of MYL-1401H, US-licensed Neulasta, and EU-approved Neulasta, following a single 2 mg subcutaneous (SC) dose. The 90% confidence intervals (CI) for all three pairwise comparisons of the PK and PD endpoints were within the pre-specified limits of 80 to 125%. The results of the study established the PK and PD similarity between MYL-1401H and US-licensed Neulasta based on the primary PK endpoints of C_{max} and $AUC_{0-\infty}$ and PD endpoints of ANC_{max} and $ANC_{AUEClast}$. The study also established the PK and PD portion of the scientific bridge between MYL-1401H, US-licensed Neulasta, and EU-approved Neulasta, which supports the use of EU-approved Neulasta in the comparative clinical Study MYL-1401H-3001.

The incidence of anti-drug antibodies (ADAs) was compared in Study MYL-1401H-3001, a comparative clinical study with MYL-1401H and EU-approved Neulasta in patients with stage II/III breast cancer receiving neoadjuvant or adjuvant chemotherapy. The incidence of immunogenicity for MYL-1401H and US-licensed Neulasta following two doses of 6 mg SC was also compared in an open-label, randomized, parallel-arm study (MYL-1401H-1002) in 50 healthy subjects. The results of the studies indicate similar incidence and titers of ADA for MYL-1401H vs. EU-approved Neulasta and MYL-1401H vs. US licensed Neulasta. The assessment of the impact of ADA on PK, PD, efficacy, and safety are limited due to insufficient PK data collected and a limited number of subjects who were ADA-positive. Therefore, the data indicate that there is no increase in immunogenicity risk for MYL-1401H as compared to US-licensed Neulasta. The OBP immunogenicity reviewer determined that data from Study MYL-1401H-3001 support that there are no clinically meaningful differences with respect to immunogenicity between MYL-1401H and EU-approved Neulasta.

Overall, Study MYL-1401H-1001 supports a demonstration of PK and PD (ANC) similarity between MYL-1401H and US-licensed Neulasta, as well as the PK and PD portion of the scientific bridge between MYL-1401H, US-licensed Neulasta, and EU-approved Neulasta. The scientific bridge (PK and analytical similarity data comparing the three products) allows for relying on data from the study using EU-approved Neulasta as a comparator product for the overall biosimilarity assessment. Clinical pharmacology's conclusion is that the PK and PD results support a demonstration of no clinically meaningful difference between MYL-1401H and US-licensed Neulasta and add to the totality of the evidence to support a demonstration of biosimilarity MYL-1401H and US-licensed Neulasta.

Study MYL-1401H-3001 was also used to assess efficacy of MYL-1401H compared to EU-approved Neulasta. The study was conducted on 194 patients with Stage II/III breast cancer receiving chemotherapy. The clinical reviewer determined that the primary efficacy endpoint was the duration of severe neutropenia (DSN) in Cycle 1, defined as days with $ANC < 0.5 \times 10^9/L$. In the MYL-1401H treatment group, the mean ($\pm$ SD) DSN was 1.2 (± 0.93) days. In the EU-approved Neulasta treatment group, the mean ($\pm$ SD) DSN was 1.2 (± 1.10) day. The primary endpoint of DSN was assessed by ANOVA and the equivalence margin was met. Based on

statistical analyses, the results from this study along with the scientific bridge contribute to an assessment of no clinically meaningful differences between MYL-1401H and US-licensed Neulasta.

The safety of MYL-1401H was assessed in two clinical studies in healthy subjects (MYL-1401H-1001 and MYL-1401H-1002) and in subjects with breast cancer (MYL-1401H-3001). MYL-1401H was generally well tolerated and the safety profile was acceptable. There were no deaths related to study treatment in any of the clinical studies. There were no events of splenomegaly, adult respiratory distress syndrome, capillary leak syndrome, or severe allergic reactions, which are rare but serious events known to be associated with pegfilgrastim treatment. The overall safety profile of MYL-1401H was similar to US-licensed Neulasta with bone pain as the most frequently reported treatment related TEAE.

As indicated above, comparative analytical and PK data between MYL-1401H, US-licensed Neulasta, and EU-approved Neulasta established a scientific bridge between the products. The bridge allows for relying on data from the study using EU-approved Neulasta as a comparator product for the overall biosimilarity assessment. The data from the three clinical studies described above support a conclusion that there are no clinically meaningful differences in the between MYL-1401H and US-licensed Neulasta.

Based on the totality of evidence reviewed and from the analytical perspective, MYL-1401H is expected to have a similar benefit/risk profile as US-licensed Neulasta.

A. Recommendation on Phase 4 (Post-Marketing) Commitments

PMC #1. To include [REDACTED] (b) (4) as an in-process parameter in Section 3.2.P.3.4 of the BLA. [REDACTED] (b) (4) acceptance criteria in [REDACTED] (b) (4) should be within the parameters validated during the [REDACTED] (b) (4) studies.

II. Summary of Quality Assessments

A. Identification, Risk and Lifecycle Knowledge Management

Table 1 below provides a summary of critical quality attributes identification and risk management. For the purposes of this table, critical quality attributes are limited to attributes intrinsic to the active pharmaceutical ingredient.

B. Drug Substance Quality Summary

Table 2 provides a summary of the identification, risk, and lifecycle knowledge management for drug substance CQAs that derive from the drug substance manufacturing process and general drug substance attributes, including process-related impurities.

Table 1: Drug Substance CQA Identification, Risk and Lifecycle Knowledge Management

CQA	Type ¹	Risk	Introduction	Control Strategy
Potency	Potency	Directly linked to efficacy (very high criticality) In vitro biological activity is dependent on both the quality and quantity of the drug substance used for DP formulation.	Intrinsic to the molecule Directly linked to efficacy (high criticality) In vitro potency includes a measure of receptor binding	(b) (4)
Target Receptor Binding	Potency	Directly linked to efficacy (very high criticality)	Intrinsic to the molecule	
Higher Order Structure	Potency	Directly linked to efficacy and MOA (very high criticality)	Intrinsic to the molecule	
Primary Sequence	Identity	Directly linked to efficacy and MOA (very high criticality)	Intrinsic to the molecule	

¹ The sponsor did not provide data to determine whether product related species are product-related substances or product-related impurities. Therefore these species are considered impurities.

Aggregates, including dimers and other HMW species	Product-related impurities	Potential impact on safety and efficacy (high criticality)	During manufacturing and storage	(b) (4)
Oxidized species	Product-related impurities	Potential impact on safety and efficacy (high criticality)		
Deamidated species	Product-related impurities	Potential impact on safety and efficacy (high criticality)	During manufacturing and storage	
Pegylation variants (including di-PEG GCSF and Des-PEG-GCSF)	Product-related impurities	Potential impact on PK (moderate criticality)	Pegylation Very low levels of Des-PEG GCSF may form during storage (based on accelerated stability data)	
N-terminal truncated species	Product-related impurities	Potential impact on PK (moderate criticality)	(b) (4) Very low levels may form during storage, particularly via Des-PEG (based on accelerated stability data)	

Table 2: Drug Substance CQA Process Risk Identification and Lifecycle Knowledge Management

CQA	Source	Risk	Control Strategy
GCSF variants (norleucine, oxidized, F-met, gluconoylated, MGO, cysteinylated, reduced, and dimer)	(b) (4) Oxidized, reduced, and dimeric forms can be formed during manufacture and storage.	Safety and efficacy	(b) (4)
Free m-PEG	Chemical Introduced during pegylation reaction or during storage	Safety	
Host Cell DNA	Host cell (<i>E.coli</i>) (b) (4)	Immunogenicity and Safety	
Host Cell Proteins	Host cell (<i>E.coli</i>) (b) (4)	Immunogenicity and Safety	

Bacterial endotoxin	Host cell (<i>E.coli</i>). Potentially introduced during entire process, raw materials (e.g. (b) (4)), environmental and equipment.	Safety
Bioburden	Host cell (<i>E.coli</i>). Potentially introduced during entire process, raw materials (e.g. (b) (4)), environmental and equipment.	Safety
(b) (4)	(b) (4)	Safety
(b) (4)		Safety
(b) (4)		Safety
(b) (4)		Safety

(b) (4)

	(b) (4)		(b) (4)
(b) (4)		Safety	
(b) (4)		Safety	
(b) (4)		Safety	

B. Drug Substance Quality Summary (cont.)

a. Names:

- Proprietary Name: MYL-1401H
 - Trade Name: Fulphila (proposed)
 - Non-Proprietary/USAN: pegfilgrastim-jmdb²

 - CAS name: N-(3-hydroxypropyl) methionyl colony-stimulating factor (human), 1-ether with α -methyl- ω -hydroxypoly (oxyethylene)

 - Common name: MYL-1401H
 - INN Name: TBD
 - Compendial Name: N/A
 - OBP systematic name: CONJ: RPROT P09919 (CSF3_HUMAN); PEG [MYL-1401H]³
- b. Pharmacology category: Leukocyte growth factor

c. Description

MYL-1401H is a non-glycosylated recombinant methionyl human granulocyte colony stimulating factor (Met-GCSF) produced in *E. coli*. The primary sequence (175 amino acids) of MYL-1401H is identical to natural GCSF, except for an additional methionine residue at the N-terminus. MYL-1401H has a mostly alpha helical protein structure and two disulfide bridges between Cys37-Cys43 and between Cys65-Cys75. MYL-1401H is also pegylated with a 20kDa PEG molecule at the N-terminus and a molecular weight of approximately 38kDa. MYL-1401H is a proposed biosimilar to US-licensed Neulasta.

The composition of drug substance is (b) (4). Drug substance is stored at (b) (4) °C.

d. Mechanism of action

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

² This USAN name is the proposed name, which will be finalized upon approval of the application

³ The OBP systematic name allows searching for related products in OBP's database and in the Document Archiving, Reporting & Regulatory Tracking System (DARRTS) for safety reasons and it is different from the nonproprietary name. The tag at the end is used to separate products from different sponsors and it is generally the name used by sponsors to refer to the proposed product in their submissions.

blood, which is an relevant pharmacodynamic (PD) marker for neutropenia, and enhanced neutrophil function¹.

e. Potency Assay

Potency of MYL-1401H is determined by measuring its ability to stimulate proliferation of NFS-60 cells compared to a reference standard. The NSF-60 cell line, which carries the GCSF receptor, was derived from mouse myelogenous leukemia cells. The cells are incubated with a MTS⁴ and PMS⁵ solution. NADPH or NADH, produced by dehydrogenase enzymes in metabolically active cells, reduces MTS to a purple formazan compound. The quantity of formazan produced is measured by absorbance at 490 nm and is proportional to the number of viable cells present. PLA software is used to assess parallelism and determine potency relative an in-house reference standard.

f Reference material(s)

The sponsor is currently implementing a two-tier reference standard system. A protocol for qualification and stability testing of in-house primary and working RSs was reviewed and found acceptable. The qualification of RSs includes calibration of potency using an international RS for GCSF. The current RS (b) (4) [REDACTED] is acceptable because it was properly qualified and calibrated against NIBSC international standard for PEG-GCSF lot no. 12/188.

g. Critical starting materials or intermediates

[REDACTED] (b) (4)

h. Manufacturing process summary

[REDACTED] (b) (4)

¹ Panopoulos, A.D. and Watowich, S.S. (2008). Cytokine 42: 277-288

⁴ 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium

⁵ phenazine methosulfate, serves as an electron acceptor

(b) (4)

i. Container closure

(b) (4)

j. Dating period and storage conditions: (b) (4) months at (b) (4)C.

C. MYL-1401H Drug Product Quality Summary

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

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

CQA	Source	Risk	Control Strategy
Identity	General CQA intrinsic to the molecule Dependent on the identity of the drug substance used for DP formulation.	Safety and Efficacy (High Criticality)	(b) (4)
Protein Content	General CQA. Protein concentration is influenced by the formulation and mixing steps.	Safety and Efficacy (High Criticality)	
Container closure integrity and sterility	Microbial contamination. Contaminants can be introduced during DP manufacturing or with a Container closure integrity failure. Container closure integrity is a sterility assurance CQA.	Safety (high criticality)	
Bacterial endotoxin	Microbial contamination. Contaminants can be introduced during DP manufacturing or with a Container closure integrity failure.	Safety (high criticality)	
Particulate matter and sub-visible particles	Particulate matter could contain product / process related impurities and aggregates. Particulate matter can form during DP manufacturing and storage.	Safety (capillary occlusion) and Immunogenicity (high criticality)	

Appearance	General CQA. Appearance may be impacted by the formulation process. May indicate product degradation or other changes to the product.	Safety and efficacy
Extractable Volume	General CQA. (b) (4)	Safety and efficacy.
Gliding Force	Device CQA. May impact ease of administration	Safety (moderate criticality)
Break Loose Force	Device CQA. May impact ease of administration	Safety (moderate criticality)
Actuation of needle guard	Device CQA.	Safety (low criticality)
pH	General CQA. Potential influence by formulation and mixing. Potential impact on stability, especially above the proposed pH range.	Safety and efficacy (high criticality)
Sorbitol	(b) (4)	Safety (high criticality)
(b) (4)		Safety (low criticality)

(b) (4)

Osmolality	General CQA. (b) (4) [Redacted] [Redacted].	(Moderate criticality)
Polysorbate 20	(b) (4) [Redacted] [Redacted].	Safety and efficacy. Polysorbate 20 levels control product aggregation and oxidation
Leachables from process materials in DP manufacture and primary package	Container closure system and materials used in the DP process.	Safety (low criticality)
HMWP and dimers	Product-related impurities that could form due to several manipulations in the DP manufacturing process e.g. (b) (4) [Redacted] etc.	Potential impact on safety and efficacy (moderate criticality)
Oxidized species	Product-related impurities. Introduced during manufacturing process and storage.	Potential impact on safety and efficacy (high criticality)
Deamidated species	Product related impurities. Introduced during manufacturing process and storage.	Potential impact on safety and efficacy (moderate criticality)



(b) (4)

MYL-1401H Drug Product Quality Summary (Cont.)

a. Description and Strength:

MYL-1401H (injection) is supplied in a pre-filled syringe (PFS) for S.C. administration at a strength of 6 mg/0.6 mL. MYL-1401H has the same strength as US-licensed Neulasta.

b. Summary of Product Design

MYL-1401H was developed as a biosimilar to US-licensed Neulasta and targets the quality profile of the US-licensed Neulasta.

c. List of Excipients

D-sorbitol (30 mg), polysorbate 20 (0.024 mg), acetate (0.7 mg), sodium (0.01 mg) pH 4.0 (b) (4)

d. Reference material(s),

The same reference materials are used for DS and DP.

e. Manufacturing process summary

(b) (4)

f. Container closure

MYL-1401H DP is filled (b) (4) glass syringes with a (b) (4) 29 gauge stacked stainless steel needle. (b) (4)

(b) (4)
(b) (4)
The needle guard is a (b) (4) Ultrasafe Plus passive needle guard and passive plunger rod (b) (4).

g. Dating period and storage conditions: 24 months at $5 \pm 3^\circ\text{C}$.

h. List of co-packaged components: not applicable

D. Any Special Product Quality Labeling Recommendations

- Protect from light
- Avoid freezing
- Do not shake

Lifecycle Knowledge Management

a. Drug Substance

- i. Protocols approved:
 1. Qualification of future MCBs and WCBs.
 2. Validation of (b) (4) at commercial scale and concurrent release of PPQ lots.
 3. Re-qualification/annual retesting of primary DS reference standard. A protocol for qualification of subsequent primary reference standards was not provided in the submission.
 4. Qualification of subsequent secondary reference standards and re-qualification/annual retesting of secondary DS reference standard.
 5. Post-approval DS stability protocol.
- ii. Outstanding review issues/residual risk: none
- iii. Future inspection points to consider: See 483 observations in the establishment information section and DIA review.

b. Drug Product

- i. Protocols approved:
 1. Post-approval DP stability protocol
 2. Prospective shipping validation protocol for DP
- ii. Outstanding review issues/residual risk: none
- iii. Future inspection points to consider: See 483 observations in the establishment information section and DIA review.

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Susan
Kirshner

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Date: 5/25/2018 12:02:40PM

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

LABELS AND LABELING REVIEW

Date:	May 22, 2018
Reviewer:	Vicky Borders-Hemphill, PharmD Labeling Review Specialist Office of Biotechnology Products (OBP)
Through:	Paul Kirwan, PhD, Product Quality Reviewer OBP/Division of Biotechnology Review and Research III
Application:	BLA 761075
Applicant:	Mylan GmbH
Submission Date:	January 22, 2018
Product:	Fulphila (pegfilgrastim-jmdb)
Dosage form(s):	Injection
Strength and Container-Closure:	6 mg/0.6 mL in a single-dose prefilled syringe
Indication, dose, route, and frequency of administration:	A leukocyte growth factor indicated to 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: 6 mg administered subcutaneously once per chemotherapy cycle. Use weight based dosing for pediatric patients weighing less than 45 kg
Background and Summary Description:	The Applicant, Mylan GmbH, resubmitted BLA 761075 Fulphila (pegfilgrastim-jmdb) on January 22, 2018 as a proposed biosimilar to US-licensed Neulasta.
Recommendations:	The prescribing information/patient labeling information, and Instructions for Use (submitted on May 10, 2018), and container labels and carton labeling (submitted on March 28, 2018) for Fulphila (pegfilgrastim-jmdb) injection, 6 mg/0.6 mL single-dose prefilled syringe for subcutaneous use are acceptable from a quality perspective.

Materials Considered for this Label and Labeling Review	
Materials Reviewed	Appendix Section
Proposed Labels and Labeling	A
Other	B (n/a)
Evaluation Tables	C
Acceptable Labels and Labeling	D

n/a = not applicable for this review

DISCUSSION and CONCLUSION

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

The prescribing information/patient labeling information, and Instructions for Use (submitted on May 10, 2018), and container labels and carton labeling (submitted on March 28, 2018) for Fulphila (pegfilgrastim-jmdb) injection, 6 mg/0.6 mL single-dose prefilled syringe for subcutaneous use were reviewed and found to comply with relevant regulations (21 CFR 610.60 through 21 CFR 610.67; 21 CFR 201.2 through 21 CFR 201.25; 21 CFR 201.50 through 21 CFR 201.57; 21 CFR 201.100) and USP standards.

APPENDICES

Appendix A: Proposed Labeling

- Prescribing Information/Patient Information submitted January 22, 2018 ([\\cdsesub1\evsprod\bla761075\0044\m1\us\114-labeling\draft\labeling\draft-labeling-text-pdf.pdf](#))
- Instructions for Use submitted January 22, 2018 ([\\cdsesub1\evsprod\bla761075\0044\m1\us\114-labeling\draft\labeling\draft-instructions-for-use-pdf.pdf](#))

2 Page(s) of Draft Labeling have been Withheld in Full as B4 (CCI/TS) immediately following this page

Appendix B: Other**Appendix C: Evaluation Tables:** Label^{1,2} and Labeling³ Standards**Container⁴ Label Evaluation**

Regulations, Guidance and CDER Best Labeling Practices	Conforms
Proper Name (21 CFR 610.60, 21 CFR 201.50, 21 CFR 201.10) <i>for container of a product capable of bearing a full label</i>	☐ No ☐ Yes ☒ N/A
Manufacturer name, address, and license number (21 CFR 610.60) <i>for container of a product capable of bearing a full label</i> Comment/Recommendation: partial label	☐ No ☐ Yes ☒ N/A
Lot number or other lot identification (21 CFR 610.60, 21 CFR 201.18, 21 CFR 201.100)	☐ No ☒ Yes ☐ N/A

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

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

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

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

Expiration date (21 CFR 610.60, 21 CFR 201.17)	☐ No ☒ Yes ☐ N/A
Multiple dose containers (recommended individual dose) 21 CFR 610.60	☐ No ☐ Yes ☒ N/A
Statement: "Rx only" 21 CFR 610.60 21 CFR 201.100	☐ No ☒ Yes ☐ N/A
Medication Guide 21 CFR 610.60 21 CFR 208.24	☐ No ☐ Yes ☒ N/A
No Package for container 21 CFR 610.60	☐ No ☐ Yes ☒ N/A
Partial label 21 CFR 610.60 21 CFR 201.10 Comment/ Recommendation: Revise the manufacturer information so that the US license number appears directly underneath the manufacturer's name. <i>The Applicant revised as requested</i> Consider adding the dosage form "Injection" to appear underneath the proper name as follows: Fulphila (pegfilgrastin-jmdb) Injection 6 mg/0.6 mL <i>The Applicant revised as requested</i>	☒ No ☐ Yes ☐ N/A
No container label 21 CFR 610.60	☐ No ☐ Yes ☒ N/A
Ferrule and cap overseal	☐ No ☐ Yes ☒ N/A
Visual inspection 21 CFR 610.60	☐ No ☐ Yes ☒ N/A
NDC numbers 21 CFR 201.2 21 CFR 207.35 Comment/ Recommendation: This is considered a partial label and the NDC number is not required information. Remove or reduce the prominence of the NDC number to make space for more important information such as route of administration. <i>The Applicant revised as requested</i>	☐ No ☒ Yes ☐ N/A

Route of administration 21 CFR 201.5 21 CFR 201.100	☒ No ☐ Yes ☐ N/A
Comment/Recommendation: Add the route of administration "For subcutaneous use" to appear above the package type term "single-dose prefilled syringe" <i>The Applicant revised as requested</i>	
Preparation instructions 21 CFR 201.5	☐ No ☐ Yes ☒ N/A
Package type term 21 CFR 201.5	☐ No ☒ Yes ☐ N/A
Drugs Misleading statements 21 CFR 201.6	☐ No ☒ Yes ☐ N/A
Strength 21 CFR 201.10 21 CFR 201.100 Comment/Recommendation: For injectable products ensure that the strength, expressed as strength per total volume, is the primary and prominent expression on the principal display panel (PDP). See USP General Chapters <7> Labeling. Revise the strength presentation to appear as 6 mg/0.6 mL. Remove (b) (4) as it is not the proper presentation of strength for this dosage form or revise it to appear as 6 mg/0.6 mL. <i>The Applicant revised as requested</i>	☒ No ☐ Yes ☐ N/A
Drugs Prominence of required label statements 21 CFR 201.15 Comment/Recommendation: To ensure the required or important information such as strength, dosage form and route of administration appears on the label with the appropriate placement and prominence, remove (b) (4) per 21 CFR 201.15 (b)(1). You may consider the recommendation for the manufacturer statement (b) (4) <i>The Applicant revised as requested</i>	☒ No ☐ Yes ☐ N/A
Spanish-language (Drugs) 21 CFR 201.16	☐ No ☐ Yes ☒ N/A
FD&C Yellow No. 5 and/or FD&C Yellow No. 6 21 CFR 201.20	☐ No ☐ Yes ☒ N/A
Phenylalanine as a component of aspartame 21 CFR 201.21	☐ No ☐ Yes ☒ N/A

<u>Sulfites; required warning statements</u> 21 CFR 201.22	☐ No ☐ Yes ☒ N/A
<u>Bar code label requirements</u> 21 CFR 201.25 21CFR 610.67	☐ No ☒ Yes ☐ N/A
<u>Strategic National Stockpile (exceptions or alternatives to labeling requirements for human drug products)</u> 21 CFR 610.68 21 CFR 201.26	☐ No ☐ Yes ☒ N/A
<u>Net quantity</u> 21 CFR 201.51	☐ No ☒ Yes ☐ N/A
<u>Usual dosage statement</u> 21 CFR 201.55 21 CFR 201.100 Comment/Recommendation: partial label	☐ No ☐ Yes ☒ N/A
<u>Inactive ingredients</u> 21 CFR 201.100 Comment/Recommendation: partial label	☐ No ☐ Yes ☒ N/A
<u>Storage requirements</u> Comment/Recommendation: partial label	☐ No ☐ Yes ☒ N/A
<u>Dispensing container</u> 21 CFR 201.100	☐ No ☐ Yes ☒ N/A

Package Label⁵ Evaluation

<u>Regulations, Guidance and CDER Best Labeling Practices</u>	<u>Conforms</u>
<u>Proper name</u> (21 CFR 610.61, 21 CFR 201.50, 21 CFR 201.10)	☐ No ☒ Yes ☐ N/A
<u>Manufacturer name, address, and license number</u> 21 CFR 610.61 Comment/Recommendation: On the tray lidding, ensure the US license No. appears with and underneath the licensed manufacturer name and address as follows per 21 CFR 610.61(b):	☒ No ☐ Yes ☐ N/A

⁵ Per 21 CFR 600.3(cc) *Package* means the immediate carton, receptacle, or wrapper, including all labeling matter therein and thereon, and the contents of the one or more enclosed containers. If no package, as defined in the preceding sentence, is used, the container shall be deemed to be the package. Thus this includes the carton, prescribing information, and patient labeling.

Manufactured by: Mylan GmbH

(b) (4) Switzerland CH- (b) (4)

US License No. xxxx

The Applicant revised as requested

Lot number or other lot identification

21 CFR 610.61

- No
 Yes
 N/A

Expiration date

21 CFR 610.61

21 CFR 201.17

- No
 Yes
 N/A

Preservative

21 CFR 610.61

- No
 Yes
 N/A

Number of containers

21 CFR 610.61

- No
 Yes
 N/A

Strength/volume

21 CFR 610.61

21 CFR 201.10

21 CFR 201.100

- No
 Yes
 N/A

Comment/Recommendation:

For the tray lidding and the carton labeling: for injectable products ensure that the strength expressed as strength per total volume is the primary and prominent expression on PDP and all carton panels see USP General Chapters <7> Labeling. Revise the strength presentation to appear as 6 mg/0.6 mL. Consider removing (b) (4) it is not the proper presentation of strength for this dosage form or revise it to appear as 6 mg/0.6 mL.

The Applicant revised as requested

Add the dosage form "Injection" to appear underneath the proper name wherever it appears on the blister and carton labeling as follows:

Fulphila
(pegfilgrastin-xxxx)
Injection
6 mg/0.6 mL

The Applicant revised as requested

Storage temperature/requirements

21 CFR 610.61

- No
 Yes
 N/A

Comment/Recommendation: Revise the storage information from "Store at 2° to 8°C (36° to 46°F). Do Not Freeze or Shake. Protect from Light." to read "Store refrigerated at 2° to 8°C (36° to 46°F) in original carton to Protect from Light. Do Not Freeze or Shake."

The Applicant revised as requested

Handling: "Shake Well", "Do not Freeze" or equivalent

(21 CFR 610.61)

- No
 Yes
 N/A

Multiple dose containers (recommended individual dose) 21 CFR 610.61	☐ No ☐ Yes ☒ N/A
Route of administration 21CFR 610.61 21 CFR 201.5 21 CFR 201.100	☐ No ☒ Yes ☐ N/A
Known sensitizing substances 21CFR 610.61	☐ No ☐ Yes ☒ N/A
Inactive ingredients 21 CFR 610.61 21 CFR 201.100	☐ No ☒ Yes ☐ N/A
Source of the product 21 CFR 610.61	☐ No ☐ Yes ☒ N/A
Minimum potency of product 21 CFR 610.61	☐ No ☒ Yes ☐ N/A
Rx only 21CFR 610.61 21 CFR 201.100	☐ No ☒ Yes ☐ N/A
Divided manufacturing 21 CFR 610.63	☐ No ☐ Yes ☒ N/A
Distributor 21 CFR 610.64	☐ No ☒ Yes ☐ N/A
Bar code 21 CFR 610.67 21 CFR 201.25	☐ No ☒ Yes ☐ N/A
Strategic National Stockpile (exceptions or alternatives to labeling requirements for human drug products) 21 CFR 610.68 21 CFR 201.26	☐ No ☐ Yes ☒ N/A
NDC numbers 21 CFR 201.2 21 CFR 207.35	☐ No ☒ Yes ☐ N/A
Preparation instructions 21 CFR 201.5	☐ No ☐ Yes ☒ N/A
Package type term 21 CFR 201.5	☐ No ☒ Yes ☐ N/A
Drugs Misleading statements 21 CFR 201.6	☐ No ☐ Yes ☒ N/A

Drugs Prominence of required label statements 21 CFR 201.15	☐ No ☒ Yes ☐ N/A
Spanish-language (Drugs) 21 CFR 201.16	☐ No ☐ Yes ☒ N/A
FD&C Yellow No. 5 and/or FD&C Yellow No. 6 21 CFR 201.20	☐ No ☐ Yes ☒ N/A
Phenylalanine as a component of aspartame 21 CFR 201.21	☐ No ☐ Yes ☒ N/A
Sulfites; required warning statements 21 CFR 201.22	☐ No ☐ Yes ☒ N/A
Net quantity 21 CFR 201.51	☐ No ☒ Yes ☐ N/A
Usual dosage statement 21 CFR 201.55 21 CFR 201.100	☐ No ☒ Yes ☐ N/A
Dispensing container 21 CFR 201.100	☐ No ☐ Yes ☒ N/A
Medication Guide 21 CFR 610.60 21 CFR 208.24	☐ No ☐ Yes ☒ N/A

Prescribing Information and Patient Labeling Evaluation

Regulations	Conforms
PRESCRIBING INFORMATION	
Highlights of prescribing information	
PRODUCT TITLE 21 CFR 201.57(a)(2)	☐ No ☒ Yes ☐ N/A
DOSAGE AND ADMINISTRATION 21 CFR 201.57(a)(7)	☐ No ☒ Yes ☐ N/A
DOSAGE FORMS AND STRENGTHS 21 CFR 201.57(a)(8)	☐ No ☒ Yes ☐ N/A
Full Prescribing Information	
2 DOSAGE AND ADMINISTRATION 21 CFR 201.57(c)(3)	☐ No ☒ Yes ☐ N/A
3 DOSAGE FORMS AND STRENGTHS	☒ No

21 CFR 201.57(c)(4) Comment/Recommendation: Added identifying characteristics per 21 CFR 201.57(c)(4) <i>The Applicant revised as requested</i>	☐ Yes ☐ N/A
6.2 IMMUNOGENICITY Draft Guidance for Industry: Labeling for Biosimilar Products Comment/Recommendation: Relocated and revised standard language to appear at the beginning of this subsection see draft Guidance for Industry: Labeling for Biosimilar Products <i>The Applicant revised as requested</i>	☒ No ☐ Yes ☐ N/A
11 DESCRIPTION (21 CFR 201.57(c)(12), 21 CFR 610.61 (m), 21 CFR 610.61(o), 21 CFR 610.61 (p), 21 CFR 610.61 (q)) Comment/Recommendation: Revised the second paragraph for readability. <i>The Applicant revised as requested</i>	☐ No ☒ Yes ☐ N/A
16 HOW SUPPLIED/ STORAGE AND HANDLING 21 CFR 201.57(c)(17) Comment/Recommendation: Added the dosage form and identifying characteristics per 21 CFR 201.57(c)(17) <i>The Applicant revised as requested</i>	☒ No ☐ Yes ☐ N/A
MANUFACTURER INFORMATION For BLAs: 21 CFR 610.61, 21 CFR 610.64 For NDAs: 21 CFR 201.1 Comment/Recommendation: Ensure the licensed manufacturer appears as the listed Applicant on the submitted Form FDA 356h and includes a placeholder for the US license No. to appear as follows per 21 CFR 610.61(b): Manufactured by: Mylan GmbH (b) (4), Switzerland CH- (b) (4) US License No. xxxx <i>The Applicant revised as requested</i>	☒ No ☐ Yes ☐ N/A
INSTRUCTIONS FOR USE, AND PATIENT INFORMATION	
TITLE (NAMES AND DOSAGE FORM)	☐ No ☒ Yes ☐ N/A
STORAGE AND HANDLING	☐ No ☒ Yes ☐ N/A
INGREDIENTS	☐ No ☒ Yes ☐ N/A
MANUFACTURER INFORMATION 21 CFR 610.61, 21 CFR 610.64 Comment/Recommendation: Ensure the licensed manufacturer appears here as the listed Applicant on the submitted Form FDA 356h and includes a placeholder for the US license No.	☒ No ☐ Yes ☐ N/A

Revise from "[REDACTED] (b) (4)

[REDACTED].” to appear as follows per 21 CFR 610.61(b):

Manufactured by: Mylan GmbH

(b) (4), Switzerland CH (b) (4)

US License No. xxxx

The Applicant revised as requested

APPENDIX D. Acceptable Labels and Labeling

Prescribing Information/Patient Information (submitted on May 10, 2018

<\\cdsesub1\evsprod\bla761075\0051\m1\us\114-labeling\draft\labeling\draft-labeling-text-clean-pdf.pdf>)

Instructions for Use (submitted on May 10, 2018

<\\cdsesub1\evsprod\bla761075\0051\m1\us\114-labeling\draft\labeling\draft-labeling-text-instructions-for-use-clean-pdf.pdf>)

Container Labels (submitted March 28, 2018)



1 Page(s) of Draft Labeling has been Withheld in Full as B4 (CCI/TS)
immediately following this page



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Borders-Hemphill

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Date: 5/22/2018 11:54:48AM
GUID: 50814c700007a3d59329f660d8ddf02



James (Paul)
Kirwan

Digitally signed by James (Paul) Kirwan
Date: 5/23/2018 08:32:21AM
GUID: 54caac3300077d97e4a094617777ee9a

Memorandum of Review:

Submission Tracking Number (STN):	761075
Subject:	Drug product (DP) quality review addendum to address data to support the label claim in section 16 of the United States Product Insert (USPI) for the 351(k) BLA application
Review/Revision Date:	5/14/2018
Primary Reviewer:	J. Paul Kirwan, Ph.D.
Secondary Reviewer:	Maria-Teresa Gutierrez-Lugo, Ph.D.
RPM:	Katie Chon
Applicant:	Mylan
Product:	MYL-1401H; proposed biosimilar to U.S.-licensed Neulasta
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
Action Due Date:	6/4/2018

Recommendation: I recommend approval of this BLA from the OBP drug product quality perspective.

Summary: This addendum is for the review of Mylan's 351(k) BLA for MYL-1401H, proposed biosimilar to U.S.-licensed Neulasta. The intent is to add clarifying comments to the primary review memo for drug product quality regarding data submitted by the sponsor to support the label claim in section 16 of the United States Product Insert (USPI) for the 351(k) BLA application for MYL-1401H. See the Panorama Informatics Platform for the complete quality review memos.

There are two label claims included in the USPI for which the reviewer is adding clarifying comments:

1. Discard syringes stored at room temperature for more than 72 hours.
2. Avoid freezing; if frozen thaw in the refrigerator before administration. Discard syringe if frozen more than once.

Reviewer Comment and Conclusion: *The sponsor's submission included stability data for MYL-1401H DP batches BS15006245 and BS15006419 (see Table 3.2.P.8.3/41 and Table 3.2.P.8.3/42) which support the stability of MYL-1401H DP in the final container closure system at 30°C for up to 72 hours.*

The sponsor did not provide data to support the stability of MYL-1401H DP after freezing. However, in the analytical similarity review, MYL-1401H was found to be analytically highly similar to U.S.-licensed Neulasta, including a similar stability profile. In addition, the formulation of MYL-1401H and US-licensed Neulasta is the same. The

current USPI for U.S.-licensed Neulasta¹ includes identical language to claim 2 above. Therefore, the reviewer concludes that this label claim is also appropriate for MYL-1401H DP based on analytical similarity and similar formulation.

¹ https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/125031s189s191lbl.pdf



James (Paul)
Kirwan

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Maria
Gutierrez Lugo

Digitally signed by Maria Gutierrez Lugo
Date: 5/17/2018 11:01:36AM
GUID: 50757b3d000038f82ef48db08ba1ceea



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

Date: 05/11/2018
To: Administrative File, STN 761075/resub-43
From: Laura Fontan, Consumer Safety Officer, CDER/OPQ/OPF/DIA
Endorsement: Peter Qiu, Ph.D., Branch Chief, CDER/OPQ/OPF/DIA
Subject: BLA 761075-resub-43
US License: 2062
Applicant: Mylan GmbH.
Mfg Facility: Drug Substance: (b) (4)
Drug Product: (b) (4)
Product: pegfilgrastim (MYL-1401H) Injection
Dosage: 6 mg/0.6 mL sterile liquid for subcutaneous injection
Indication: Treatment to 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
Due Date: 6/04/2018

RECOMMENDATION: The facility recommendation for the drug substance manufacturing site is acceptable based on the (b) (4) PLI inspection of the (b) (4) conducted during the first review cycle for BLA 761075. There were no changes for this review cycle.

For the proposed drug product manufacturing site, a PLI inspection was conducted for (b) (4). A seven-item Form FDA 483 was issued to the firm. The responses from the firm were deemed acceptable and the inspection was classified as VAI. The drug product site is acceptable for the production of MYL-1401H.

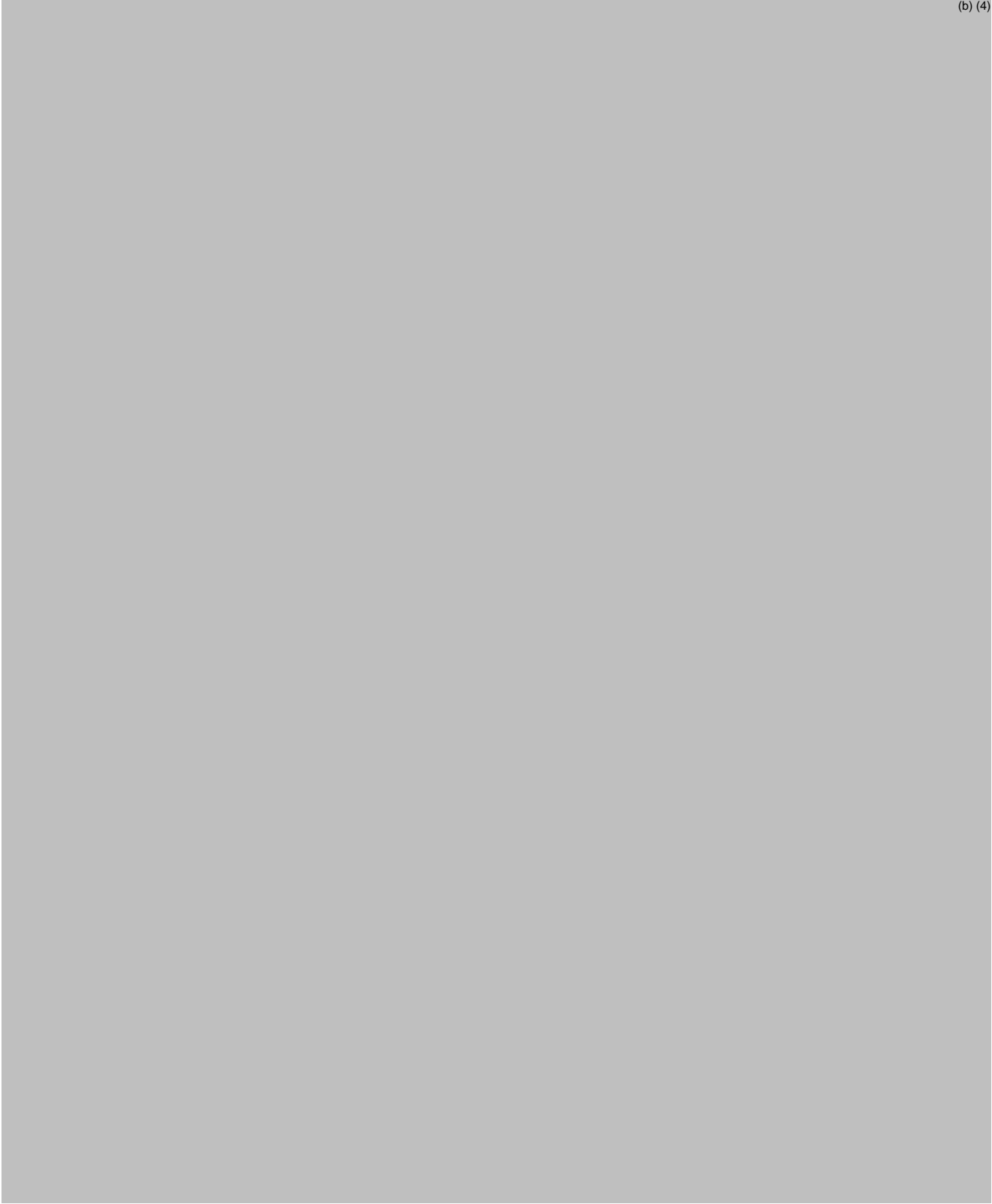
SUMMARY

The subject BLA proposes manufacture of pegfilgrastim DS and DP at the following facilities. The drug substance is proposed to be manufactured at (b) (4). This site is currently acceptable from a facilities compliance standpoint. The drug product is proposed to be filled into syringes at 6mg/0.6 mL strength at (b) (4).

(b) (4) This site is currently acceptable from a facilities compliance standpoint.

ASSESSMENT

(b) (4)



(b) (4)



CONCLUSION

Adequate descriptions were provided for the following sites proposed for Pegfilgrastim DS and DP manufacture:

- [Redacted] (b) (4)
- [Redacted] (b) (4)

The proposed drug product manufacturing ([Redacted] (b) (4)) is recommended for approval from a facilities assessment standpoint.

Laura Fontan
Consumer Safety Officer
OPF Division of Inspectional Assessment
Branch 1

Laura
Fontan -S

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ou=FDA, ou=People, cn=Laura Fontan -
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Zhihao Peter Qiu, Ph.D.
Branch Chief
OPF Division of Inspectional Assessment
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Laura
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Zihao Peter
Qiu

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Date: 5/11/2018 03:08:41PM
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Memorandum of Review:

Submission Tracking Number (STN):	761075
Subject:	Mylan's response to Complete Response Letter issued 10/06/2017 for their 351(k) BLA application
Stamp Date:	12/04/2017
Review/Revision Date:	01/09/2018; 5/1/2018
Primary Reviewer:	J. Paul Kirwan, Ph.D.
Secondary Reviewer:	Maria-Teresa Gutierrez-Lugo, Ph.D.
RPM:	Katie Chon
Applicant:	Mylan
Product:	MYL-1401H; proposed biosimilar to U.S.-licensed Neulasta
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
Primary Review Due Date:	5/11/2018
Action Due Date:	6/4/2018

Recommendation: I recommend approval of this BLA from the OBP drug product quality perspective.

Background: During the review of Mylan's 351(k) BLA for MYL-1401H, proposed biosimilar to U.S.-licensed Neulasta, the following CMC CR issue was listed as an item in FDA's Complete Response (CR) Letter issued 10/6/2017:



[REDACTED] (b) (4)

Mylan's Response to the CR Letter:

The sponsor agrees [REDACTED] (b) (4)

[REDACTED]

Reviewer Comment and Conclusion: The sponsor's response is acceptable [REDACTED] (b) (4)

[REDACTED]

[REDACTED]. *The reviewer verified that the following BLA sections/documents were revised accordingly:*

- 3.2.P.3.2
- 3.2.P.3.3
- 3.2.R.1
- DP Batch Manufacturing Record [REDACTED] (b) (4)

The sponsor's response resolves the CMC CR issue cited in the 10/6/2017 CR Letter and is acceptable from the CMC perspective.



James (Paul)
Kirwan

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Maria
Gutierrez Lugo

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Date: 5/02/2018 11:30:49AM
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Recommendation: Complete Response

BLA 761075

Review 1

Review Date: September 26, 2017

Drug Name/Dosage Form	MYL-1401H/injection
Strength/Potency	6 mg/0.6
Route of Administration	Subcutaneous
Rx/OTC Dispensed	Rx
Indications	MYL-1401H is proposed for the following indication: Decrease the incidence of infection, as manifested by febrile a neutropenia, in patients with non-myeloid malignancies receiving myelosuppressive anti-cancer drugs associated with a clinically significant incidence of febrile neutropenia
Applicant/Sponsor	Mylan GmbH

Product Overview

MYL-1401H is a pegylated recombinant granulocyte colony stimulating factor (PEG-G-CSF). G-CSF is a growth factor that stimulates production of neutrophils and neutrophil precursors by binding the G-CSF receptor on blood cells of the neutrophilic granulocyte. This binding initiates signal transduction that leads to the survival, proliferation, and differentiation of neutrophil progenitor cells into mature neutrophils.

Quality Review Team

DISCIPLINE	REVIEWER	DIVISION
Drug Substance	Merry Christie	DBRR III
Drug Product and Analytical Similarity	Paul Kirwan	DBRR III
Facilities	Laura Fontan/Michael Shanks/Peter Qiu	DIA
Microbiology	Scott Nichols/Lyndsey Brown/Lakshmi Narasimhan/Maria Reyes Candauchacon/Patricia Hughes	DMA
Immunogenicity	Zhenzhen Liu/ M.T. Gutierrez-Lugo/Susan Kirshner	DBRR III
Labeling	Vicky Borders-Hemphill	OBP
Application Technical Lead	Maria-Teresa Gutierrez-Lugo	DBRR III
Tertiary Reviewer	Susan Kirshner	DBRR III

Multidisciplinary Review Team

DISCIPLINE	REVIEWER	OFFICE/DIVISION
RPM	Beatrice Kallungal	OHOP/DHP
RBPM	Keith Olin	OHOP/DHP
CDTL/Medical Officer	Nicole Gormley	OHOP/DHP

Clinical Reviewer	Rachel Ershler	OHOP/DHP
Pharm/Tox	Natalie E Simpson/Christopher Sheth	OHOP/DABT
Clinical Pharmacology	Saeho Chong /Sarah Schreiber	OCP/DCP V
CMC Statistics	Yaping Wang /Meiyu Shen	OB/DBVI

Submissions Reviewed

Submission:	Date Received:	Review Completed (yes or no)
STN 761075/0000	12/09/2016	Yes
STN 761075/0003 (Response to IR #1)	02/03/2017	Yes
STN 761075/0005 (Response to IR #2)	02/08/2017	Yes
STN 761075/0008 (Partial response to IR #3)	02/28/2017	Yes
STN 761075/0010 (Partial response to IR #3)	03/21/2017	Yes
STN 761075/0013 (Partial response to IR #4)	04/27/2017	Yes
STN 761075/0014 (Partial response to IR #4)	05/11/2017	Yes
STN 761075/0015 (Response to IR #5)	05/11/2017	Yes
STN 761075/0016 (Partial response to IR #4)	05/12/2017	Yes
STN 761075/0019 (Partial response to IRs #4)	05/25/2017	Yes
STN 761075/0020 (DS and DP stability update)	06/16/2017	Yes
STN 761075/0024 (Partial response to IR #6)	07/17/2017	Yes
STN 761075/0025 (Partial response to IR #6)	07/25/2017	Yes
STN 761075/0024 (Response to IR #7)	07/25/2017	Yes
STN 761075/0027 (Response to IR #8)	07/31/2017	Yes
STN 761075/0029 (Response to IR #9)	08/10/2017	Yes
STN 761075/0030 (Response to IR #10)	08/18/2017	Yes
STN 761075/0033 (Response to IR #11)	08/29/2017	Yes
STN 761075/0034 (Response to IR #12)	08/30/2017	Yes
STN 761075/0035 (Partial response to IRs #13 14)	09/05/2017	Yes
STN 761075/0036 (Response to IRs #14 and 15)	9/06/2017	Yes
STN 761075/0038 (Response to IRs #16)	9/18/2017	Yes

Quality Review Data Sheet

1. LEGAL BASIS FOR SUBMISSION: 351(k)

2. RELATED/SUPPORTING DOCUMENTS:

A. DMFs:

DMF#	DMF Holder	Item Referenced	Letter of Cross-Reference	Comments (status)
(b) (4)			Yes	Sufficient information in application
			Yes	Sufficient product quality information in application. For device functionality, defer CDRH/ODE review
			Yes	Sufficient product quality information in application. For device functionality, defer CDRH/ODE review
			Yes	Sufficient product quality information in application. For device functionality, defer CDRH/ODE review
			Yes	Defer DMA review
			N/A	Sufficient product quality information in application. For device functionality, defer CDRH/ODE review
			N/A	Sufficient product quality information in application. For device functionality, defer CDRH/ODE review

3. CONSULTS:

Latoya Oliver-Powell (CDRH/OC) and Steven Basile (CDRH/ODE)

Executive Summary

I. Recommendations

A. Recommendation and Conclusion on Approvability

a. Recommendation

The Office of Biotechnology Products, OPQ, CDER, completed review of BLA 761075 for MYL-1401H manufactured by (b) (4) a CMO for Mylan. MYL-1401H is a proposed biosimilar to US-licensed Neulasta.

The analytical similarity data submitted by the applicant support a determination that MYL-1401H is highly similar to US-licensed Neulasta notwithstanding minor differences in clinically inactive components. The data also support that the proposed MYL-1401H drug product has the same strength as US-licensed Neulasta. The manufacturing data and information provided in the submission are insufficient to support a determination that the manufacturing process of MYL-1401H is well controlled and leads to a product that is pure and potent for the duration of the product shelf life. FDA identified deficiencies in MYL-1401H drug product manufacturing, including cGMP deficiencies identified at the (b) (4) FEI: (b) (4) the proposed drug product manufacturing facility. Therefore, OPQ recommends a complete response be issued to the applicant.

b. Summary of Complete Response Issues

1. The proposed MYL-1401H drug product manufacturing facility, (b) (4) FEI: (b) (4) has GMP deficiencies. Refer to Establishment Inspection Report and Form FDA 483 from a pre-license inspection associated with this application.
2. Insufficient assurance of drug product quality and control, including:
 - a. Insufficient assurance of drug product sterility
 - b. Insufficient assurance of product homogeneity

c. Complete Response Letter Draft Language





d. Additional comments (non-CR issues):

Drug Substance – Microbiology and Product Quality

1. Only one batch of bulk drug substance (DE-B14-03-000018) was used to qualify the bioburden test. Conduct the qualification of the (b) (4) bioburden test for the bulk drug substance using two additional batches.
2. Perform an extractables and leachables study and a safety risk assessment for the (b) (4) used as a component of the drug substance container closure system to confirm low risk of leachables and low safety risk.

Drug Product – Microbiology

5. Clarify the container closure integrity test conditions used during shipping validation and justify the use of a (b) (4) μm positive control. In addition, indicate the test acceptance criteria in mg/L of dye solution.
6. The endotoxin recovery studies included in amendment 0022 did not include quantitative recovery data. Repeat the study using different dilutions of the product in order to determine the endotoxin recovery in each sample.

e. Benefit/Risk Considerations

MYL-1401H is a proposed biosimilar to US-licensed Neulasta. The applicant requested the neutropenia indication for which US-licensed Neulasta is approved (see page 1 of this review).

The manufacturing data and information provided in the submission are insufficient to support a determination that the manufacturing process of MYL-1401H is well controlled and leads to a product that is pure and potent for the duration of the product shelf life. Deficiencies were identified in MYL-1401H drug product manufacturing, including cGMP deficiencies identified at the (b) (4) FEI: (b) (4) the proposed drug product manufacturing facility.

As part of demonstration of biosimilarity, Mylan conducted analytical similarity studies. Up to 24 lots of Neulasta, 12 of MYL-1401H drug product (DP), and 31 of EU-approved Neulasta were evaluated, including MYL-1401H lots used in the PK/PD similarity and additional clinical studies that were manufactured by the proposed commercial manufacturing process. This assessment was supported by comparative statistical analysis. To determine the comparative analyses that would be used to support a demonstration of highly similar through analytical testing, quality attributes were ranked into categories of high, medium and low criticality using information about the importance of that attribute to product safety, potency, PK and immunogenicity. The sponsor then selected the two high criticality attributes one of which is most important for the mechanism of action of the product. Analytical similarity of these attributes, referred as Tier 1 attributes, was evaluated using statistical equivalence testing. Per FDA advice, if a Tier 1 attribute was measured using more than one analytical method, results from only one of the methods need to be evaluated using statistical equivalence testing. Results from other attributes were assessed using “quality ranges” and comparison of graphical data, referred as Tier 2 and Tier 3 attributes, respectively. The methods and results of the analytical similarity assessment are summarized in Table A below and are discussed in detail in the OBP primary review.

Briefly, the analytical similarity assessment of MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta is extensive and robust and included multiple orthogonal methods that adequately evaluated the necessary quality attributes of the products. The characterization of the products is consistent with FDA Guidance: Quality Considerations in Demonstrating Biosimilarity of a Therapeutic Protein Product to a Reference Product, April 2015. The observed differences between MYL-1401H and U.S.-licensed Neulasta were adequately addressed as follows, and would not preclude a demonstration of highly similar:

- Data submitted for near-UV-CD analyses to compare the tertiary structure of MYL-1401H and U.S.-licensed Neulasta were inadequate to establish similarity because the experiments were not conducted appropriately. However, the sponsor provided 1D-NMR, intrinsic and extrinsic fluorescence, and differential scanning calorimetry data to support a similar tertiary structure between the products.
- Levels of high molecular weight proteins (HMWP) in MYL-1401H detected by SE-HPLC and identified as aggregates by the sponsor were slightly higher than the quality range for both U.S.-licensed Neulasta and E.U.-approved Neulasta. The difference in HMWP species between MYL-1401H and the comparator products is ~0.19%. On the other hand, AUC and MFI results show similar low levels of aggregates and particles, respectively. The difference in HMWP species identified by SEC is justified by their low levels of aggregates and a risk assessment of potential impact on product quality.
- A photostability study showed higher levels of oxidation species, corresponding to methionine oxidation, by RP-HPLC in MYL-1401H as compared to U.S.-licensed Neulasta

and E.U.-approved Neulasta. This has a low risk of impacting product quality because the photoexposed samples showed similar relative potency values for MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta.

Table A. Analytical Similarity Summary

Quality Attribute	Method/Test	Supports a Conclusion of Highly Similar	Supports an Analytical Bridge	Residual Differences	Notes
Protein Content	UV Abs 280 nm	Yes	Yes		Met equivalence testing; Theoretical Extinction Coefficient
Potency	Mouse NFS-60 cell-based proliferation assay	Yes	Yes		Met equivalence testing Ref Stand Lots bridged
G-CSF Receptor Binding	SPR	Yes	Yes		
Primary Sequence	Peptide Mass Fingerprinting Glu-C and Trypsin	Yes	Yes		
	MALDI-TOF MS	Yes	Yes		
Secondary and Higher Order Structure	Non-reduced Peptide Mass Fingerprint, Glu-C	Yes	Not Tested		Far UV-CD & 1D-NMR supports analytical bridge
	Far-UV-CD	Yes	Yes		$\theta_{220}/\theta_{222}$ ratio supports similarity & analytical bridge
	FT-IR	Yes	Yes		
Free Cysteine	Ellman's Assay	Yes	Yes	Minor differences due to method variability	peptide mapping supports Cys similarity
	Extrinsic Fluorescence	Yes	Yes		
HMWP - Aggregates	Near-UV-CD	---	---	Spectrum deconvolution inconsistent with literature	1D-NMR supports similar tertiary structure & analytical bridge
	DSC	Yes	Yes		
	1D-NMR (Fingerprint)	Yes	Yes		
	Intrinsic Fluorescence	Yes	Yes		
HMWP - Aggregates	SEC-MALS	Yes	Yes	MYL-1401H lots outside quality ranges 0.55% vs 0.33%	AUC (aggregates) & MFI (particles) support similarity & analytical bridge; HMWP levels are low; Immunogenicity risk historically low
Molecular Weight	AUC	Yes	Yes		
	SEC-MALS	Yes	Yes		
PEG Polydispersity	SEC-MALS	Yes	Yes		
Hydrodynamic radius	SEC-DLS	Yes	Yes		
Dimer	RP-HPLC	Yes	Yes		
N-term PEG confirmation	Peptide Mass Fingerprinting Glu-C and Trypsin	Yes	Yes		
	CNBr, MALDI, MS/MS	Yes	Not Tested		Peptide Mapping Glu-C and Trypsin supports analytical bridge
Di-PEG-G-CSF	CIEX-HPLC	Yes	Yes		
Deamidation Q108	RP-HPLC	Yes	Yes		
LMWP	SEC-MALS	Yes	Yes		
DES-PEG-G-CSF	RP-HPLC	Yes	Yes		
Methionine Oxidation	RP-HPLC	Yes	Yes		Met122, 127, 138
Free PEG	RP-HPLC-CAD	Yes	Yes		
pI	cIEF	Yes	Yes		
Sub-Visible Particles	MFI	Yes	Yes		
Size Variants	CE-SDS	Yes	Yes		
Stability Profile	Multiple Methods	Yes	Yes		

Note: Gray boxes are used to facilitate readability of the information contained in this table.

The totality of the analytical similarity exercise supports a determination that MYL-1401H is highly similar to US-licensed Neulasta notwithstanding minor differences in clinically inactive components. The pair-wise analytical comparisons of MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta, support an analytical bridge based on the extensive pair-wise analytical characterization. The analytical similarity data do not raise residual uncertainties about the conclusion that MYL-1401H is highly similar to U.S.-licensed Neulasta.

The assessment of other review disciplines is as follows:

The totality of the nonclinical data indicates that MYL-1401H is similar to US-licensed Neulasta. This assessment was based on two studies in rats. One Sprague-Dawley rat study assessed the pharmacodynamics (PD), toxicity, and toxicokinetics of MYL-1401H and EU-approved Neulasta. The second study, intended to support a bridge between MYL-1401H, EU-approved and US-licensed Neulasta, compared PD of the three products in neutropenic rats. The animal model, dose, route of administration and dosing regimen used in these studies were found adequate from non-clinical perspective.

Clinical studies MYL-1401H-1001, MYL-1401H-1002, and MYL-1401H-3001 were submitted by the applicant to support a determination of no clinically meaningful differences between MYL-1401H and US-licensed Neulasta. The following is clinical pharmacology's assessment of these studies.

Study MYL-1401H-1001 was a single-dose, randomized, double-blind, 3-period, 3-treatment, 3-way crossover study in 216 healthy subjects designed to determine the PK and PD (absolute neutrophil count (ANC)) similarity of MYL-1401H, US-licensed Neulasta, and EU-approved Neulasta, following a single 2 mg subcutaneous (SC) dose. The 90% confidence intervals (CI) for all three pairwise comparisons of the PK and PD endpoints were within the pre-specified limits of 80 to 125%. The results of the study established the PK and PD similarity between MYL-1401H and US-licensed Neulasta based on the primary PK endpoints of C_{max} and AUC_{0-inf} and PD endpoints of ANC_{max} and ANC AUEClast. The study also established the PK and PD portion of the scientific bridge between MYL-1401H, US-licensed Neulasta, and EU-approved Neulasta, which supports the use of EU-approved Neulasta in the comparative clinical Study MYL-1401H-3001.

The incidence of anti-drug antibodies (ADAs) was compared in Study MYL-1401H-3001, a comparative clinical study with MYL-1401H and EU-approved Neulasta in patients with stage II/III breast cancer receiving neoadjuvant or adjuvant chemotherapy. The incidence of immunogenicity for MYL-1401H and US-licensed Neulasta following two doses of 6 mg SC was also compared in an open-label, randomized, parallel-arm study (MYL-1401H-1002) in 50 healthy subjects. The results of the studies indicate similar incidence and titers of ADA for MYL-1401H vs. EU-approved Neulasta and MYL-1401H vs. US licensed Neulasta. The assessment of the impact of ADA on PK, PD, efficacy, and safety are limited due to insufficient PK data collected and a limited number of subjects who were ADA-positive. Therefore, the data indicate that there is no increase in immunogenicity risk for MYL-1401H as compared to US-licensed Neulasta. The OBP immunogenicity reviewer determined that data from Study MYL-

1401H-3001 support that there are no clinically meaningful differences with respect to immunogenicity between MYL-1401H and EU-approved Neulasta.

Overall, Study MYL-1401H-1001 supports a demonstration of PK and PD (ANC) similarity between MYL-1401H and US-licensed Neulasta, as well as the PK and PD portion of the scientific bridge between MYL-1401H, US-licensed Neulasta, and EU-approved Neulasta. The scientific bridge (PK and analytical similarity data comparing the three products) allows for relying on data from the study using EU-approved Neulasta as a comparator product for the overall biosimilarity assessment. Clinical pharmacology's conclusion is that the PK and PD results support a demonstration of no clinically meaningful difference between MYL-1401H and US-licensed Neulasta and add to the totality of the evidence to support a demonstration of biosimilarity MYL-1401H and US-licensed Neulasta.

Study MYL-1401H-3001 was also used to assess efficacy of MYL-1401H compared to EU-approved Neulasta. The study was conducted on 194 patients with Stage II/III breast cancer receiving chemotherapy. The clinical reviewer determined that the primary efficacy endpoint was the duration of severe neutropenia (DSN) in Cycle 1, defined as days with ANC $<0.5 \times 10^9/L$. In the MYL-1401H treatment group, the mean ($\pm$ SD) DSN was 1.2 (± 0.93) days. In the EU-approved Neulasta treatment group, the mean ($\pm$ SD) DSN was 1.2 (± 1.10) day. The primary endpoint of DSN was assessed by ANOVA and the equivalence margin was met. Based on statistical analyses, the results from this study along with the scientific bridge contribute to an assessment of no clinical meaningful differences between MYL-1401H and US-licensed Neulasta.

The safety of MYL-1401H was assessed in two clinical studies in healthy subjects (MYL-1401H-1001 and MYL-1401H-1002) and in subjects with breast cancer (MYL-1401H-3001). MYL-1401H was generally well tolerated and the safety profile was acceptable. There were no deaths related to study treatment in any of the clinical studies. There were no events of splenomegaly, adult respiratory distress syndrome, capillary leak syndrome, or severe allergic reactions, which are rare but serious events known to be associated with pegfilgrastim treatment. The overall safety profile of MYL-1401H was similar to US-licensed Neulasta with bone pain as the most frequently reported treatment related TEAE.

As indicated above, comparative analytical and PK data between MYL-1401H, US-licensed Neulasta, and EU-approved Neulasta established a scientific bridge between the products. The bridge allows for relying on data from the study using EU-approved Neulasta as a comparator product for the overall biosimilarity assessment. The data from the three clinical studies described above support a conclusion that there are no clinically meaningful differences in the between MYL-1401H and US-licensed Neulasta.

Based on the totality of evidence reviewed and from the analytical perspective, MYL-1401H is expected to have a similar benefit/risk profile as US-licensed Neulasta.

II. Summary of Quality Assessments

A. Identification, Risk and Lifecycle Knowledge Management

Table 1 below provides a summary of critical quality attributes identification and risk management. For the purposes of this table, critical quality attributes are limited to attributes intrinsic to the active pharmaceutical ingredient.

B. Drug Substance Quality Summary

Table 2 provides a summary of the identification, risk, and lifecycle knowledge management for drug substance CQAs that derive from the drug substance manufacturing process and general drug substance attributes, including process-related impurities.

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

CQA	Type ¹	Risk	Introduction	Control Strategy
Potency	Potency	Directly linked to efficacy (very high criticality) In vitro biological activity is dependent on both the quality and quantity of the drug substance used for DP formulation.	Intrinsic to the molecule Directly linked to efficacy (high criticality) In vitro potency includes a measure of receptor binding	(b) (4)
Target Receptor Binding	Potency	Directly linked to efficacy (very high criticality)	Intrinsic to the molecule	
Higher Order Structure	Potency	Directly linked to efficacy and MOA (very high criticality)	Intrinsic to the molecule	
Primary Sequence	Identity	Directly linked to efficacy and MOA (very high criticality)	Intrinsic to the molecule	
Aggregates, including dimers	Product-related	Potential impact on safety and efficacy (high	During manufacturing	

¹ The sponsor did not provide data to determine whether product related species are product-related substances or product-related impurities. Therefore these species are considered impurities.

and other HMW species	impurities	criticality)	and storage	(b) (4)
Oxidized species	Product-related impurities	Potential impact on safety and efficacy (high criticality)		
Deamidated species	Product-related impurities	Potential impact on safety and efficacy (high criticality)	During manufacturing and storage	
Pegylation variants (including di-PEG GCSF and Des-PEG-GCSF)	Product-related impurities	Potential impact on PK (moderate criticality)	Pegylation Very low levels of Des-PEG GCSF may form during storage (based on accelerated stability data)	
N-terminal truncated species	Product-related impurities	Potential impact on PK (moderate criticality)	(b) (4). Very low levels may form during storage, particularly via Des-PEG (based on accelerated stability data)	

Table 2: Drug Substance CQA Process Risk Identification and Lifecycle Knowledge Management

CQA	Source	Risk	Control Strategy
GCSF variants (norleucine, oxidized, F-met, gluconoylated, MGO, cysteinylated, reduced, and dimer)	(b) (4) Oxidized, reduced, and dimeric forms can be formed during manufacture and storage.	Safety and efficacy	(b) (4)
Free m-PEG	Chemical Introduced during pegylation reaction or during storage	Safety	
Host Cell DNA	Host cell (<i>E.coli</i>) (b) (4)	Immunogenicity and Safety	
Host Cell Proteins	Host cell (<i>E.coli</i>) (b) (4)	Immunogenicity and Safety	
Bacterial endotoxin	Host cell (<i>E.coli</i>). Potentially introduced during entire process, raw materials (e.g. (b) (4) r), environmental and equipment.	Safety	

Bioburden	Host cell (<i>E.coli</i>). Potentially introduced during entire process, raw materials (e.g. (b) (4)), environmental and equipment.	Safety	(b) (4)
(b) (4)	(b) (4)	Safety	
(b) (4)		Safety	
(b) (4)		Safety	
(b) (4)		Safety	
(b) (4)		Safety	
(b) (4)		Safety	

	(b) (4)		
(b) (4)		Safety	(b) (4)

B. Drug Substance Quality Summary (cont.)

a. Names:

- Proprietary Name: MYL-1401H
 - Trade Name: Fulphila (proposed)
 - Non-Proprietary/USAN: pegfilgrastim-jmdb²

 - CAS name: N-(3-hydroxypropyl) methionyl colony-stimulating factor (human), 1-ether with α -methyl- ω -hydroxypoly (oxyethylene)

 - Common name: MYL-1401H
 - INN Name: TBD
 - Compendial Name: N/A
 - OBP systematic name: CONJ: RPROT P09919 (CSF3_HUMAN); PEG [MYL-1401H]³
- b. Pharmacology category: Leukocyte growth factor

c. Description

MYL-1401H is a non-glycosylated recombinant methionyl human granulocyte colony stimulating factor (Met-GCSF) produced in *E. coli*. The primary sequence (175 amino acids) of MYL-1401H is identical to natural GCSF, except for an additional methionine residue at the N-terminus. MYL-1401H has a mostly alpha helical protein structure and two disulfide bridges between Cys37-Cys43 and between Cys65-Cys75. MYL-1401H is also pegylated with a 20kDa PEG molecule at the N-terminus and a molecular weight of approximately 38kDa. MYL-1401H is a proposed biosimilar to US-licensed Neulasta.

The composition of drug substance is (b) (4)
Drug substance is stored at (b) (4)
°C.

d. Mechanism of action

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

² This USAN name is the proposed name, which will be finalized upon approval of the application

³ The OBP systematic name allows searching for related products in OBP's database and in the Document Archiving, Reporting & Regulatory Tracking System (DARRTS) for safety reasons and it is different from the nonproprietary name. The tag at the end is used to separate products from different sponsors and it is generally the name used by sponsors to refer to the proposed product in their submissions.

blood, which is an relevant pharmacodynamic (PD) marker for neutropenia, and enhanced neutrophil function¹.

e. Potency Assay

Potency of MYL-1401H is determined by measuring its ability to stimulate proliferation of NFS-60 cells compared to a reference standard. The NSF-60 cell line, which carries the GCSF receptor, was derived from mouse myelogenous leukemia cells. The cells are incubated with a MTS⁴ and PMS⁵ solution. NADPH or NADH, produced by dehydrogenase enzymes in metabolically active cells, reduces MTS to a purple formazan compound. The quantity of formazan produced is measured by absorbance at 490 nm and is proportional to the number of viable cells present. PLA software is used to assess parallelism and determine potency relative an in-house reference standard.

f. Reference material(s)

The sponsor is currently implementing a two-tier reference standard system. A protocol for qualification and stability testing of in-house primary and working RSs was reviewed and found acceptable. The qualification of RSs includes calibration of potency using an international RS for GCSF. The current RS ^{(b) (4)} is acceptable because it was properly qualified and calibrated against NIBSC international standard for PEG-GCSF lot no. 12/188.

g. Critical starting materials or intermediates

^{(b) (4)}

h. Manufacturing process summary

^{(b) (4)}

¹ Panopoulos, A.D. and Watowich, S.S. (2008). Cytokine 42: 277-288

⁴ 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium

⁵ phenazine methosulfate, serves as an electron acceptor

(b) (4)

i. Container closure

(b) (4)

j. Dating period and storage conditions: (b) (4) months at (b) (4)C.

C. MYL-1401H Drug Product Quality Summary

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

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

CQA	Source	Risk	Control Strategy
Identity	General CQA intrinsic to the molecule Dependent on the identity of the drug substance used for DP formulation.	Safety and Efficacy (High Criticality)	(b) (4)
Protein Content	General CQA. Protein concentration is influenced by the formulation and mixing steps.	Safety and Efficacy (High Criticality)	
Container closure integrity and sterility	Microbial contamination. Contaminants can be introduced during DP manufacturing or with a Container closure integrity failure. Container closure integrity is a sterility assurance CQA.	Safety (high criticality)	
Bacterial endotoxin	Microbial contamination. Contaminants can be introduced during DP manufacturing or with a Container closure integrity failure.	Safety (high criticality)	
Particulate matter and sub-visible particles	Particulate matter could contain product / process related impurities and aggregates. Particulate matter can form during DP manufacturing and storage.	Safety (capillary occlusion) and Immunogenicity (high criticality)	

Appearance	General CQA. Appearance may be impacted by the formulation process. May indicate product degradation or other changes to the product.	Safety and efficacy	(b) (4)
Extractable Volume	General CQA. (b) (4)	Safety and efficacy.	
Gliding Force	Device CQA. May impact ease of administration	Safety (moderate criticality)	
Break Loose Force	Device CQA. May impact ease of administration	Safety (moderate criticality)	
Actuation of needle guard	Device CQA.	Safety (low criticality)	
pH	General CQA. Potential influence by formulation and mixing. Potential impact on stability, especially above the proposed pH range.	Safety and efficacy (high criticality)	
Sorbitol	(b) (4)	Safety (high criticality)	

(b) (4)	(b) (4)	Safety criticality) (low	(b) (4)
Osmolality	General CQA. (b) (4)	(Moderate criticality)	
Polysorbate 20	(b) (4)	Safety and efficacy. Polysorbate 20 levels control product aggregation and oxidation	
Leachables from process materials in DP manufacture and primary package	Container closure system and materials used in the DP process.	Safety criticality) (low	
HMWP and dimers	Product-related impurities that could form due to several manipulations in the DP manufacturing process e.g. (b) (4)	Potential impact on safety and efficacy (moderate criticality)	

Oxidized species	Product-related impurities. Introduced during manufacturing process and storage.	Potential impact on safety and efficacy (high criticality)	 (b) (4)
Deamidated species	Product related impurities. Introduced during manufacturing process and storage.	Potential impact on safety and efficacy (moderate criticality)	

D. MYL-1401H Drug Product Quality Summary (Cont.)**a. Description and Strength:**

MYL-1401H (injection) is supplied in a pre-filled syringe (PFS) for S.C. administration at a strength of 6 mg/0.6 mL. MYL-1401H has the same strength as US-licensed Neulasta.

b. Summary of Product Design

1

MYL-1401H was developed as a biosimilar to US-licensed Neulasta and thus to target the quality profile of the US-licensed Neulasta.

c. List of Excipients

D-sorbitol (30 mg), polysorbate 20 (0.024 mg), acetate (0.7 mg), sodium (0.01 mg) pH 4.0 (b) (4)

d. Reference material(s),

The same reference materials are used for DS and DP.

e. Manufacturing process summary

(b) (4)

Deficiencies in the (b) (4) process were identified. These include:

(b) (4)

In addition to these deficiencies, inspection of the manufacturing facility identified cGMP deficiencies. Refer to the facilities section of this review and the primary facilities review in Panorama.

f. Container closure

MYL-1401H DP is filled (b) (4) glass syringes with a (b) (4) 29 gauge stacked stainless steel needle. (b) (4)

The needle guard is a (b) (4) Ultrasafe Plus passive needle guard and passive plunger rod (b) (4).

g. Dating period and storage conditions: 24 months at $5 \pm 3^{\circ}\text{C}$.

h. List of co-packaged components: not applicable

E. Any Special Product Quality Labeling Recommendations

- Protect from light
- Do not freeze
- Do not shake

G. Lifecycle Knowledge Management

a. Drug Substance

- i. Protocols reviewed:
 1. Qualification of future MCBs and WCBs.
 2. Validation of (b) (4) at commercial scale and concurrent release of PPQ lots.
 3. Re-qualification/annual retesting of primary DS reference standard. A protocol for qualification of subsequent standards was not provided in the submission.
 4. Qualification of subsequent secondary reference standards and re-qualification/annual retesting of secondary DS reference standard.
 5. Post-approval DS stability protocol.
- i. Outstanding review issues/residual risk: See CR comments in section I.A.c. and additional comments.
- ii. Future inspection points to consider: See 483 observations in the establishment information section and DIA review.

b. Drug Product

- i. Protocols reviewed:
 1. Post-approval DP stability protocol
 2. Prospective shipping validation protocol for DP
- ii. Outstanding review issues/residual risk: See CR comments in section I.A.c and additional comments
- iii. Future inspection points to consider: See 483 observations in the establishment information section and DIA review.

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Kirshner

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**Center for Drug Evaluation and Research
Office of Pharmaceutical Quality
Office of Process and Facilities
Division of Microbiology Assessment**

PRODUCT QUALITY MICROBIOLOGY REVIEW AND EVALUATION

REVIEWER: Lindsey Brown, Ph.D.

Quality Assessment Lead (acting): Reyes Candau-Chacon, Ph.D.

Date: 9/11/2017

To: Administrative File

BLA: 761075/0

Applicant: Mylan Pharmaceuticals

US License Number: 1898

Manufacturing Site:

(b) (4)

FEI:

(b) (4)

Submission Reviewed: Biological License Application

Product: Proposed biosimilar to US approved Pegfilgrastim (Fulphila), MYL-1401H

Indication: To 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

Dosage Form: Pre-filled syringe (0.6 ml, 10 mg/ml)

FDA Receipt Date: 12/09/2016

Action Date: 10/6/2017

Recommendation for Approvability:—The drug product portion of BLA 761075 is not recommended for approval from a sterility assurance and microbiology quality perspective.

The following deficiencies were identified and should be communicated to the sponsor:

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Reyes
Candau-Chacon

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Lindsey
Brown

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

Date: 09/25/2017
To: Administrative File, STN 761075/0
From: Laura Fontan, Consumer Safety Officer, CDER/OPQ/OPF/DIA
Endorsement: Peter Qiu, Ph.D., Branch Chief, CDER/OPQ/OPF/DIA
Subject: Original BLA
US License: N/A
Applicant: Mylan GmbH.
Mfg Facility: Drug Substance: (b) (4)
 Drug Product: (b) (4)
Product: pegfilgrastim (Fulphila) Injection
Dosage: 6 mg/0.6 mL sterile liquid for subcutaneous injection
Indication: Treatment to 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
Due Date: 10/06/2017

RECOMMENDATION: The facility recommendation for the drug substance manufacturing site is acceptable based on the (b) (4) PLI inspection of the (b) (4) (b) (4)). For the proposed drug product manufacturing site, two inspections were recently conducted for (b) (4); a PLI from (b) (4) and a surveillance inspection from (b) (4). Observations from both inspections were reviewed to consider potential impact to the proposed operations for pegfilgrastim drug product manufacturing. Corrective actions from the PLI conducted from (b) (4) were completed and deemed adequate.

However, corrective actions from the surveillance inspection conducted from (b) (4), (b) (4) are currently still in progress. (b) (4) proposed (b) (4). An update from the firm received (b) (4) stated that (b) (4) CAPAs are still outstanding. Some of the outstanding CAPAs are critical to confirming correct (b) (4) operation. In the CAPA status update, the firm stated that (b) (4)

(b) (4)

Since critical CAPAs are still outstanding and the firm is currently not ready to manufacture, BLA 761075 for manufacture of pegfilgrastim is not recommended for approval.

(b) (4)

BLA 761075 MYL-1401H (Biosimilar Pegfilgrastim) DS and DP Manufacture

Laura Fontan
Consumer Safety Officer
OPF Division of Inspectional Assessment
Branch 1

Zhihao Peter Qiu, Ph.D.
Branch Chief
OPF Division of Inspectional Assessment
Branch 1



Center for Drug Evaluation and Research
WO Building 22
10903 New Hampshire Ave.
Silver Spring, MD 20993

PRODUCT QUALITY MICROBIOLOGY REVIEW AND EVALUATION

Date: September 8, 2017
To: Administrative File, 761075/0
From: Scott Nichols, Ph.D., Reviewer, OPQ/OPF/DMA/BIV
Through: Patricia Hughes, Ph.D., Branch Chief, OPQ/OPF/DMA/BIV
Subject: New 351(k) Biosimilar Biologics License Application (BLA)
US License: 2062
Sponsor: Mylan GmbH
Facilities: (b) (4)
Product: Fulphila (proposed), MYL-1401H
Dosage: sterile liquid for pre-filled syringe for subcutaneous injection (6 mg/0.6 mL)
Indication: decrease incidence of infection as manifested by febrile neutropenia, in patients with non-myeloid malignancies receiving myelosuppressive anti-cancer drugs associated with a clinically significant increase of febrile neutropenia
Action Date: October 6, 2017

Conclusion and Approvability Recommendation:

The drug substance portion of BLA 761075, as amended, is recommended for approval from a microbial control and a microbiological product quality perspective. However, the following should be added to the Complete Response Letter as an additional comment.

Only one batch of bulk drug substance (DE-B14-03-000018) was used to qualify the bioburden test. Conduct the qualification of the (b) (4) bioburden test for the bulk drug substance using two additional batches of bulk drug substance.

Please refer to the review memo from Dr. Lindsey Brown for an assessment of microbial control and microbiological product quality for the drug product (DP) portions of the BLA.



Scott
Nichols

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Patricia
Hughes Troost

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Drug Product Review
BLA STN 761075
Proposed Product USAN Name: pegfilgrastim-jmdb¹
Fulphila ("MYL-1401H"²)
Sponsor: Mylan GmbH
Manufacturer: (b) (4)

¹ This USAN name is the proposed name, which will be finalized upon approval of the application

² Nonproprietary name: referred to as "MYL-1401H" by the applicant*

*For purposes of this review, we generally refer to Mylan's proposed product by the Mylan descriptor "MYL-1401H."

OBP CMC Review Data Sheet

1. BLA#: 761075
2. Review Date: 09/05/2017
3. Primary Review Team:
 - a. Medical Officer: Rachel Ershler, Nicole Gormley
 - b. Pharm/Tox: Natalie Simpson, Christopher Sheth
 - c. Product Quality Team: Merry Christie (DS), Paul Kirwan (DP), Maria-Teresa Gutierrez-Lugo (ATL), Susan Kirshner (RC)
 - d. Immunogenicity: Zhenzhen Liu, Maria-Teresa Gutierrez-Lugo, and Susan Kirshner
 - d. Facilities: Laura Fontan, Michael Shanks, Peter Qiu
 - e. Clinical Pharmacology: Saeho Chong
 - f. Statistics: Yaping Wang
 - g. OBP Labeling: Vicky Borders-Hemphill
 - h. RBPM: Keith Olin
 - i. Microbiology: Scott Nichols, Lyndsey Brown, Lakshmi Narasimhan, Maria Reyes Candau-Chacon, Patricia Hughes

4. Major GRMP Deadlines:

- a. Filing Meeting: 01/17/2017
- b. Mid-cycle meeting: 05/09/17
- c. Wrap-up meeting: 08/29/17
- d. Primary review due: 09/05/17
- e. Secondary review due: 09/18/17
- f. PDUFA action date: 10/09/17

5. Communications with Sponsor and OND:

Communication/Document:	Date:
BPD 2 Meeting (Teleconference)	02/17/2015
BPD 2 Meeting (Teleconference)	10/22/2015
BPD 2 &4 Meeting	06/01/2016
Information Request (IR) #1	01/31/2017
IR #2	02/02/2017
IR #3	02/22/2017
IR #4	04/19/2017
IR #5	05/10/2017
IR #6	06/14/2017
IR#7	07/12/2017
IR #8	07/24/2017
IR #9	08/01/2017
IR #10	08/09/2017
IR #11	08/18/2017
IR #12	08/22/2017

IR #13	08/28/2017
IR #14	08/30/2017
IR #15	08/31/2017

6. Submission Reviewed:

Submission:	Date Received:	Review Completed (yes or no)
STN 761075/0000	12/09/2016	Yes
STN 761075/0003 (Response to IR #1)	02/03/2017	Yes
STN 761075/0005 (Response to IR #2)	02/08/2017	Yes
STN 761075/0008 (Partial response to IR #3)	02/28/2017	Yes
STN 761075/0010 (Partial response to IR #3)	03/21/2017	Yes
STN 761075/0013 (Partial response to IR #4)	04/27/2017	Yes
STN 761075/0014 (Partial response to IR #4)	05/11/2017	Yes
STN 761075/0015 (Response to IR #5)	05/11/2017	Yes
STN 761075/0016 (Partial response to IR #4)	05/12/2017	Yes
STN 761075/0019 (Partial response to IRs #4)	05/25/2017	Yes
STN 761075/0020 (DS and DP stability update)	06/16/2017	Yes
STN 761075/0024 (Partial response to IR #6)	07/17/2017	Yes
STN 761075/0025 (Partial response to IR #6)	07/25/2017	Yes
STN 761075/0024 (Response to IR #7)	07/25/2017	Yes
STN 761075/0027 (Response to IR #8)	07/31/2017	Yes
STN 761075/0029 (Response to IR #9)	08/10/2017	Yes
STN 761075/0030 (Response to IR #10)	08/18/2017	Yes
STN 761075/0033 (Response to IR #11)	08/29/2017	Yes
STN 761075/0034 (Response to IR #12)	08/30/2017	Yes
STN 761075/0035 (Partial Response to IR #13 & 14)	09/05/2017	Yes

7. Drug Product Name/Code/Type:
- a. Proprietary Name: Fulphila (proposed)
 - b. Trade Name:
 - c. Proposed Non-Proprietary Name/USAN: pegfilgrastim-jmdb (to be finalized upon application approval)
 - d. CAS Name: N-(3-hydroxypropyl) methionyl colony-stimulating factor (human), 1-ether with α -methyl- ω -hydroxypoly (oxyethylene)
 - e. Common Name:
 - f. INN Name
 - g. Compendial Name
 - h. OBP systematic name: CONJ: RPROT P09919 (CSF3_HUMAN); PEG [MYL1401H]
 - i. Other names: "MYL-1401H;" Pegylated Granulocyte colony stimulating factor (PEG-GCSF); (b) (4)

8. Pharmacological Category: Leukocyte growth factor

9. Dosage Form: Injection

10. Strength/Potency:
- (i): 6 mg/0.6 mL
 - (ii): Cell proliferation assay

11. Route of Administration: Subcutaneous injection

12. Referenced Drug Master Files (DMF):

DMF#	DMF Holder	Item Referenced	Letter of Cross-Reference	Comments (status)
		(b) (4)	Yes	Sufficient information in application
			Yes	Sufficient product quality information in application. For device functionality, defer CDRH/ODE review
			Yes	Sufficient product quality information in application. For device functionality, defer CDRH/ODE review
			Yes	Sufficient product quality information in application. For device functionality, defer CDRH/ODE review
			Yes	Defer DMA review
			N/A	Sufficient product quality information in application. For device functionality, defer CDRH/ODE review

(b) (4)	N/A	Sufficient product quality information in application. For device functionality, defer CDRH/ODE review

13. Inspectional Activities:

PAI was conducted from (b) (4) at the DS and DP facilities. Information about the facilities and FDA personnel involved in the inspections are shown below:

Firm:
Location:

Type of Establishment:
FEI Number:
Dates of Inspection:
Days in Facility:
FDA participants:



An eleven-item 483 form was issue at the end of the DS facility inspection (Appendix II of the DS review)

Firm:
Location:

Type of Establishment:
FEI Number:
Dates of Inspection:
Days in Facility:
FDA participants:



An eight-item 483 form was issued at the end of the DP facility inspection (See Appendix VII)

14. Consults Requested by OBP:
Latoya Oliver-Powell (CDRH/OC) and Steven Basile (CDRH/ODE)

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 (Used for process characterization)
X	Risk Assessment/Risk Management (Used for process characterization)
	Multivariate Statistical Process Control
	Process Analytical Technology
	Expanded Change Protocol

16. Precedents: None

Summary of Quality Assessments

I. Primary Reviewer Summary Recommendation

I do not recommend approval of this 351(k) BLA application for MYL-1401H (Fulphila) as a biosimilar to US-licensed Neulasta for the reasons listed below.

II. List of Deficiencies to be Communicated (draft)



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

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

A categorical exclusion is requested by Mylan under 21 CFR Part 25.31(a). As stated in 21 CFR Part 25.31(a), if "action on an application for marketing approval of a biologic product does not increase the use of the active moiety." Approval of this submission will not increase the overall use of the active moiety, therefore, exclusion may be granted.

V. Primary Container Labeling Review
Refer to review by Vicky Borders-Hemphill

VI. Review of Common Technical Document- Quality Module 3.2
See below

VII. Review of Immunogenicity Assays- Module 5.3.1.4

Refer to review by Zhenzhen Liu, Maria-Teresa Lugo-Gutierrez, and Susan Kirshner

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P: DRUG PRODUCT

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

MYL-1401H drug product (DP) 6 mg/0.6 mL solution (protein concentration (b)(4) mg/mL), injection is a clear colorless, preservative-free sterile solution for subcutaneous administration. Its composition is described in Table 3.2.P.1/1. MYL-1401H is supplied in a single-use prefilled syringe (PFS) ((b)(4) ItraSafe™ Plus passive needle guard), at a pH of 4.0 (b)(4). No diluents are required for reconstitution. The selection of components of MYL-1401H, compatibility with excipients, and compatibility with the contain closure system are discussed below.

Table 3.2.P.1/ 1: Composition of the Drug Product

Component	Function	Quantity per PFS	Reference to Standards
MYL-1401H	Active ingredient	6 mg	In- House
D-Sorbitol	(b)(4)	30 mg	Ph. Eur./USP
Polysorbate 20		0.024 mg	Ph. Eur./USP
Acetate ^a		0.7 mg	Ph. Eur./USP
Sodium ^a		0.01 mg	Ph. Eur./USP
Water for injection		q.s to 0.6 mL	Ph. Eur./USP

Abbreviations: Ph. Eur. = European Pharmacopeia; q.s.=quantity sufficient; USP = United States Pharmacopeia (b)(4)

3.2.P.2 Pharmaceutical Development

(b)(4)

3.2.R. ANALYTICAL SIMILARITY

The data and information presented in this section was provided by (b) (4) on behalf of Mylan and represents the sponsor’s analyses of the proposed biosimilar product and U.S.-licensed Neulasta. Unless otherwise noted, all figures and tables referred to in this section were selected from Mylan’s 351(k) BLA submission.

The proposed biosimilar product, MYL-1401H, was developed as a liquid for subcutaneous injection, filled in a prefilled syringe in the same strength (6 mg/0.6 mL) as U.S.-licensed Neulasta. A comparison of the formulation of MYL-1401H and US-licensed Neulasta is provided below.

Table E. Drug Product Formulation Comparison

Component	MYL-1401H	US-Licensed Neulasta
MYL-1401H	6 mg	
Pegfilgrastim		6 mg
D-Sorbitol	30.0 mg	30.0 mg
Polysorbate 20	0.024 mg	0.02 mg
Acetate / (b) (4)	0.7 ma	0.35 ma (b) (4)
Sodium / (b) (4)**	0.01 mg	(b) (4) mg (b) (4)
Water for Injection (WFI)	Quantity Sufficient	Quantity Sufficient
pH	4.0	4.0

Note: The table was prepared by the CMC reviewer

The clinical development program for MYL-1401H included three studies. Study MYL-1401H-1001 was conducted in healthy subjects and was intended to assess PK/PD similarity between MYL-1401H and US-licensed Neulasta and also served as a PK bridge between MYL-1401H, US-licensed Neulasta, and EU-approved Neulasta. Study MYL-1401H-1002 was conducted in healthy subjects and was intended to assess immunogenicity of MYL-1401H in comparison to US-licensed Neulasta. Study MYL-1401H-3001 was conducted in patients with breast cancer and was intended to assess safety, efficacy, and immunogenicity of MYL-1401H in comparison to E.U.-approved Neulasta. These studies are proposed to support that there are no clinically meaningful differences between MYL-1401H and U.S.-licensed Neulasta.

To support the use of E.U.-approved Neulasta as a comparator in clinical study MYL-1401H-3001 and use the resulting data to support a demonstration that MYL-1410H is biosimilar to U.S.-licensed Neulasta, the sponsor intends to establish a scientific bridge between MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta. The analytical portion of the scientific bridge is discussed in this review.

The non-clinical program included five studies: BDL/TR/BR.14.5003/16/001, BDL/TR/BR.14.5003/15/001, and DDL/TR/BR.14.5003/16/003 which compared MYL-1401H and U.S.-licensed Neulasta; PHA 071-001 which compared MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta; and TOX 071-001 which compared MYL-1401H and E.U.-approved Neulasta.

The clinical and proposed commercial processes for MYL-1410H are the same. The analytical similarity assessment included MYL-1401H lots (BS14005356 and BS14009031) that were used in the clinical studies (Table F) and process validation lots supporting the proposed commercial product.

Table F. Clinical lots of MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta Used for Analytical Similarity Assessment

Product	Lots Used for Analytical Similarity Assessment	Clinical Study
MYL-1401H	BS14005356*	MYL-1401H-1001 MYL-1401H-1002
	BS14009031*	MYL-1401H-3001
U.S.-licensed Neulasta	1044948	MYL-1401H-1001 MYL-1401H-1002
E.U.-approved Neulasta	1047632A [‡]	MYL-1401H-1001 MYL-1401H-1002
	1048603D	MYL-1401H-3001
	1053573B	

*The MYL-1401H lots used in the clinical studies were also representative of the commercial process

[‡]E.U.-approved Neulasta lot not included in potency analysis

All lots except 1047632A were included in both potency and protein content analysis

Note: The table was prepared by the CMC reviewer

Based on the sponsor estimation of the age of the products, the U.S.-licensed Neulasta and E.U.-approved Neulasta lots analyzed for analytical similarity ranged from 11 – 35 months of age. The MYL-1401H lots analyzed ranged from 2-29 months of age. For US-licensed Neulasta, the sponsor’s estimation of age was based on a 36 month expiry. The age of each lot was estimated from the expiration date at the time of analysis. This information is summarized in Tables 4-6 of Appendix III.

Table 5 below shows the methods used to analyze MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta, including criticality of the quality attribute tested and the tier assignments proposed for each method by the sponsor. The methods were validated or qualified at the time of testing. Table 6 shows the quality ranges assigned for each test method based on the criticality risk ranking of the attribute tested by the method and the method variability as determined by the sponsor. The criticality risk ranking considered the potential impact of individual quality attributes on efficacy, PK, immunogenicity, safety, and stability (3.2.P.2.2.3).

Table 5: Risk Ranking of Quality Attributes and Tier Assignments of the Corresponding Analytical Methods

Quality Attribute	Risk Rank	Methods/tests	Characteristics	Tier
Protein content	Very High	Ultra Violet -280	Protein Content	1
Potency	Very High	MNFS-60 cell based proliferation assay	Potency	1
G-CSFR Binding	Very High	Surface Plasmon Resonance based Binding kinetic assay	Target Receptor Binding	2
Primary sequence	Very High	Peptide mass fingerprinting Glu-C and Trypsin Digest	Peptide mass fingerprinting Site of pegylation & sequence	3
		Matrix Assisted Laser Desorption Ionization – Time of Flight	Intact Molecular Mass	3
Conformation	Very High	Non-reduced peptide mass fingerprint Glu-C	Disulfide Bridging	3
Quality Attribute	Risk Rank	Methods/tests	Characteristics	Tier
(Secondary and Higher order structure)	Very High	Far Ultra Violet Circular Dichroism	Secondary Structure Helix, Sheet, Turns & Random Coil	2
		Fourier transform infrared spectroscopy	Tertiary Structure	3
		Ellman's Assay	Free Cysteine	2
		Extrinsic Fluorescence	Hydrophobicity λ max and Intensity	3
		Near Ultra Violet Circular Dichroism	Tertiary Structure	3
		Differential Scanning Calorimetry	Melting Temp T _m (°C)	3
		1D NMR (fingerprint)	Tertiary Structure	3
High Molecular Weight species	High	Size Exclusion Chromatography – Ultra Violet	Aggregates and HMWP	2
		Analytical Ultra Centrifuge	Characterization of HMWP	3
		Size Exclusion Chromatography – Multi Angle Light Scattering	Characterization of HMWP	3
Dimer	High	Reverse Phase Chromatography	Dimers	2
Di-PEG-G-CSF	Moderate	Cation Exchange chromatography	Di-PEG-G-CSF	2
Deamidation (Q ₁₀₈)	Moderate	Reverse Phase Chromatography	Deamidation	2
LMWP	High	Size Exclusion Chromatography – Ultra Violet	Des-PEG	2
Related Protein variants	High	Reverse Phase Chromatography	Total pre-peaks Total post peaks	2
Methionine oxidation	High	Reverse Phase Chromatography	Oxidation	2
Des-PEG-G-CSF	High	Reverse Phase Chromatography	Truncation	2
Free PEG amount	High	Reverse Phase Chromatography - Charged Aerosol Detection (CAD)	Residual Free PEG amount	3
pI	High	CIEF	pI	3
Sub-visible Particles	Very High	Molecular Flow Imaging / Dynamic Light Scattering	Sub visible particles	3
Process related Impurity	Very High	ELISA	HCP	3
	Very High	PCR	Hc-DNA	3

Table 6: Quality Range Approach for similarity assessment of MYL-1401H

Method	Attribute	Quality Range	Justification	
			Criticality	Method Variability
G-CSF Receptor binding	Association Rate constant (ka)	± 2SD	Very High	>15%
	Dissociation Rate constant (kd)	± 2SD		
	Equilibrium Dissociation Constant (K _D)	± 2SD		
SEC-HPLC	Aggregates (HMWP-1)	± 2SD	High to Moderate	>15%
	HMWP 2+3	± 2SD		
	Monomer	± 2SD		
	Total HMWP	± 2SD		
	Total LMWP	± 2SD		
CIEX-HPLC	Di-Peg-G-CSF	± 3SD	Moderate	<15%
	Peg-G-CSF	± 3SD		
RP-HPLC	M ₁₃₈ Oxidized	± 3SD	High to Moderate	<15%
	Q ₁₀₈ Deamidated	± 3SD		
	Des-Peg-G-CSF	± 3SD		
	Dimer	± 3SD		
	Total Pre Peaks	± 3SD		
	Total Post Peaks	± 3SD		
	Main Peak	± 3SD		
Far-UV CD	% Alpha helix	± 2SD	Very High	>15%
	% Beta sheet	± 2SD		
	% Beta turn	± 2SD		
	% Random coil	± 2SD		
Ellman's Assay	Free Cysteine	± 2SD	Very High	<15%

Reviewer Comments: Product characterization (Table 5) is consistent with published FDA guidance⁸⁹. The tier assignments (Table 5) and quality ranges (Table 6) are adequate as described. The adequacy of risk ranking of quality attributes will be discussed in the context of the control strategy.

⁸ Quality considerations in demonstrating biosimilarity of a therapeutic protein product to a reference product, U.S. FDA, April 2015

⁹ Scientific considerations in demonstrating biosimilarity to a reference product, U.S. FDA, April 2015

PRIMARY SEQUENCE

Intact mass analysis and multiple peptide mapping approaches based on enzyme and chemical digests, followed by mass spectrometry analysis, were used to evaluate and compare the primary sequences of MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta. The resulting data provided detailed information on the composition of the G-CSF moiety from the molecular masses of the digested peptide fragments, including the location of the PEG moiety at the N-terminal methionine in sequence position 1.

Intact Mass – MALDI-TOF

The intact mass of MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta was evaluated by MALDI-TOF mass spectrometry. Figure 28 shows a comparison of representative spectra from the tested lots.

Figure 28: Intact Molecular Mass Spectra overlay for MYL-1401H, US-licensed Neulasta, and EU-approved Neulasta

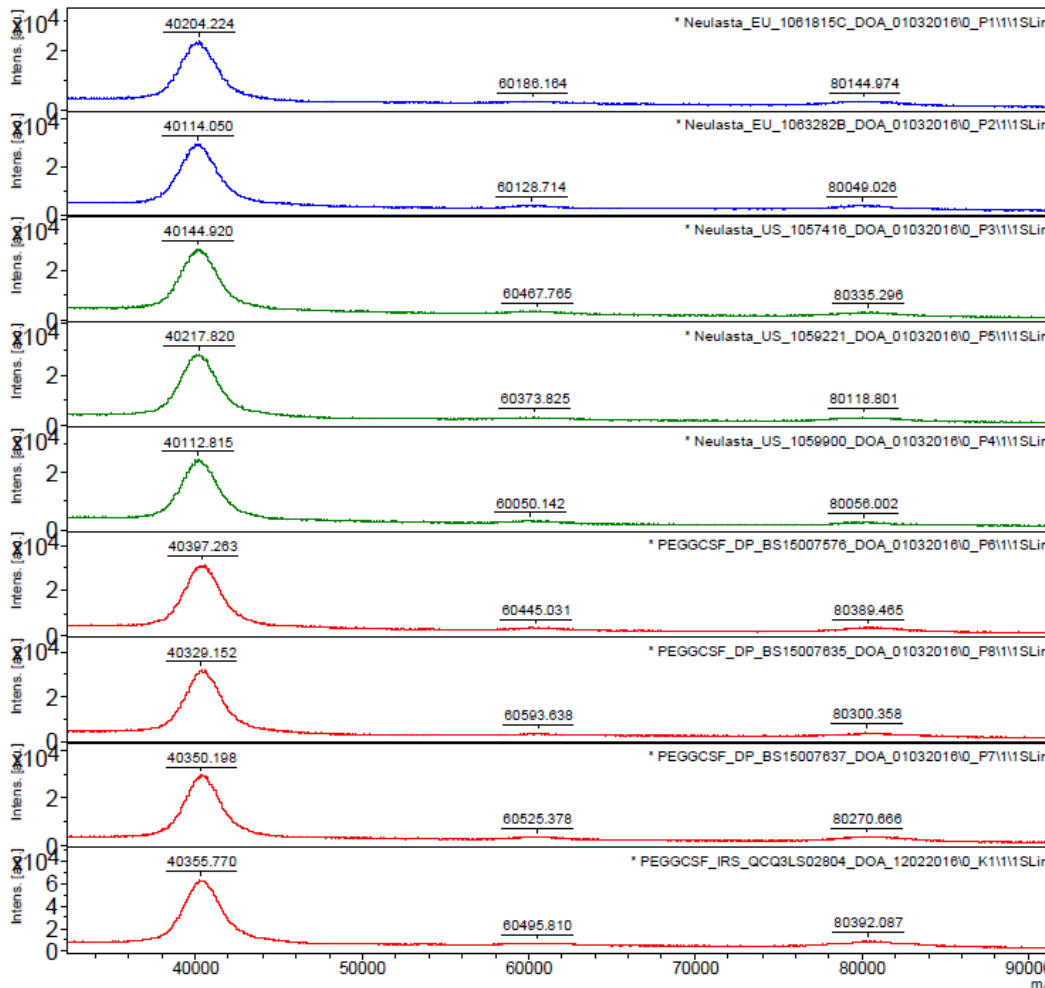
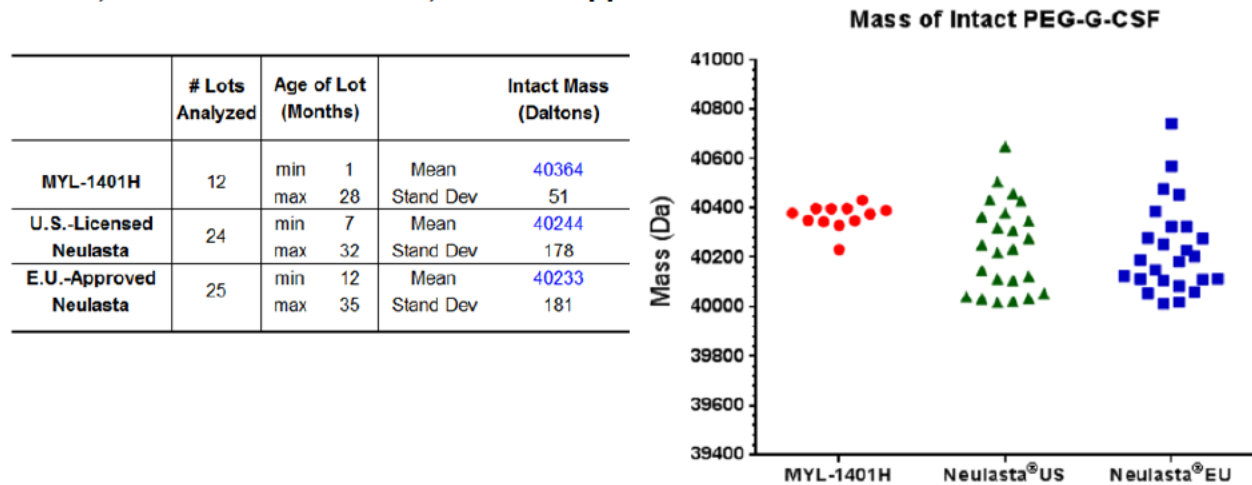


Figure 29. Tabulated summary and scatter plot results of intact mass analysis of MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta.



Note: The table was prepared and the figure adapted by the CMC reviewer

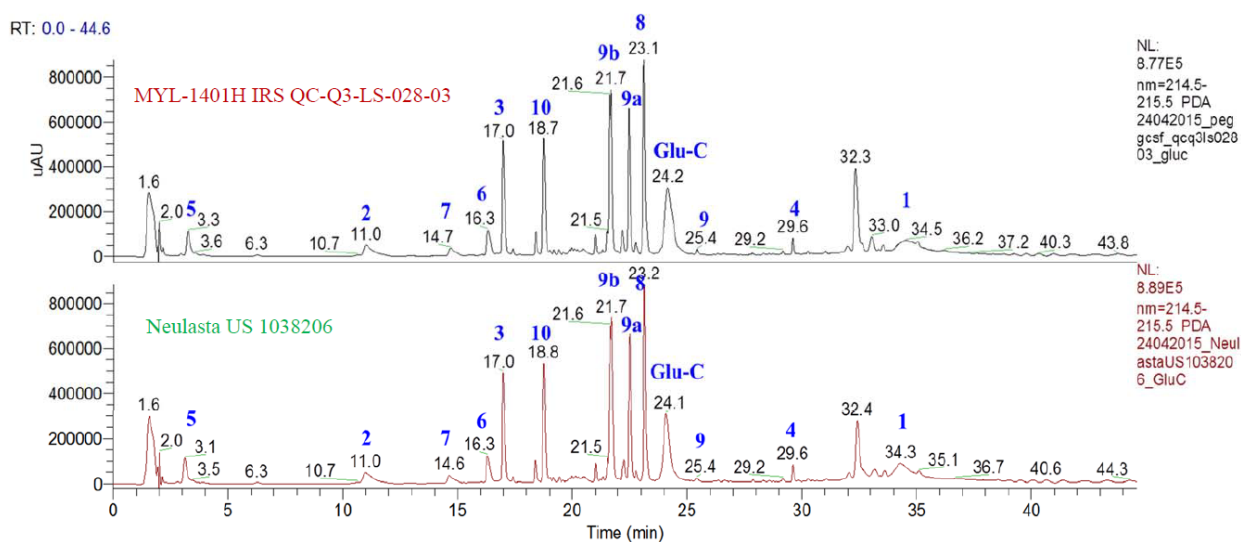
Reviewer Comments: The expected theoretical molecular weight of the non-PEGylated Met-rhG-CSF 175 amino acid component of MYL-1401H is 18802.85 Da (based on the ExPASy Bioinformatics Resource Portal ProtParam tool). When conjugated with the average molecular weight of the PEG moiety of 20,000 Da (b) (4) the expected molecular weight of MYL-1401H is 38,803 Da (b) (4). The results shown in Figures 28 and 29 show that the three products have similar molecular mass and the masses are consistent with the expected mass range of 38,803 Da (b) (4). The MALDI-TOF results also give an indication of the variation of PEG dispersion which is similar between the products.

In addition to the main peaks, broad peaks are visible near m/z values of 60,000 and 80,000 in the intact mass spectra for the three products (Figure 28). These masses are consistent with Di-PEG species and PEG-G-CSF dimers described as product related impurities by the sponsor. The sponsor supported the identity of these species by mass spectrometry data (refer to the characterization section 3.2.S.3). The reviewer notes that the 60,000 and 80,000 m/z values peaks are present in the MALDI spectra for MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta at similar levels.

Non-Reduced Peptide Mapping with Glu-C and Trypsin Digestion

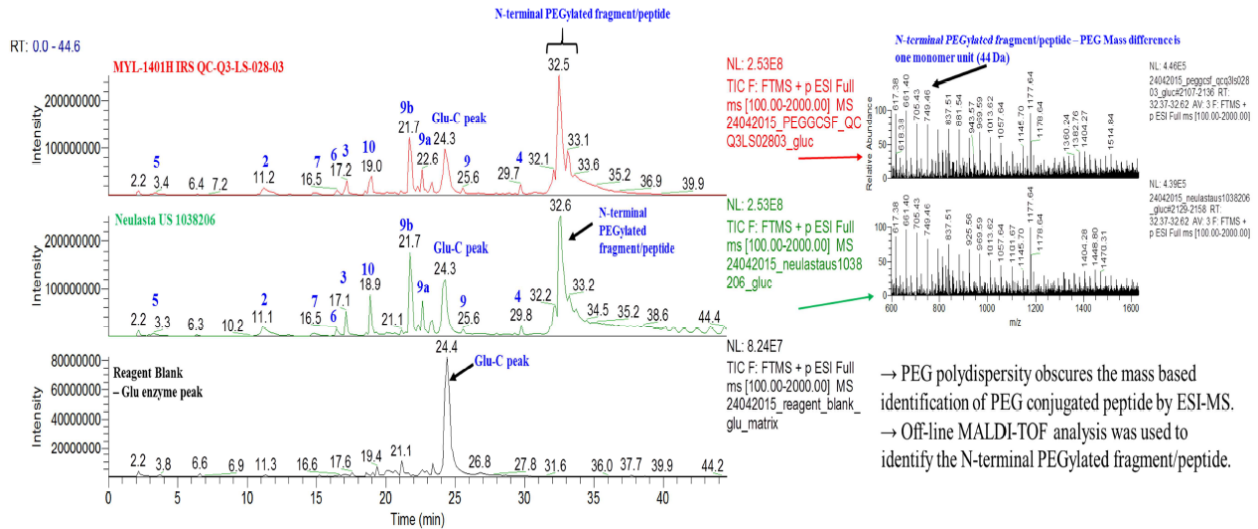
Multiple lots of MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta were subjected to two separate digestion reactions, one with Glu-C enzyme (cleaves proteins on the C-terminal side of Asp or Glu residues) and another with trypsin enzyme (cleaves proteins on the C-terminal side of Lys or Arg residues). Following digestion, the resulting peptide fragments were separated by RP-HPLC and analyzed by LTQ-Orbitrap mass spectrometry or ESI-MS/MS. The N-terminal fragments (PEG-Fragment 1) from each digest for each sample were analyzed by MALDI-TOF to confirm the presence of the PEG moiety (see *N-Terminal PEGylation Confirmation* below). Figure 30 shows representative RP-HPLC profiles for peptides from Glu-C digests of MYL-1401H and U.S.-licensed Neulasta. Figures 31 and 32 in the submission show overlaid chromatograms for six Glu-C and trypsin digests each for MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta.

Figure 30: Overlay of UV (PDA) Profile of Peptide Map for MYL-1401H and US-licensed Neulasta



Reviewer Comments: Figure 30 shows similar RP-HPLC chromatograms for peptides from Glu-C digests of MYL-1401H and U.S.-licensed Neulasta. Note that an MYL-1401H internal reference standard is used in the comparison with U.S.-licensed Neulasta. This is acceptable since the reference standard is prepared from a DS lot of MYL-1401H that was also used to make DP lots included in the analytical similarity assessment. There is a peak at retention time 32.3-33.4 min that is prominent but unidentified. The peak is present in both representative chromatograms in Figure 30 and appears in the Glu-C digests of E.U.-approved Neulasta, as shown in Figure 31 in the submission. In response to an IR, the sponsor clarified that the peak at 32.3 min is N-terminal PEGylated fragment peptide with a PEG molecular weight that differs from the average PEG molecular weight due to inherent polydispersity. It is reasonable that polydispersity could result in retention time changes for minor peaks from lot to lot. The nature of the peak near 32.2 min was confirmed by its mass (Figure 8 below) which shows m/z repeats of 44, characteristic of the PEG monomer unit.

Figure 8: Electro spray Ionization Mass Spectrometry – Overlaid Total Ion Chromatogram Profile of Peptide Map for MYL-1401H, US-Licensed Neulasta, and Reagent Blank



The Glu-C and Trypsin digested peptide map chromatograms in Figures 31 and 32 (not shown) in the submission are visually similar between MYL-1401H (6 lots), U.S.-licensed Neulasta (6 lots), and E.U.-approved Neulasta (6 lots).

Tables 30 and 31 Lists the peptide fragments generated for MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta by Glu-C and trypsin digestion, respectively, and includes their expected monoisotopic and experimentally observed masses.

Table 30: List of Peptide Fragments and Their Masses Monitored by Peptide Mass Fingerprinting (representative batch as all other batch have same peptide mass)

Fragments	AA Range	Theoretical Mono [M+H] Mass	Sequence	EU-APPROVED NEULASTA 1051893B	US-LICENSED NEULASTA 1038206	MYL-1401H IRS QCQ3LS02803
1	[A1-A20]	22131.1	PEG-MTPLGPASSLPQSFLKCLE(1*Oxidized(SS).)	N-terminal Peptide mass absent in Peptide map and is confirmed using MALDI-TOF MS (Figure 33)		
2	[A21-A34]	1512.8	QVRKIQGDGAALQE	1512.82	1512.82	1512.82
3*	[A35-A47]	1532.7	KLCATYKLCHPPEE	1532.73	1532.73	1532.73
4*	[A48-A94]	4940.6	LVLLGHSGLIPWAPLSSCPQALQLAGCLSQLHSGFLYQGLLQALE	4940.65	4940.65	4940.65
5	[A95-A99]	502.2	GISPE	502.25	502.25	502.25
6	[A100-A105]	615.3	LGPTLD	615.34	615.34	615.34
7	[A106-A110]	589.3	TLQLD	589.32	589.32	589.32
8	[A111-A124]	1668.8	VADFATTIWQQMEE	1668.77	1668.77	1668.77
9	[A125-A163]	4027.1	LGMAPALQPTQGAMPAFASAFQRRAGGVLVASHLQSFLE	4027.12	4027.12	4027.12
9a	[A125-A142]	1771.9	LGMAPALQPTQGAMPAFA	1771.90	1771.90	1771.90
9b	[A143-A163]	2273.2	SAFQRRAGGVLVASHLQSFLE	2273.24	2273.24	2273.24
10	[A164-A175]	1438.8	VSYRVLRLHAQP	1438.84	1438.84	1438.84

Table 31: List of Peptide Fragments and Their Masses Monitored by Peptide Mass Fingerprinting using Trypsin (representative batch as all other batch have same peptide mass)

NO	FRAGMENT	THEORETICAL [M+H] ⁺ [DA]	SEQUENCE	EU- APPROVED NEULASTA 1056942B	US- LICENSED NEULASTA 1057133	MYL1401H IRS QCQ3LS0280 4
1	A1 - A17		PEG-O-MTPLGPASSLPQSFLK	Fragment 1 mass was analysed using MALDI-TOF MS (Figure 33)		
2	A18 - A23	746.8	CLEQVR	747.39	747.39	747.39
3	A24	147.1	K	-	-	
4	A25 - A35	1130.2	IQGDGAALQEK	1129.60	1129.60	1129.60
5	A36 - A147	11993.7	LCATYKLCHPPEELV LLGHSLGIPWAPLSS CPSQALQLAGCLSQ LHSGFLYQGLLQA LEGISPELGPPTLDTL QLDVADFATTIWQQ MEELGMAPALQPTQ GAMPAFASAFQR	11994.03	11994.03	11994.03
	36-41/42-147 with disulphide linkage Cys37-Cys43					
6	A148	175.2	R	-	-	
7	A149 - A167	2034.2	AGGVLVASHLQSFL EVSYR	2034.12	2034.12	2034.12
8	A168 - A170	387.4	VLR	387.28	387.28	387.28
9	A171 - A175	565.6	HLAQP	565.32	565.32	565.32

Reviewer Comments: Tables 30 and 31 confirm that all expected theoretical monoisotopic masses of the digested fragments 2-10 (Glu-C, Table 30) and fragments 2-9 (trypsin, Table 31) were observed experimentally and are similar between the three products. The experimental masses are also consistent with their theoretical monoisotopic mass. The PEG-Fragment 1 was identified separately by MALDI-TOF MS following both digests.

In the Glu-C digest (Table 30), fragment 3 includes Cys37 and Cys43, and fragment 4 includes Cys65 and Cys75. The reported monoisotopic masses of these fragments are similar between the products and are consistent with the presence of disulfide bonds (Table 30B).

Table 30B. Sample calculation of expected monoisotopic masses of selected Glu-C and Trypsin digest fragments of MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta

Monoisotopic Mass - Reduced	Monoisotopic Mass – ExPASy [M+H] ⁺ (+1 Da)	Disulfide Bond Mass Difference (-2 Da)	Reported Result
Glu-C Frag 3	1533.73	1531.73	1532.7
Glu-C Frag 4	4941.63	4939.63	4940.6

Table prepared by the CMC reviewer using the ExPASy Bioinformatics Resource Portal PeptideMass tool

In the trypsin digest (Table 31), Fragment 5 contains four of five cysteines and therefore two disulfide bonds. The reported mass of this fragment (11994.03) differs from the theoretical

reduced mass of 11991.04 Da.¹⁰ However, the results of all other peptide fragment masses listed in Table 31 were consistent with the expected theoretical masses. In addition, the presence of disulfide bonds was confirmed by peptide mapping with Glu-C. Overall, the peptide mapping results using both Glu-C and trypsin enzymes resulted in 100% sequence coverage, with all expected peptides identified. These data support the conclusion that MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta have the same amino acid sequence and disulfide bonds.

N-Terminal PEGylation Confirmation – Glu-C and Trypsin Digests - MALDI-TOF

Analysis of the N-terminal PEG-Fragment 1 peptides resulting from separate Glu-C and trypsin digests of MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta was performed using MALDI-TOF MS and is shown in Figure 33. Table 32 and Figure 34 show tabulated means and scatter plots of the results. The sequences and theoretical molecular weights of the PEG-Fragment 1 peptides are as follows:

Glu-C digestion: **Fragment 1:** PEG-O-MTPLGPASSLPQSFLKCLE
PEG (20,000 Da (b) (4)) + Fragment 1 (2,132.5 Da)
~ 22,133 Da (b) (4)

Trypsin digestion: **Fragment 1:** PEG -O-MTPLGPASSLPQSFLK
PEG (20,000 Da (b) (4)) + Fragment 1 (1,788.0 Da)
~ 21,788 Da (b) (4)

¹⁰ ExPASy Bioinformatics Resource Portal PeptideMass Tool, http://web.expasy.org/peptide_mass/

Figure 33: Molecular Mass Spectra of (PEG + Fragment 1) digested with Glu-C (left panel) and Trypsin (right panel) for MYL-1401H lots, US-licensed Neulasta and EU-approved Neulasta

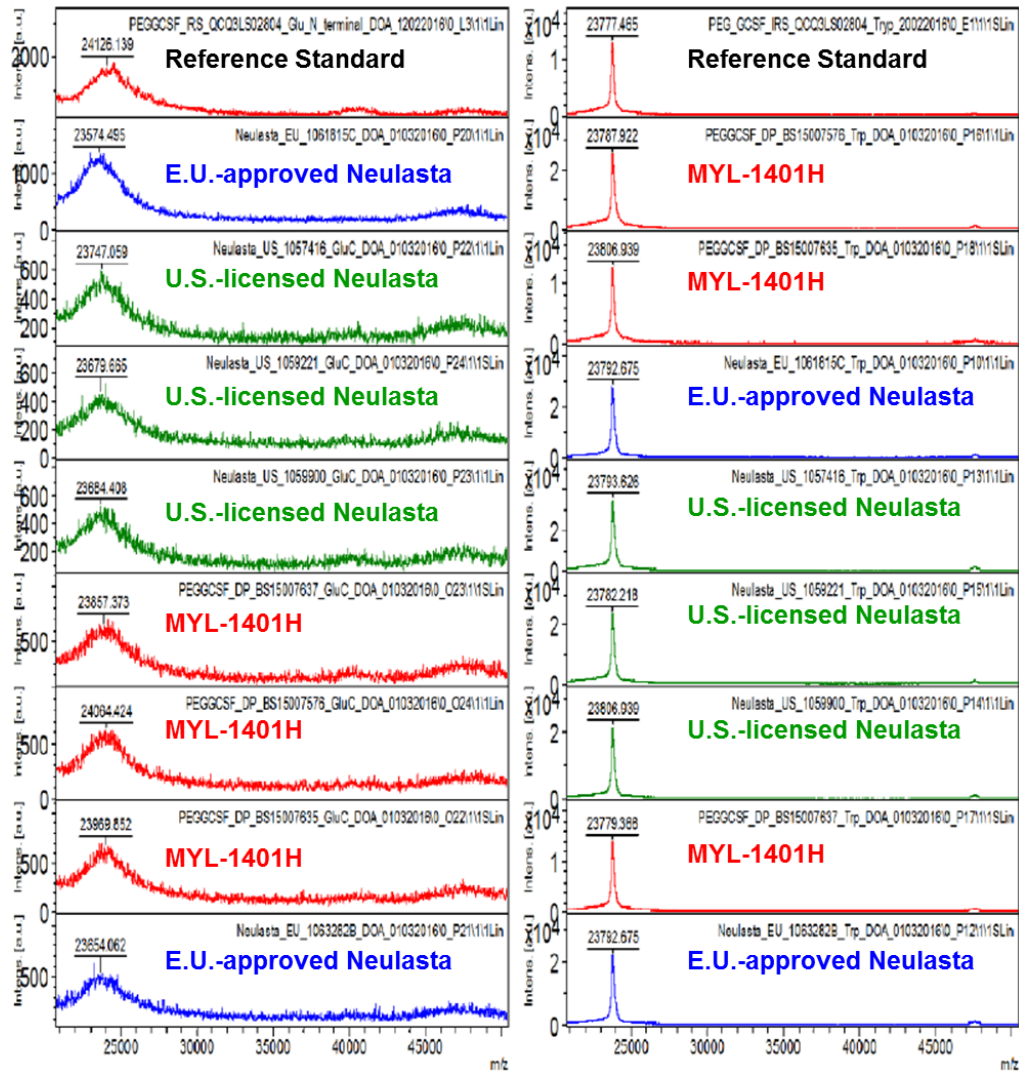
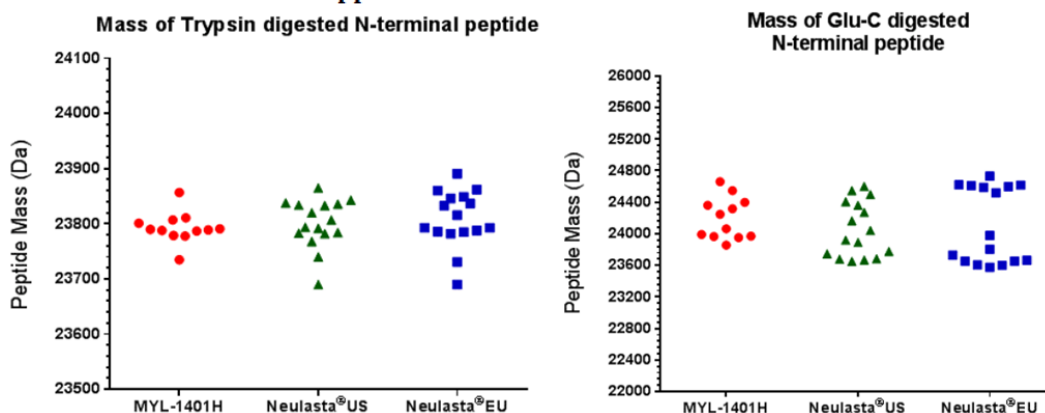


Table 32: Data for N-terminal mass in both Glu-C and Trypsin digested MYL-1401H, US-licensed Neulasta and EU-approved Neulasta

	# Lots Analyzed	Age of Lot (Months)		N-term Peptide Mass (Da)		
				Glu-C Digested	Trypsin Digested	
MYL-1401H	12	min	1	Mean	24195	23793
		max	28	Stand Dev	264	28
U.S.-Licensed Neulasta	16	min	7	Mean	24057	23801
		max	28	Stand Dev	348	44
E.U.-Approved Neulasta	16	min	13	Mean	24097	23809
		max	35	Stand Dev	480	51

Note: The table was prepared by the CMC reviewer

Figure 34: Scatter Plot of N-terminal Mass using MALDI-TOF-MS for the N-terminal peptide (PEG + Fragment 1) in both Glu-C and Trypsin digestion for MYL-1401H, US-licensed Neulasta and EU-Approved Neulasta.



Note: The plots were combined as a single figure by the CMC reviewer

Reviewer Comment: The MALDI-TOF MS analysis was performed off-line on PEG-Fragment 1 fractions isolated by RP-HPLC. The reported mean N-terminal PEG-Fragment 1 peptide masses are similar between MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta for both Glu-C and trypsin digests (Table 32). The scatter plots in Figure 34 show a visual comparison of the reported results for each lot analyzed and reflect similar spread of the data points for each product.

The mean observed masses for the trypsin digested PEG-Fragment 1 peptide are similar for all three products (23,793 – 23,809 Da) and are consistent with the expected theoretical mass of 21,788 Da ^{(b) (4)}. Similarly, the reported mean observed masses (24,057 – 24,195 Da) for the Glu-C digested PEG-Fragment 1 peptide are similar between all three products and are consistent with the expected theoretical mass of (22,133 Da ^{(b) (4)}

The mass spectra in Figure 33 show peaks at $m/z \sim 47,500$ Da present in spectra from both Glu-C and trypsin digests for all three products. The reviewer thinks it is possible that these peaks

indicate the presence of PEG-Fragment-1 dimer¹¹ or a dipegylated fragment at positions M1 and K17. However, the sponsor did not provide data to directly address these species. Since these peaks are present in all three products, at similarly low abundance, and at high m/z region of the spectrum where resolution is diminished, it is reasonable to conclude that PEG-Fragment 1 is similar for MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta. These data support the location of the PEG moiety at Met1 in the three products.

The sponsor was asked to address the similarity of the chemical linkage between the PEG moiety and the G-CSF molecule in MYL-1401H as it compares to U.S.-licensed Neulasta. Mylan justified similarity of the chemical linkage by indicating that the chemical reaction for the PEG linkage in MYL-1401H is based on the same chemistry used for U.S.-licensed Neulasta as per publicly available information.¹² Additional data (not shown) for FT-IR analysis of the PEG raw material compared to overlaid FT-IR spectra of MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta also supports the similarity of the PEG moiety in all three products. The sponsor also provided an assessment of the effect of a CNBr-Trypsin double digest on the PEG linkage in MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta (see the review of these data in the section below), which showed similarity between the PEG-Met1 fragment of MYL-1401H and U.S.-licensed Neulasta. The reviewer concludes that sponsor has provided enough data and information to support a similar PEG linker between MYL-1401H and U.S.-licensed Neulasta.

N-Terminal Pegylation Confirmation – CNBr + Trypsin Double Digestion MALDI-TOF-MS and ESI-MS

The sponsor utilized an additional cyanogen bromide (CNBr) plus trypsin double digest method to further confirm N-terminal PEGylation. The PEG-Fragment 1 peptides generated from Glu-C and trypsin digests both contain a lysine residue. Although the probability of pegylation at K17 is low due to the PEGylation conditions favoring reaction at methionine, the possibility of PEG ligation at lysine cannot be excluded. CNBr treatment is expected to cleave the M1 peptide at the C-terminal side of the N-terminal Met1 residue, allowing the resulting PEG-Met fragment to be analyzed by MALDI-TOF-MS. Trypsin generates smaller fragments that are more amenable to mass spectrometry (Thiede et al., 2005, Methods **35**:237–247) and are analyzed by LC-MS-MS (Figure 34B). Table 33 show the summary mean molecular weight values of the PEG-Met₁ fragment.

¹¹ Johnson et. al., 1997. Curr. Biol. **22**:939-950

¹² Turecek et al., 2106, J. Pharm. Sci., **105**:460-475

Figure 34 B. Representative CID MS-MS Spectra of N-terminal fragment T₂PLGPASSLPQSFLK₁₇ derived after CNBr-Trypsin digestion of MYL-1401H and U.S.-licensed Neulasta.

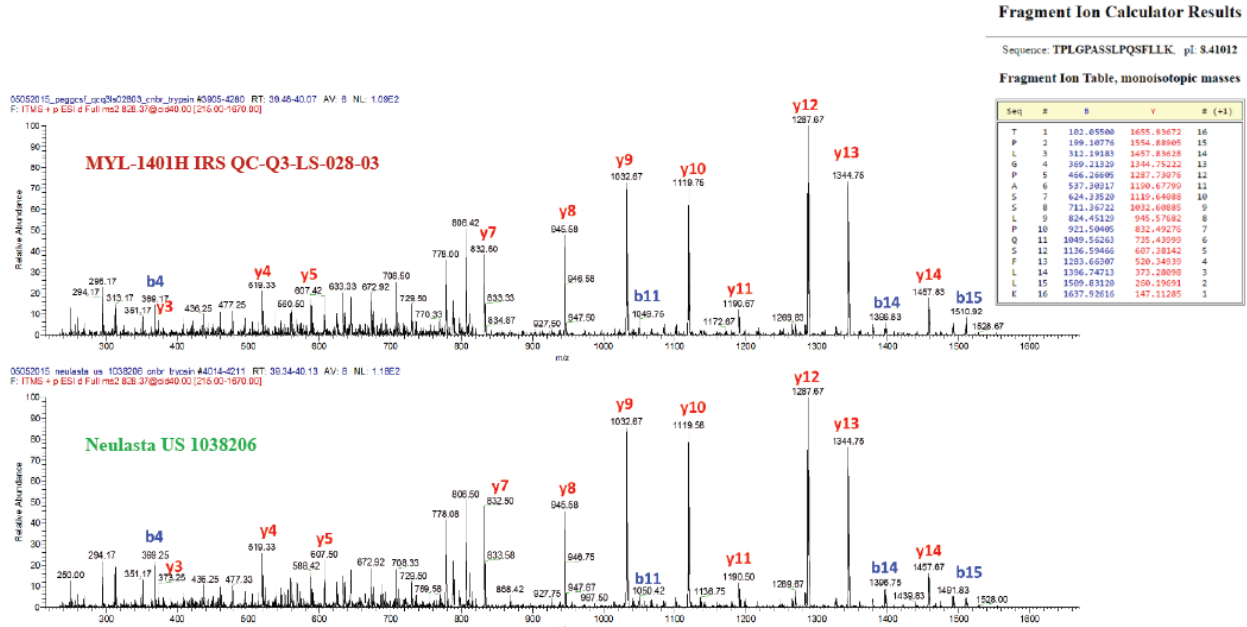


Table 33. Average Molecular Mass and Polydispersity Index for the PEG-Met (N-terminal) Fragment determined by MALDI-TOF of MYL-1401H-1401H and U.S.-licensed Neulasta

	# Lots Analyzed	Age of Lot (Months)		Avg Mol Wt (Da)	Polydispersity Index
MYL-1401H	6	min 5	Mean	21057	1.002
		max 6	Stand Dev	64	0.000
U.S.-Licensed Neulasta	6	min 12	Mean	20868	1.002
		max 18	Stand Dev	81	0.001
E.U.-Approved Neulasta	NA	min NA	Mean	NA	NA
		max NA	Stand Dev	NA	NA

Note: The table was prepared by the CMC reviewer

Reviewer Comments: Figure 34B shows the MS/MS spectrum from collision induced dissociation (CID) of the N-terminal peptide fragment of amino residues 2-17 that resulted from the CNBr-trypsin digestion of MYL-1401H and U.S.-licensed Neulasta. The image shows the representative spectra and a table of y-ions (C-terminal fragment ions cleaved at the peptide bond) and b-ions (N-terminal fragment ions cleaved at the peptide bond) resulting from CID (Steen & Mann, 2004, Nat. Rev. Mol. Cell Biol., 5:699-711). The results show spectra that compare closely and include the same y- and b-ion m/z values between MYL-1401H and U.S.-licensed Neulasta. The calculated masses of the y- and b-ions for both products also support the conclusion that K₁₇ is not PEGylated (e.g. y₁₂ measured m/z = 1287.67, theoretical m/z = 1287.52, using the ExPASy Bioinformatics Resource Portal ProtParam tool).

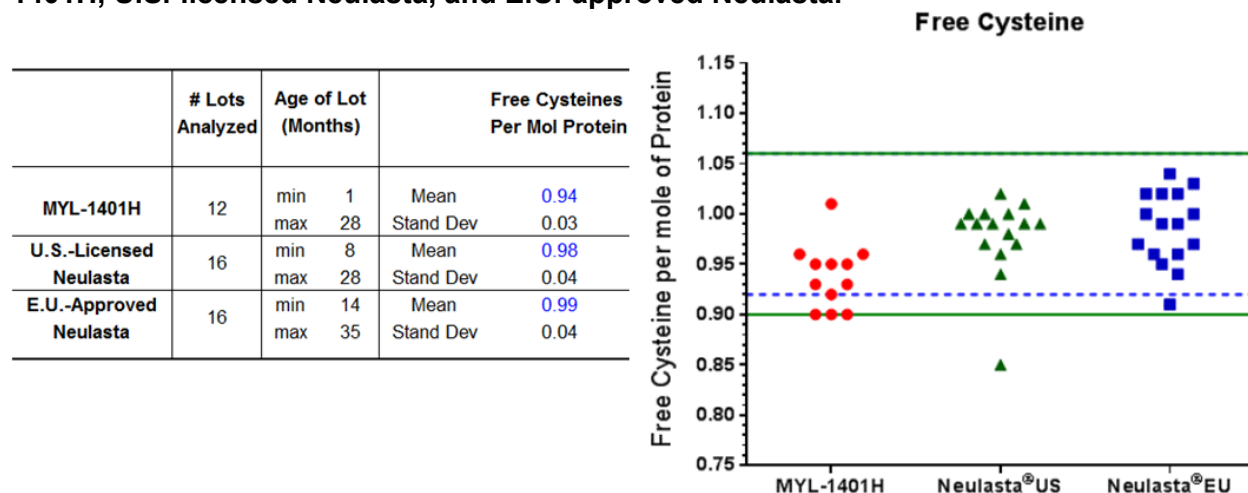
The data in Table 33 show that although the average molecular weight for the PEG-Met fragment is slightly higher by ~189 Da for MYL-1401H compared to U.S.-licensed Neulasta, the calculated mass values for both products are consistent with the expected molecular weight of PEG + methionine (20,149 ^{(b) (4)}). The dispersity index also indicates the presence of only one PEG moiety with this fragment (Table 33).

Overall, the results from CNBr-trypsin digestion mass spectrometry analysis support that MYL-1401H is similar to U.S.-licensed Neulasta with respect to the location of the PEG moiety and the presence of a single pegylation site at M1.

Free Cysteine Analysis and Disulfide Analysis

The presence of free cysteine was measured using Elman's Reagent DTNB (5,5-dithio-bis-(2-nitrobenzoic acid) in a colorimetric assay used to quantify the molar ratio of thiol functional groups in a sample.¹³ Figure 27 shows the results of free cysteine analysis of MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta.

Figure 27. Tabulated summary and scatter plot results of free cysteine analysis of MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta.



¹³ Elman, 1959, Arch. Biochem. Biophys., 82:70-77

Solid green line: U.S.-licensed Neulasta quality range

Dashed blue line: E.U.-approved Neulasta quality range

Note: The table was prepared and the figure adapted by the CMC reviewer

Reviewer Comment: *The tabulated results show that the number of free cysteines is similar between MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta. The scatter plot of the individual lot results shows that MYL-1401H and E.U.-approved Neulasta are both within the quality range of U.S.-licensed Neulasta. However, three of twelve lots of MYL-1401H are outside the quality range of E.U.-approved Neulasta by a margin of 0.02 cysteines per mol of protein. Our current expectation is that 90% of the proposed biosimilar product lots tested fall within the quality range for a given test. The free cysteine analysis by DTNB treatment show 100% of MYL-1401H lots within the U.S.-licensed Neulasta quality range and 75% of MYL-1401H lots within the E.U.-approved Neulasta quality range. However, considering the scale of the data, the low accuracy of the colorimetric assay, and the results of the Glu-C and trypsin digest peptide mapping analysis (Tables 30 and 31), the results of the free cysteine analysis by DTNB treatment have less impact. The peptide mapping results showed m/z values for enzyme digest peptide fragments consistent with the presence of two disulfide bonds at Cys37 and Cys43 and Cys65 and Cys75, as well as a single reduced Cys18 residue for MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta. The peptide mapping results are more accurate and provide adequate support for the presence of a single cysteine residue in the three products.*

Primary Sequence Isoelectric Point - cIEF

Capillary isoelectric focusing (cIEF) was used to determine the isoelectric point (pI) for MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta. The pI of a protein is governed by the amino acid composition of the primary sequence due to surface charge and is altered by modifications like PEGylation. Mobilization and focusing are achieved by applying a current across a capillary containing the proteins of interest and protein pI standards mixed with anolyte, catholyte, and mobilizing solutions. The results were obtained by spiking samples with reference standards of known pI values (10.0, 9.5, and 4.1) and correlating the standard and sample peak mobilization times with a linear calibration plot (Figures 46 and 47).

Figure 46: Representative overlay of MYL-1401H, US-licensed Neulasta and EU-approved Neulasta

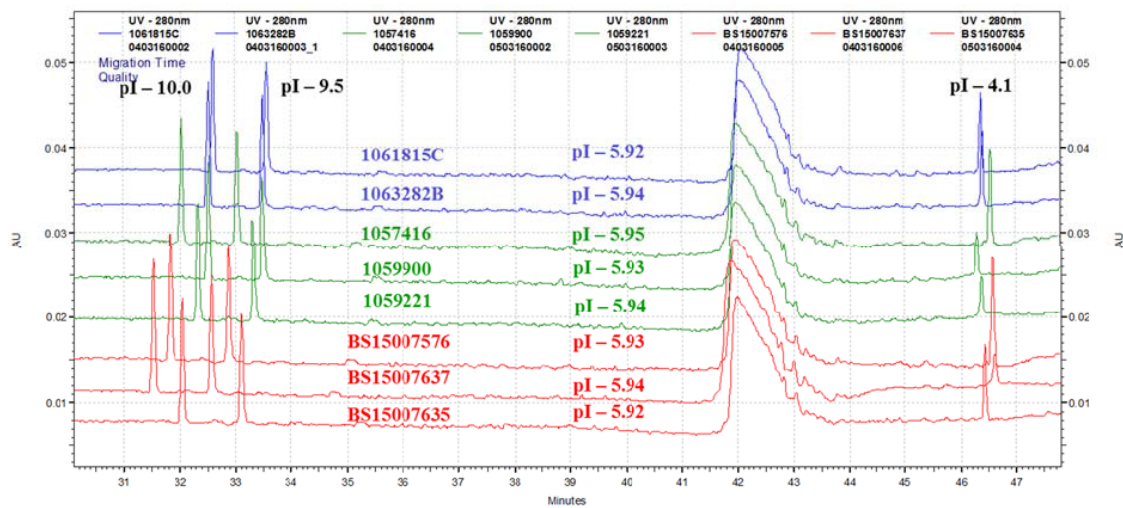
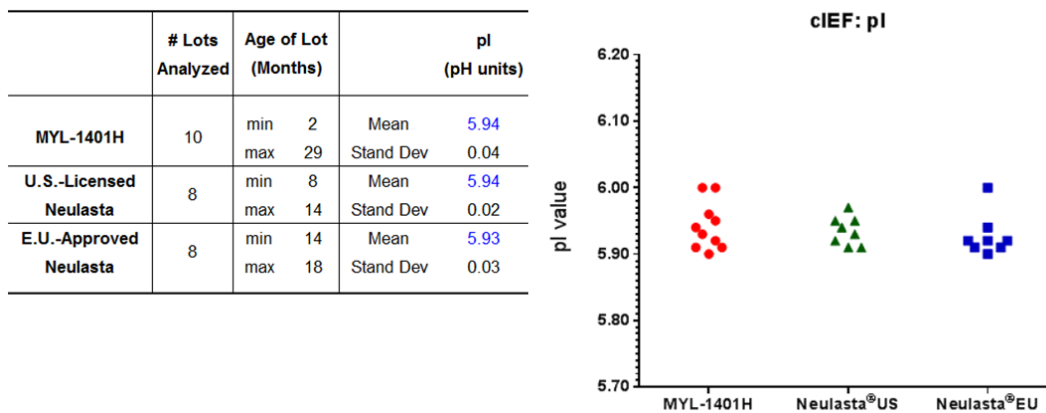


Figure 47. Tabulated summary and scatter plot results of pI values for MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta.



Note: The table was prepared and the figure adapted by the CMC reviewer

Reviewer Comment: Figure 46 shows overlaid electropherograms for MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta. Peaks for pI standards of 4.1, 9.5 10.0 are visible for each trace along with a major peak at a mobilization time of 41.5 – 42 minutes for the

three products. The reported tabulated mean pI values and scatter plot results shown in Figure 47 indicate that MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta have similar isoelectric points (pI values).

The sponsor indicated that a linear pH gradient was used for their calibration plot but did not provide additional supporting information initially. In addition, the sponsor did not explain the differences in the peak mobilization times shown in Figure 46 for the reference standard pI markers that were spiked into sample lots. An IR was sent to the sponsor asking them to provide a method description that clearly describes the conditions and standards used in the cIEF analysis for each product, an explanation for the different mobilization times, and a qualification report for the cIEF method. Mylan's response to the IR indicated that anodic and cathodic drift can result in different mobilization times from one run to the next. This is supported by industry application publications.^{14,15} Mylan also provided a method description and qualification report confirming a linear correlation between the pI standards and their resulting mobilization times. The pI of the three products is then calculated relative to the pI standards plot generated from the same run. Therefore, the sponsor's response is acceptable. The cIEF results indicate that the pI values are similar between MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta, which further supports a conclusion that the primary sequences of the three products are the same.

HIGHER ORDER STRUCTURE

The higher order structure of MYL-1401H was compared to U.S.-Licensed Neulasta and E.U.-approved Neulasta using far-UV-CD, near-UV-CD, FTIR, intrinsic fluorescence, extrinsic fluorescence, DSC, and 1D-¹H-NMR.

Secondary Structure Far-UV-CD

Far ultra violet (190 nm – 250 nm) circular dichroism spectroscopy (Far-UV-CD) depends upon the differential absorbance of left hand and right hand circularly polarized light. This method yields information for the prediction of secondary structure in proteins, where α -helices exhibit absorbance minima at 208 nm and 222 nm, which correspond to the $n-\pi^*$ and $\pi-\pi^*$ electronic transitions of the peptide bond, where β -sheet structure exhibits absorbance minima at 218 nm and random coil content absorbing at 195 nm.¹⁶

¹⁴ Application Guide, cIEF, PA800 plus Pharmaceutical Analysis System, Beckman, 2014

¹⁵ Principles and Applications of cIEF, Primer, Agilent, 2014

¹⁶ Greenfield, 2006, Nat Protoc, 1:2876-2890

Figure 24: Representative Overlaid of Far UV CD Spectra Profiles of MYL-1401H, US-licensed Neulasta and EU-approved Neulasta

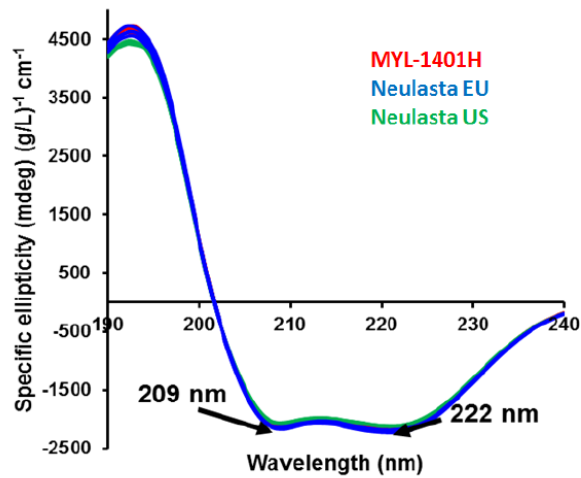


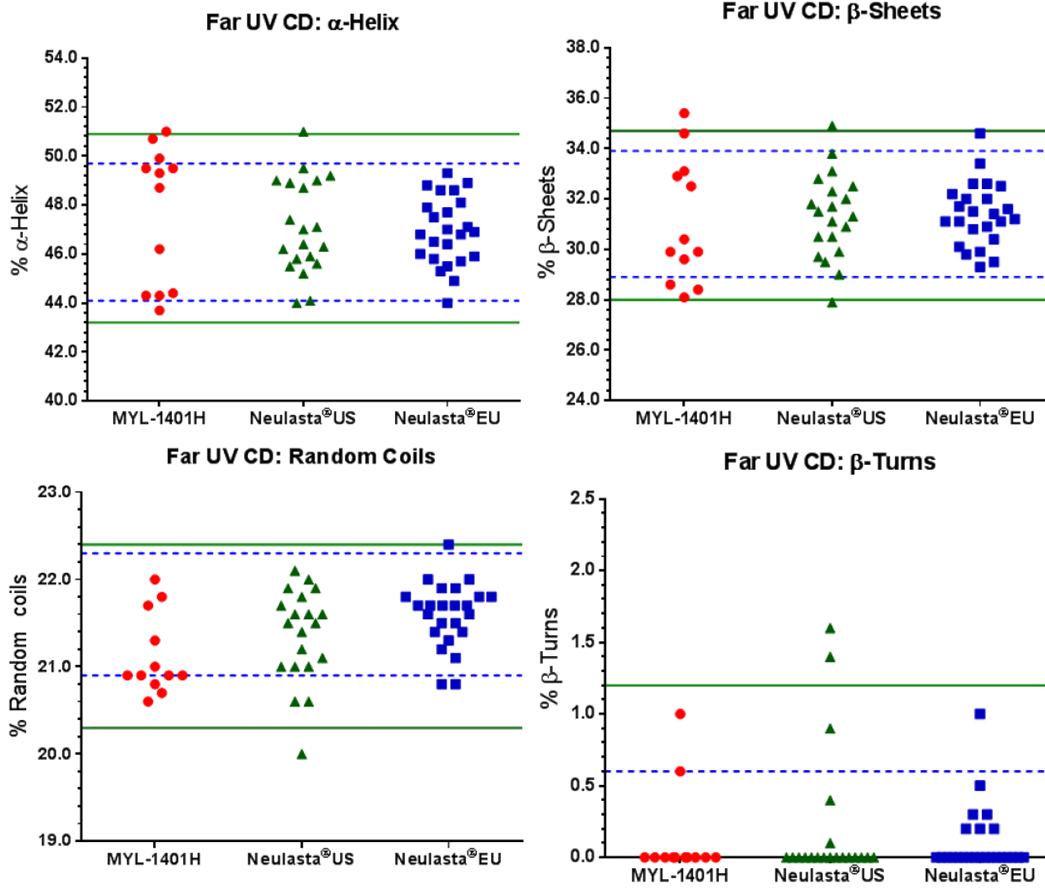
Table 24. Secondary Structure Predictions for MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta from far-UV-CD*

	# Lots Analyzed	Age of Lot (Months)			alpha-Helix (%)	Beta-Sheet (%)	Beta-Turn (%)	Random Coils (%)
MYL-1401H	12	min	2	Mean	47.6	31.1	0.1	21.1
		max	29	Stand Dev	2.8	2.5	0.3	0.5
U.S.-Licensed Neulasta	20	min	8	Mean	47.1	31.3	0.2	21.4
		max	31	Stand Dev	1.9	1.7	0.5	0.5
E.U.-Approved Neulasta	24	min	14	Mean	46.9	31.4	0.1	21.6
		max	35	Stand Dev	1.4	1.3	0.2	0.4

*25 lots of E.U.-Approved Neulasta were initially analyzed but one lot was not included in the data since the sponsor believed it was an outlier.

Note: The table was prepared by the CMC reviewer

Figure 25. Scatter Plots of Predicted Secondary Structure Content from far-UV-CD for MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta



Solid green line: U.S.-licensed Neulasta quality range
 Dashed blue line: E.U.-approved Neulasta quality range
 Note: The plots were combined into a single figure by the CMC reviewer

Table 24B. Summary of Lots Within the Quality Range for far-UV-CD

	α-Helix	β-Sheet	Random Coil	β-Turn
MYL-1401H vs U.S.-licensed Neulasta	11/12	11/12	12/12	12/12
MYL-1401H vs E.U.-approved Neulasta	8/12	7/12	9/12	11/12
E.U.-approved Neulasta vs U.S.-licensed Neulasta	24/24	24/24	24/24	24/24

Note: The table was prepared by the CMC reviewer

Reviewer Comment: Figure 24 shows that the far-UV-CD spectra of MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta overlay closely and are visually similar. However, the scatter plot of deconvoluted data in Figure 25 and the tabulated data in Table 24B show 1-5 lots of MYL-1401H falling outside the quality ranges established for the secondary structure elements when compared to E.U.-approved Neulasta. One lot of MYL-1401H was outside the U.S.-licensed Neulasta quality range for α -helix and β -sheet content, but this is acceptable since $>90\%$ data are within the quality range. Although the spectra and deconvoluted data support that MYL-1401H is similar to U.S.-licensed Neulasta, too many MYL-1401H lots are outside the quality range of E.U.-approved Neulasta.

The sponsor indicated that secondary structure content was obtained by deconvolution of the far-UV-CD data based on referenced literature.¹⁷ However, this publication describes deconvolution of secondary structure from FTIR data so the accuracy of the deconvolution for CD is questionable. In addition, the calculated quality range for E.U.-approved Neulasta is tighter than U.S.-licensed Neulasta for all secondary structure elements, with even some E.U.-approved Neulasta data points outside its own quality range. An IR was sent to the sponsor asking them address this issue. Mylan's IR response indicated that if they use a quality range of $\pm 3\sigma$ rather than $\pm 2\sigma$, the results are within the quality range. However, this conflicts with their similarity acceptance criteria.

The reviewer recommends that the assessment of far-UV-CD should be based on measured absorbance values^{18,19} at 208 nm and 222 nm rather than on the results from a deconvolution algorithm because the measured absorbance values would reduce bias introduced by the deconvolution algorithm.

A follow-up IR was sent to the sponsor asking them to assess CD data based on the absorbance values ($\Theta_{222}/\Theta_{208}$). Mylan's response to the IR included the analysis of the ratio of absorbance values for all lots of MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta initially tested by far-UV-CD for analytical similarity. The results show that all lots of MYL-1401H fall within the quality range of $\pm 2\sigma$ for U.S.-licensed Neulasta and E.U.-approved Neulasta (Tables 1 and 2, Figure 2). The results support that MYL-1401H is similar to U.S.-licensed Neulasta secondary structure by far-UV-CD analysis and the results support an analytical bridge between MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta.

¹⁷ Yang et al., 2015, Nat. Protocol., **10**:382-396

¹⁸ Cooper and Woody, 1990, Biopolymers, **30**:657-676

¹⁹ Greenfield, 2004, Methods Enzymol., **383**:282-317

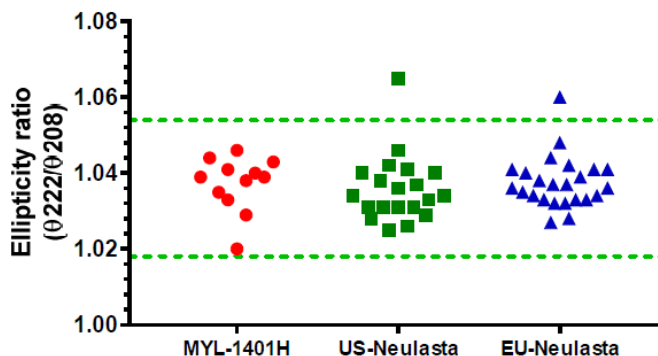
Table 1: Quality Range for Ø222/Ø208 Ratio of US-Neulasta and EU-Neulasta

Product	No. of Lots Analysed	Ø222 nm/ Ø208 nm		
		Mean	Standard Deviation	Mean ± 2 SD Range
US-licensed Neulasta	20	1.036	0.009	1.018 - 1.054
EU-approved Neulasta	24	1.038	0.007	1.024 - 1.051

Table 2: Range Observed in MYL-1401H for Ø222/Ø208 Ratio

Product	No. of Lots Analysed	Ø222 nm/ Ø208 nm		
		Mean	Standard Deviation	Range of observed ratio
MYL-1401H	12	1.037	0.007	1.020 - 1.046

Figure 2: Scatter Plot for MYL-1401H, US-Neulasta and EU-Neulasta of Ø222 nm/Ø208 nm



Note: The green dashed lines denote the mean ± 2 SD range for US-Neulasta

Secondary Structure - ATR-FTIR

Attenuated Total Reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) was used to analyze the secondary structure of MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta by assessing the vibrational energies (spectral bands) of protein functional groups. Amide I bands (1700-1600 cm⁻¹) from C=O stretching vibration coupled with in-phase bending of the N-H bond of the peptide bond are informative for the protein backbone conformation (Yang et al. 2015). Amide II bands (1510 and 1580 cm⁻¹) from in-plane N-H bending and C-N stretching vibration provide additional information. Representative overlay of IR spectra of US-licensed Neulasta, EU-approved Neulasta, and MYL-1401H is shown below (Figure 37) with tabulated summary data (Table 35) and scatter plot results for individual lots (Figure 38).

Figure 37: Overlaid IR Spectra Profiles of MYL-1401H, US-licensed Neulasta and EU-approved Neulasta

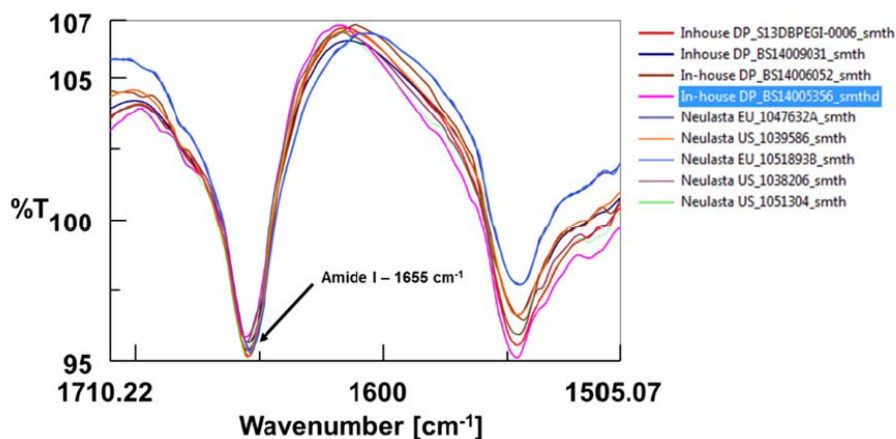
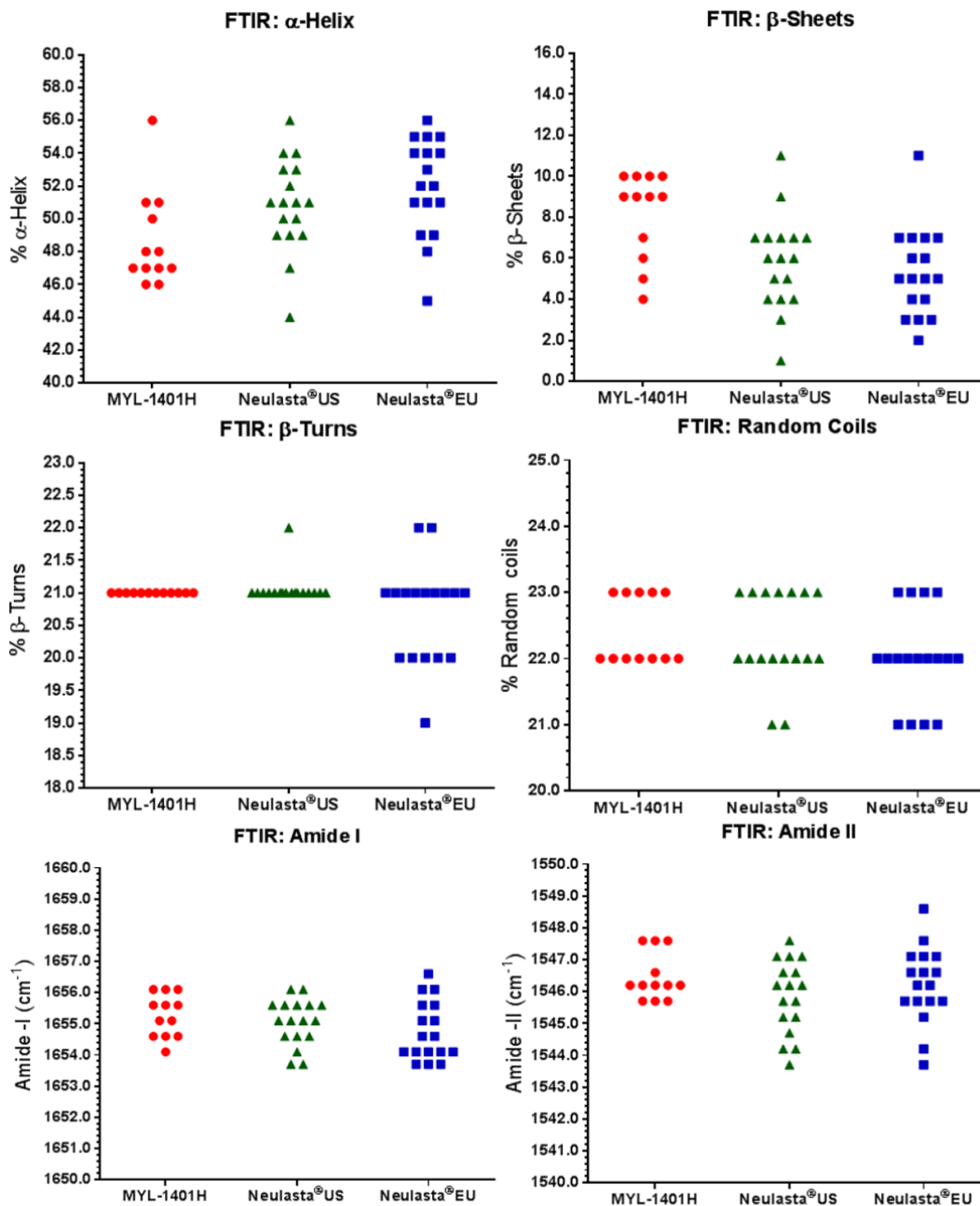


Table 35. Summary results of ATR-FTIR analysis of MYL-1401H, U.S.-licensed Neulasta, E.U.-approved Neulasta

	# Lots Analyzed	Age of Lot (Months)		α -Helix (%)	β -Sheet (%)	β -Turn (%)	Random Coils (%)	Amide I (cm^{-1})	Amide II (cm^{-1})	
MYL-1401H	12	min	2	Mean	48.7	8.2	21.0	22.4	1655.3	1546.4
		max	29	Stand Dev	3.0	2.1	0.0	0.5	0.7	0.8
U.S.-Licensed Neulasta	17	min	8	Mean	50.8	5.8	21.1	22.3	1655.1	1545.8
		max	30	Stand Dev	2.9	2.3	0.2	0.7	0.7	1.2
E.U.-Approved Neulasta	17	min	14	Mean	52.0	5.3	20.7	22.0	1654.8	1546.2
		max	35	Stand Dev	3.0	2.2	0.8	0.7	0.9	1.2

Note: The table was prepared by the CMC reviewer

Figure 38. Scatter Plots of Predicted Secondary Structure Content from ATR-FTIR for MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta



Note: The plots were combined into a single figure by the CMC reviewer

Reviewer Comment: The FT-IR spectra shown in Figure 37 overlay closely in the amide I region in both frequency and intensity but the amide II region exhibits much more variable intensity for MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta. The tabulated summary data in Table 35 and scatter plot data show agreement in the amide I and amide II frequencies for all three products but the calculated secondary structure assigns slightly less α-helix and slightly more β-sheet in MYL-1401H compared to U.S.-licensed Neulasta and E.U.-approved Neulasta. The determination of protein secondary structure from an algorithm based on spectroscopic data

includes some uncertainty. This uncertainty is addressed by other orthogonal methods for higher order structure as well as receptor binding and potency data (see below). Overall, the FT-IR data show a similar spread of vibration band frequencies and secondary structure results from lot to lot (Figure 38) for MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta.

Intrinsic and Extrinsic Fluorescence

Intrinsic fluorescence behavior of a protein derives from tryptophan, tyrosine, and phenylalanine residues that are present in a protein sequence of interest via excitation at 278 nm and scanning for emission from 300 – 400 nm. Extrinsic fluorescence relies upon the addition and non-covalent interaction of an organic fluorescent dye or molecular probe, such as 8-Anilidonaphthalene-1-sulfonic acid (ANS), with a protein of interest. For extrinsic fluorescence with ANS, excitation is initiated at 388 nm and emission is scanned from 400 nm – 660nm. Both intrinsic and extrinsic fluorescence give information about tertiary structure based on the packing or exposure of aromatic side chains. Both approaches were used to compare MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta. The results are shown in Figures 39 and 40 and Table 39.

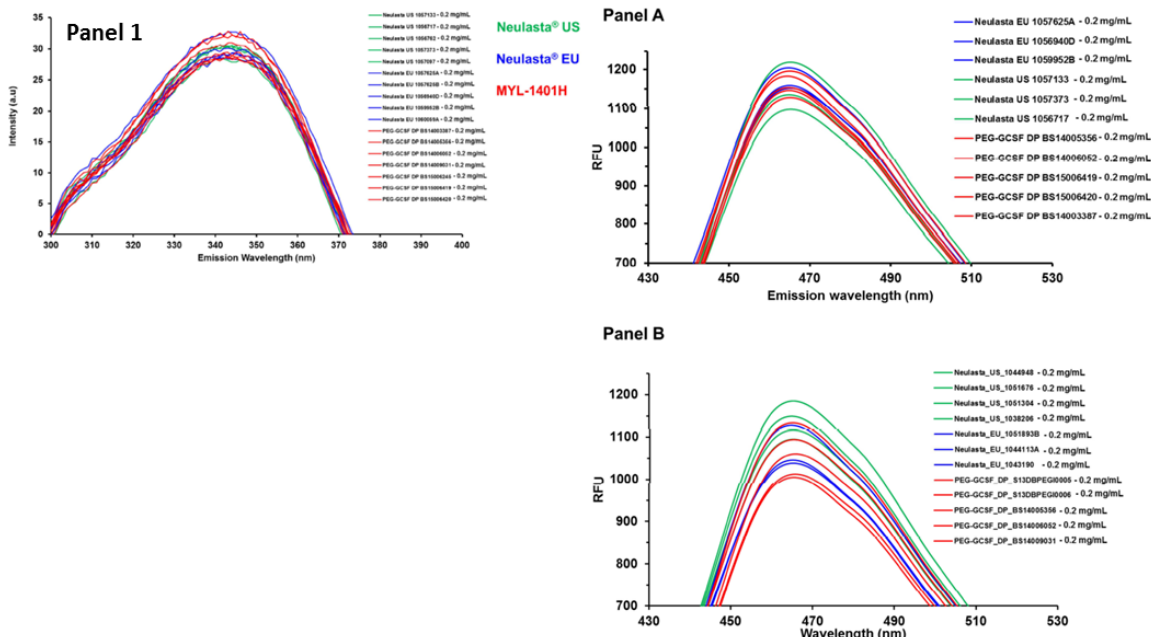


Table 39. Summary Results of Intrinsic and Extrinsic Fluorescence Analysis of MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta

	# Lots Analyzed	Age of Lot (Months)		Intrinsic λ-Max (nm)	Extrinsic λ-Max (nm)
MYL-1401H	12	min 2	Mean	342.7	466.2
		max 29	Stand Dev	1.61	0.73
U.S.-Licensed Neulasta	16	min 8	Mean	343.0	466.1
		max 28	Stand Dev	1.31	0.68
E.U.-Approved Neulasta	16	min 14	Mean	343.2	466.1
		max 35	Stand Dev	0.91	0.69

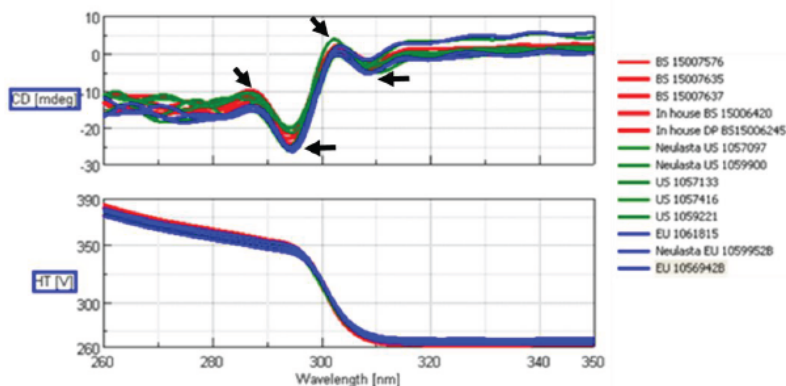
Note: The table was prepared the figure was adapted by the CMC reviewer

Reviewer Comment: All samples represented in Figures 39 and 40 were tested at 0.2 mg/mL. These figures shows intrinsic and extrinsic fluorescence spectra for MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta that overlay closely. The extrinsic fluorescence spectra show a slight absorbance or shoulder near 490nm that is clearly present in all three products. The sponsor explained that the 1-anilino-8-naphthalene sulfonate (ANS) fluorescent dye binds protein by both hydrophobic (460 nm) and ion pairing (480-490 nm) interactions, where, subtle absorbance differences in these binding modes results in the shoulder observed. Table 39 also shows similar intrinsic and extrinsic maximum absorbance wavelengths for MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta. Overall, the extrinsic fluorescence data support a similar tertiary structure for all three products.

Tertiary Structure - Near-UV-CD

Near-UV-CD spectra (260 nm – 350 nm) provide information about protein tertiary structure through the effects of changes in aromatic side chains on the differential absorbance between left handed and right handed circularly polarized light. When performed correctly, near-UV-CD data provides supporting tertiary structure information to complement fluorescence and NMR data.

Figure 41. Near-UV-CD Spectra for MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta



Reviewer Comments: The sponsor submitted the spectrum in panel B as representative of deconvoluted (by Jasco J-815 instrument software) data. However, the reviewer found no literature to support the deconvolution of Near-UV-CD for tertiary structure characterization as described by the sponsor, but instead obtained expert opinion indicating that there is no analogous deconvolution of Near-UV-CD data into structural components as is the practice for Far-UV-CD.²⁰ The results in Figure 41 are also inconsistent with published Near-UV-CD spectra of filgrastim (unpegylated). Since the PEG moiety is not optically active, it does not contribute to the CD spectrum. The reviewer concludes that Near-UV-CD analysis was not performed correctly so these data are not useful for assessment of similarity. However, other tertiary structure data from fluorescence and 1D-¹H-NMR analyses provide sufficient evidence to support that the tertiary structure between MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta are similar.

²⁰ Li et al., 2011, J. Pharm. Sci., 100:4642-4654

Thermostability – DSC

Differential scanning calorimetry (DSC) allows for a physico-chemical assessment of protein structure by measuring protein unfolding with temperature. A common parameter obtained by DSC is the temperature half-way through the unfolding transition, known as the temperature mid-point or T_m. At the T_m, approximately half the analyzed protein molecules are unfolded. Changes in T_m values or thermodynamic parameters indicate changes in the energetics of protein structure. DSC was used to compare the thermal stability of MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta.

Figure 42. Overlaid DSC Profiles for MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta

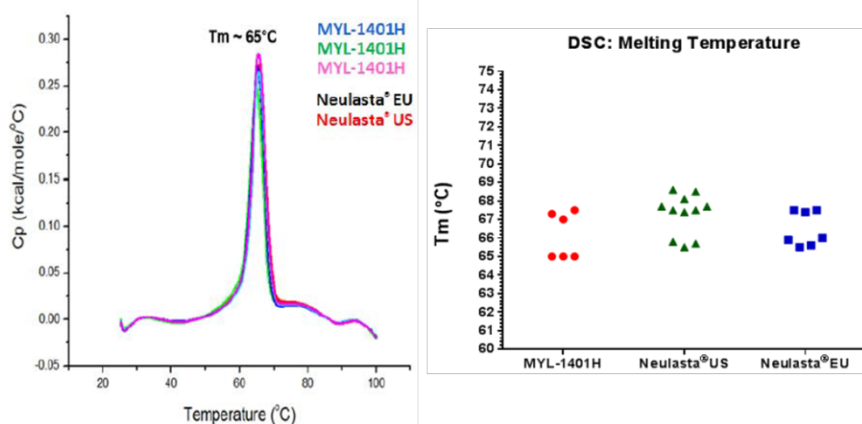


Table 38. Summary T_m Values for MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta Measured by DSC

	# Lots Analyzed	Age of Lot (Months)		T _m (°C)	
MYL-1401H	6	min	3	Mean	66.1
		max	4	Stand Dev	1.3
U.S.-Licensed Neulasta	11	min	15	Mean	67.3
		max	32	Stand Dev	1.1
E.U.-Approved Neulasta	7	min	11	Mean	66.5
		max	31	Stand Dev	0.9

Note: The table was prepared and the figure adapted by the CMC reviewer

Reviewer Comment: The overlaid DSC melting curves, the scatter plot data for individual lot results, and the tabulated summary mean T_m values support that the thermal melting profiles of MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta are similar, despite the fact that lots of U.S.-licensed Neulasta and E.U.-approved Neulasta analyzed were several months older than the MYL-1401H lots.

Secondary and Tertiary Structure - 1D-[¹H]-NMR

One dimensional nuclear magnetic resonance (1D-[¹H]-NMR) was used to characterize the protein structure of MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta. For PEGylated proteins, 2D NMR correlation methods are complicated by the presence of PEG due to line broadening from the higher molecular weight and confounding of the aliphatic region of the NMR spectrum. 1D-NMR provides an orthogonal profile of atomic resolution for proteins in solution.²¹ Figure 44 shows a representative overlay of the 1D-[¹H]-NMR spectrum of MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta lots. Figure 45 shows the NMR spectrum for methoxy-PEG (m-PEG) and depicts the chemical shift of the PEG-aldehyde end group with a methoxy signal.

Figure 44. Representative full 1D-[¹H]-NMR spectra for MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta

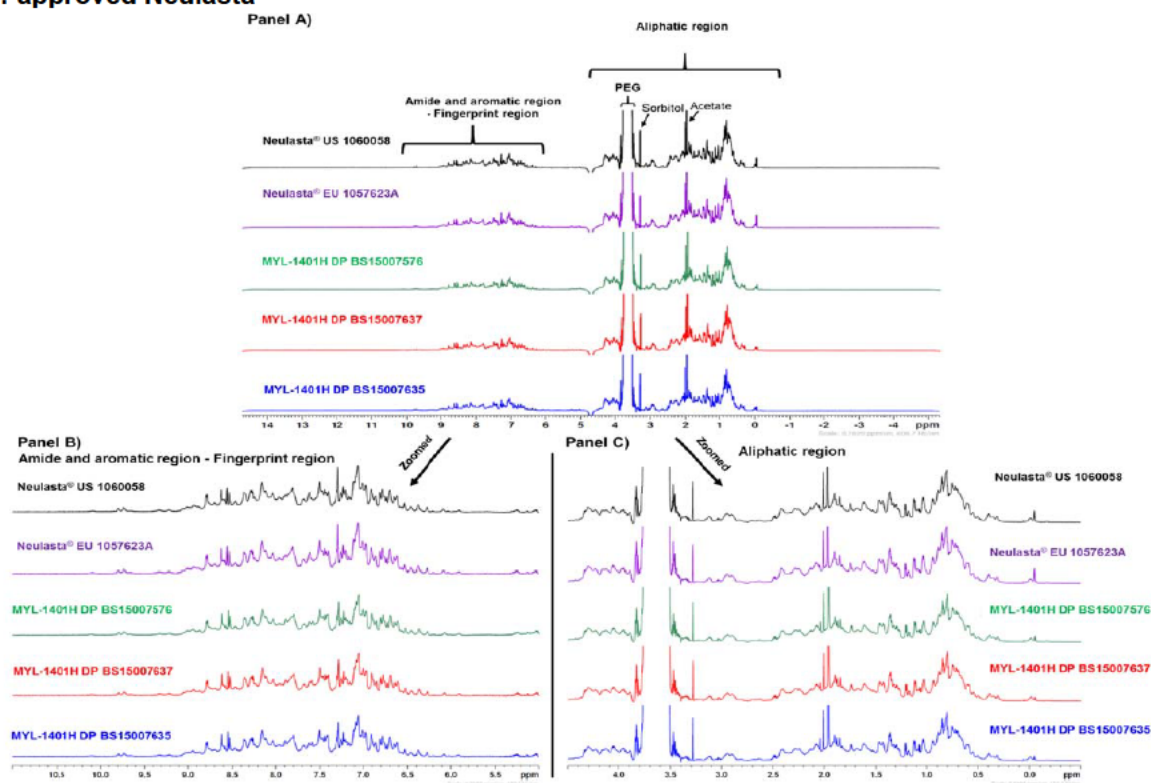
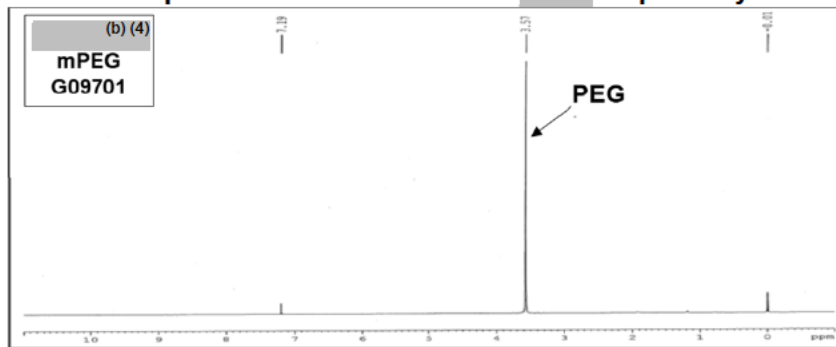


Figure 45: Representative Spectrum for m-PEG G09701 (b) (4) acquired by 400 MHz NMR



²¹ Poppe, L., et al., *Anal. Chem.* 2015. **87**:5539-5545

Reviewer Comments: Figure 44 shows closely overlaid 1D-¹H NMR spectra for MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta with amide, aromatic, and aliphatic regions that are visually similar. The spectrum shows dispersed peaks of low intensity near 8.3 ppm and beyond 8.5 ppm, both of which indicate a folded protein.²² A strong PEG signal is also observed in the expected region 3.5 – 3.8 ppm. The result qualitatively support similar secondary and tertiary structure between MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta. Figure 45 shows a 1D ¹H NMR spectrum with a pure monomethoxy-PEG (mPEG) signal for the raw material from the supplier (b) (4) with no indication of degradation products.

IMPURITIES – PRODUCT AND PROCESS-RELATED

Hydrophobic Variants – RP-HPLC

Reversed-Phase Chromatography (RP-HPLC) separates protein components on the basis of hydrophobicity, where protein sequences with greater hydrophobicity are retained on the column longer. RP-HPLC was used to separate hydrophobic variants in MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta, including sequences with chemically modified amino acids (e.g. oxidation or deamidation) and size variants. The identities of the variants were determined by mass spectrometry. Figure 21 shows an overlay of representative RP-HPLC chromatograms for all three products. Table 22 summarizes the levels of the variants identified in each product based on the individual results of all analyzed lots (Figure 22).

Figure 21: Representative RP-HPLC chromatograms overlay of MYL-1401H, US-Licensed NEULASTA and EU-approved NEULASTA

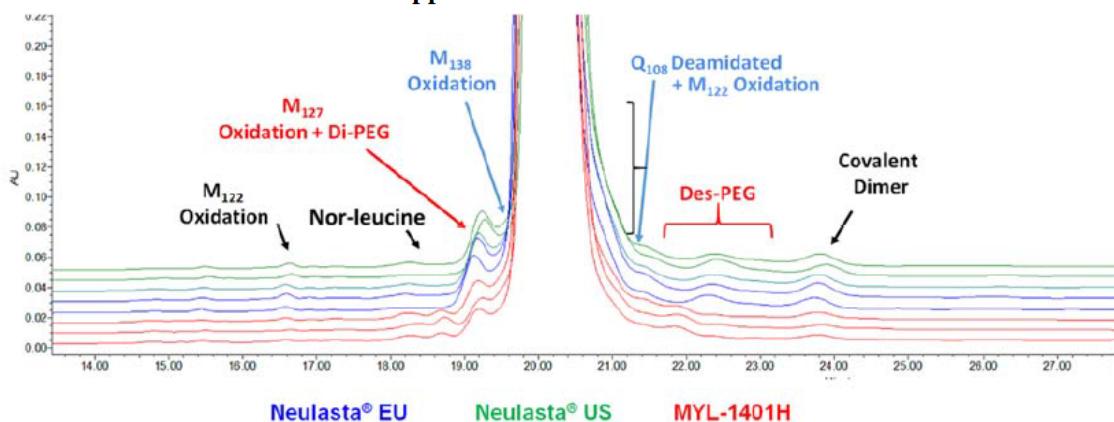


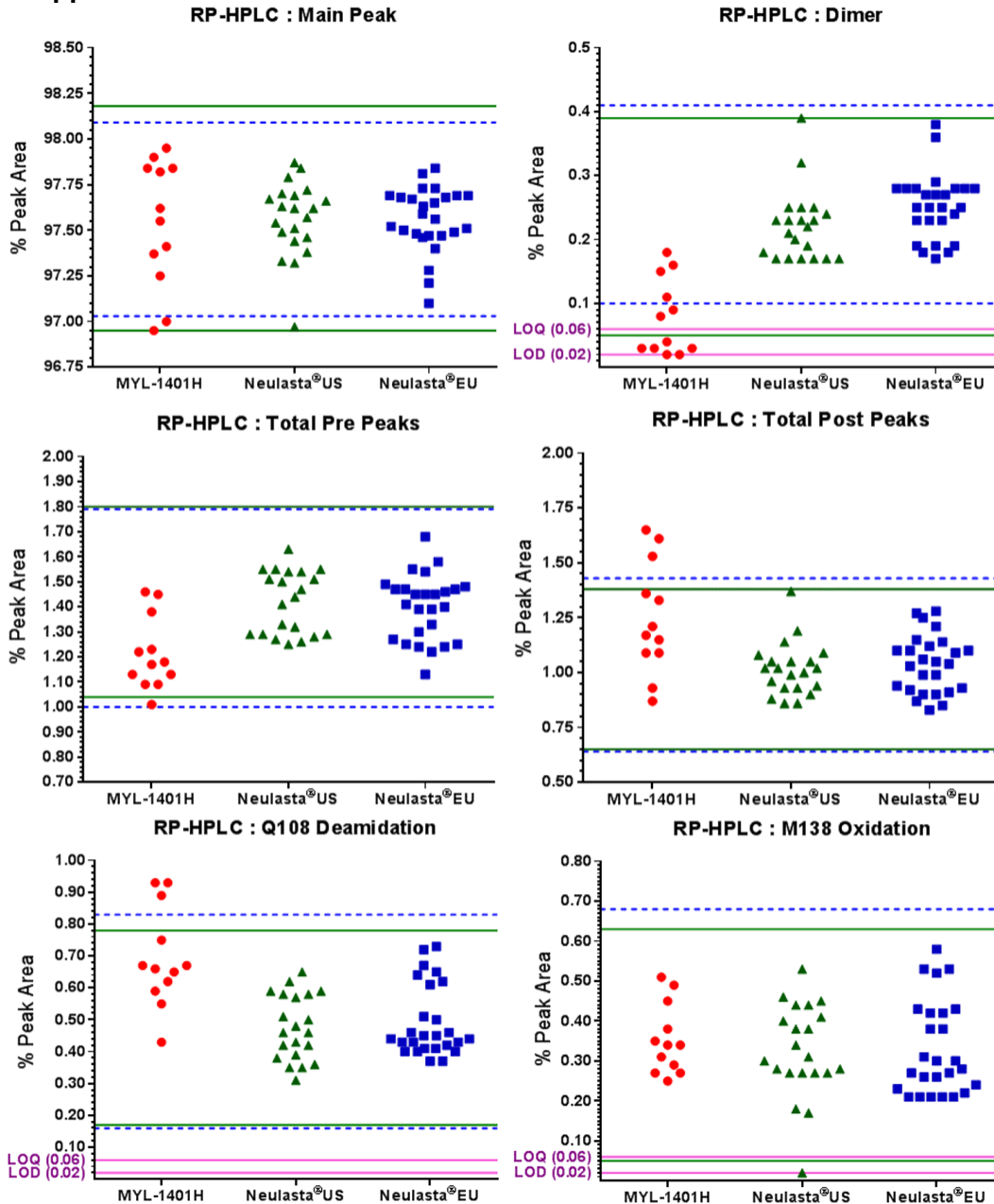
Table 22. Summary Comparison of Hydrophobic Variants by RP-HPLC

	# Lots Analyzed	Age of Lot (Months)		% Q108 Deamidated	% M138 Oxidized	% Dimer	% Des-PEG-G-CSF	% Total (Pre-Peaks)	% Total (Post-Peaks)	% Main Peak
MYL-1401H	12	min 2	Mean	0.70	0.35	0.08	0.43	1.21	1.25	97.54
		max 29	Stand Dev	0.15	0.09	0.06	0.16	0.15	0.25	0.35
U.S.-Licensed Neulasta	20	min 8	Mean	0.48	0.35	0.22	0.27	1.42	1.02	97.56
		max 28	Stand Dev	0.10	0.09	0.06	0.10	0.13	0.12	0.21
E.U.-Approved Neulasta	25	min 14	Mean	0.50	0.33	0.25	0.23	1.41	1.04	97.55
		max 35	Stand Dev	0.11	0.12	0.05	0.09	0.12	0.13	0.18

Note: The table was prepared by the CMC reviewer

²² Rehm et al., 2002. Structure. 10:1613-1618

Figure 22. Scatter Plots of Hydrophobic Variants for MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta



Solid green line: U.S.-licensed Neulasta quality range
 Dashed blue line: E.U.-approved Neulasta quality range
 Note: The plots were combined into a single figure by the CMC reviewer

Reviewer Comment: For analytical similarity of hydrophobic variants, the sponsor assessed some attributes individually and others in combination (Table 22) because some of the species coelute. The sponsor reports the following species:

- Met138 oxidation, Di-PEG (Met1 and Lys17)+M127 oxidation, Norleucine and M122 oxidation. These species are measured as Pre-peaks 1-4.

- *Gln108 deamidation + M122 oxidation, Des-PEG-G-CSF (G-CSF with the PEG moiety removed), and covalent dimer. These species are measured as Post-peaks 1-3.*

The sponsor presented peptide mapping analyses as part of analytical similarity and in the characterization section of the submission 3.2.S.3.2 that describes the identification of these attributes.

The data summarized in Figure 22 show that the levels of the reported % peak areas for main peak, dimer, total pre-peaks, and M138 oxidation in MYL-1401H are less than or within the quality ranges for U.S.-licensed Neulasta and E.U.-approved Neulasta. This is acceptable because this difference is not expected to negatively impact product quality. However, for Q108 deamidation and total post-peaks, less than 90% of the lots are within the quality ranges of both U.S.-licensed Neulasta and E.U.-approved Neulasta. In both cases 9/12 lots are within quality range. Q108 deamidation is also included in the levels of total post-peaks, so this attribute appears to be the main variant difference in MYL-1401H.

Based on Mylan's stability data, deamidation is stability indicating. The lots selected from all three products for this analysis (Table 22) are near the same age, so the results do not appear to be stability related. Since, the sponsor did not clearly define whether Q108 deamidation is a product related substance or impurity, Q108 deamidation is considered an impurity. However, the difference in levels of Q108 deamidation between the products is less than 0.5% (Table 22) and is not expected to impact potency. This assessment is supported by comparison of potency data, which met equivalence testing (see below).

For the M122, M127, and M138 oxidation species labeled in Figure 21, % peak areas were reported in the characterization section 3.2.S.3. These peak areas are lower in MYL-1401H than in U.S.-licensed Neulasta and E.U.-approved Neulasta by visual assessment of the chromatogram in Figure 21. This is significant since oxidized variants reduce G-CSF potency.²³ As discussed below potency was found to be statistically equivalent between the three products. Norleucine species are in low abundance and visually similar in the three products. Overall, the results support that hydrophobic variants are similar between MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta.

Charge Variants - CIEX-HPLC

Cation Exchange Chromatography (CIEX-HPLC) separates protein components on the basis of surface charge where proteins with greater net positive charge are retained longer on the column. PEGylation of a protein at the N-terminal alpha amino group changes the net charge of a protein as does deamidation of glutamine residues. PEGylation at other sequence positions (e.g lysine residues) creates additional charge variants that differ in net charge. A CIEX-HPLC profile was obtained for MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta to compare charge variants (Figure 18).

²³ Skrlin et al., J. Pharmaceut. Biomed. 2010. 53:262-268.

Figure 18: Representative Overlay of Cation Exchange Chromatographic Profile of MYL-1401H, US-licensed Neulasta and EU-approved Neulasta

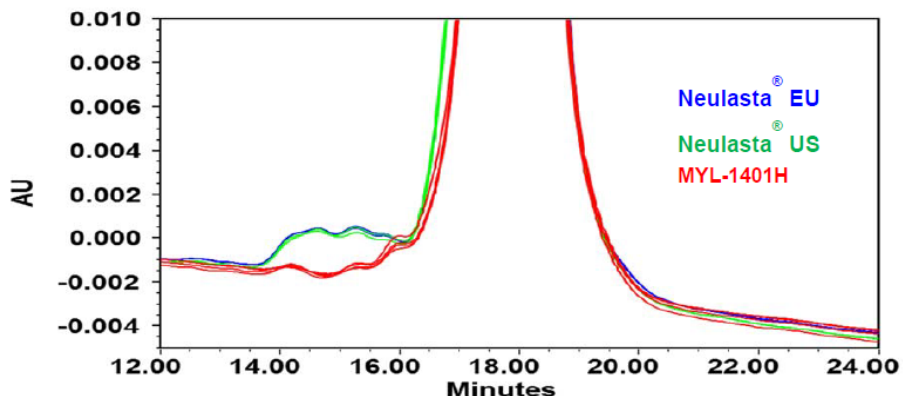
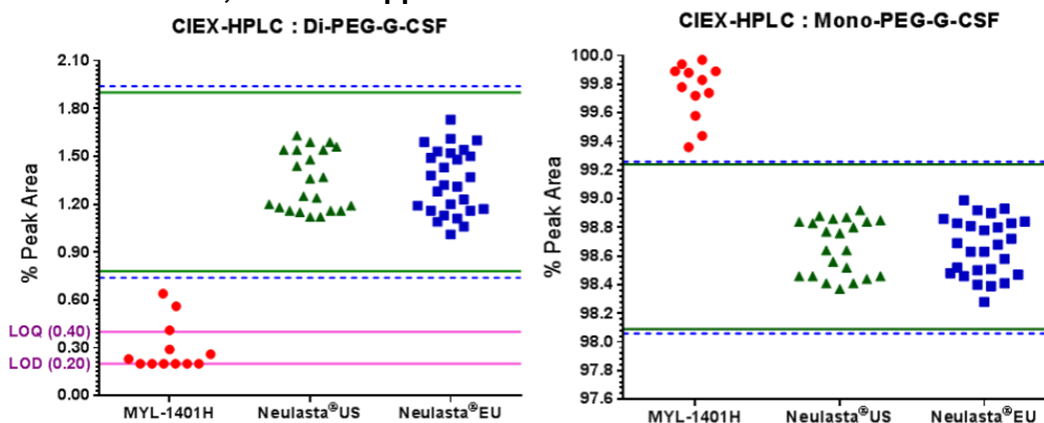


Figure 19. Scatter Plots of di-PEGylated and mono-PEGylated G-CSF for MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta



Solid green line: U.S.-licensed Neulasta quality range
Dashed blue line: E.U.-approved Neulasta quality range
Note: The plots were combined into a single figure by the CMC reviewer

Table 20. Summary of Charge Variant Levels by CIEX-HPLC for MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta

	# Lots Analyzed	Age of Lot (Months)		% Di-PEG-G-CSF		% PEG-G-CSF	
				Mean	Stand Dev	Mean	Stand Dev
MYL-1401H	12	min	2	0.36	0.18	99.75	0.20
		max	29				
U.S.-Licensed Neulasta	22	min	8	1.34	0.19	98.66	0.19
		max	33				
E.U.-Approved Neulasta	27	min	14	1.34	0.20	98.66	0.20
		max	35				

Note: The table was prepared by the CMC reviewer

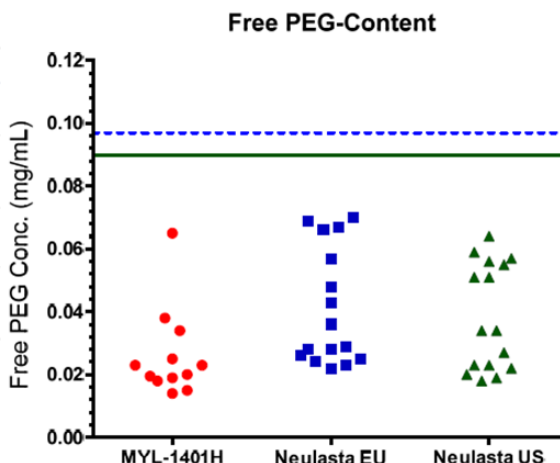
Reviewer Comments: The sponsor identifies one charge variant (% Di-PEG-G-CSF) from the CIEX-HPLC profile (Figure 18). However, the chromatographic profile shows three distinct peaks for MYL-1401H eluting in the retention time range of 14.0 – 16.5 minutes. These peaks are very low peak area, at or near the signal to noise level, and all are significantly less abundant than the main charge variant peak shown in the overlaid profiles of both U.S.-licensed Neulasta and E.U.-approved Neulasta. Mylan indicated that the three peaks are di-PEG-G-CSF with different PEG polydispersity and different charge from the main peak. Lys-C peptide mass analysis supports similar CIEX-HPLC-MS profiles of digest fragments between MYL-1401H and U.S.-licensed Neulasta and confirms the pegylation sites of the Di-pegylated species as Met₁ and Lys₁₇ (see data and comments below). All MYL-1401H lots show levels of charge variants that are lower than the quality ranges of both U.S.-licensed Neulasta and E.U.-approved Neulasta. The levels of percent mono-PEG-G-CSF (main peak) in all MYL-1401H lots are slightly higher than the quality ranges of U.S.-licensed Neulasta and E.U.-approved Neulasta (Figure 19). Although both of these data sets show MYL-1401H outside the quality ranges of U.S.-licensed Neulasta and E.U.-approved Neulasta for these attributes, lower levels of charge variants and higher purity of the MYL-1401H main peak is not expected to negatively impact product quality. Overall, the CIEX-HPLC results support that charge variants are similar between MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta.

Free PEG Content – RP-HPLC-CAD

The residual amount of methoxy-PEG (mPEG) aldehyde was monitored in MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta using RP-HPLC with Charged Aerosol Detection (CAD). CAD is a sensitive mass selective detection method based on generation and analysis of charged analyte particles that are not compatible with traditional ionization and mass spectrometry analysis methods.²⁴

Figure 36. Tabulated summary and scatter plot results of Free PEG Content analysis of MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta.

	# Lots Analyzed	Age of Lot (Months)	Free-PEG Conc. (mg/mL)
MYL-1401H	12	min 2	Mean 0.0178
		max 21	Stand Dev 0.0035
U.S.-Licensed Neulasta	16	min 8	Mean 0.038
		max 28	Stand Dev 0.017
E.U.-Approved Neulasta	16	min 14	Mean 0.041
		max 35	Stand Dev 0.019



Solid green line: U.S.-licensed Neulasta quality range

Dashed blue line: E.U.-approved Neulasta quality range

Note: The table was prepared and the figure adapted by the CMC reviewer

²⁴ Swartz et al., 2009, LCGC, 27:40-48

Reviewer Comments: The tabulated summary data and scatter plot data in Figure 36 show that the concentration of free PEG in MYL-1401H on average is 0.04 – 0.02 mg/mL lower than U.S.-licensed Neulasta and E.U.-approved Neulasta. All data are below 0.1 mg/ml and the data spread is similar between the three products. The data show that the concentration of free PEG is similar in MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta.

SDS-PAGE and CE-SDS

SDS-PAGE (Figure 48) and CE-SDS (Figure 49) were used to estimate size variants and monomer content based on electrophoretic separation. These methods complement SEC-HPLC, SEC-MALS, and AUC. Table 41 summarizes the CE-SDS results.

Figure 48: Representative SDS-PAGE for MYL-1401H, US-licensed Neulasta and EU-Approved Neulasta

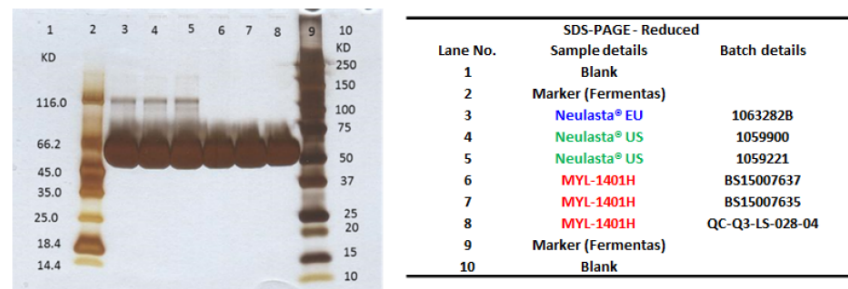


Figure 49: Representative Overlaid CE-SDS Electropherogram for MYL-1401H, US-licensed Neulasta and EU-approved Neulasta

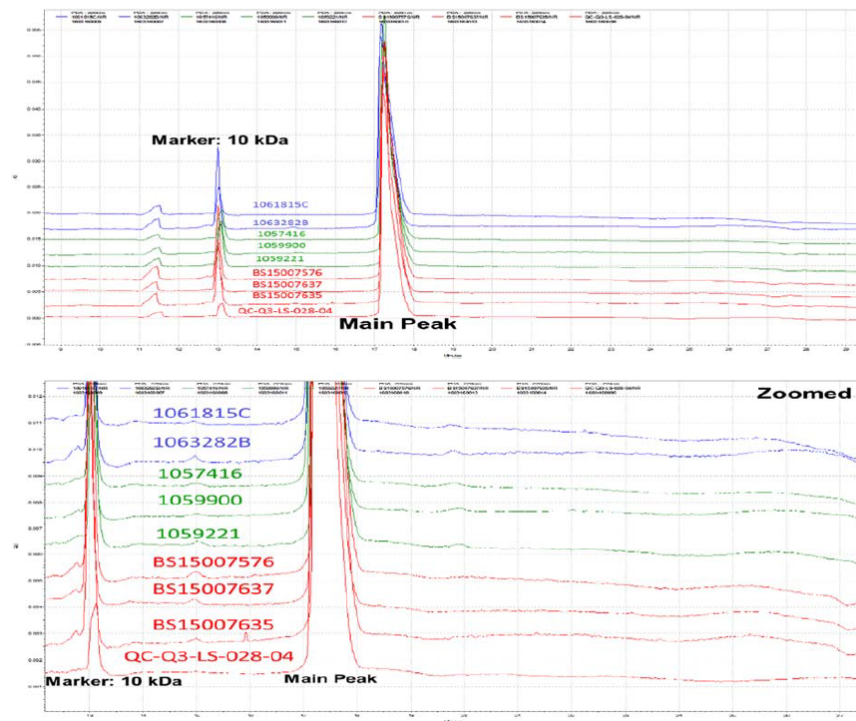


Table 41. Summary results for CE-SDS Analysis of MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta

	# Lots Analyzed	Age of Lot (Months)		Main Peak		Size Variants
				Area %	Area %	Area %
MYL-1401H	10	min	2	Mean	100.00	----*
		max	29	Stand Dev	0.00	----*
U.S.-Licensed Neulasta	8	min	8	Mean	99.80	0.26
		max	15	Stand Dev	0.11	0.08
E.U.-Approved Neulasta	8	min	14	Mean	99.80	0.33
		max	19	Stand Dev	0.18	0.09

*No size variants were detected for MYL-1401H by CE-SDS

Note: The table was prepared by the CMC reviewer

Reviewer Comment: Figure 48 shows a main band running at ~ 50 kDa, that is similar in all three products, and is consistent with PEG-G-CSF. Lanes 3-5 include one lot of E.U.-approved Neulasta and two lots of U.S.-licensed Neulasta and show high molecular weight bands running at ~ 116 kDa, which could be attributed to dimer (~77.6 kD) or dipegylated G-CSF (~58.8 kD) running at higher molecular weight in the gel due to the PEG moiety. Lanes 3-8 show a common band (PEG-G-CSF) running between 45 – 66.2 kD for an expected molecular weight of ~38.8 kD. Lanes 6-8 show lots of MYL-1401H, none of which show high molecular weight bands. The CE-SDS data in Figure 49 also show a comparison of the three products. Zoomed in CE-SDS electropherograms also shows peaks near 20 minutes for U.S.-licensed Neulasta and E.U.-approved Neulasta that are not clearly present in MYL-1401H (Figure 49, lower panel). These data indicate that MYL-1401H has lower levels of HMWS. However, orthogonal methods for assessment of HMWS provide conflicting results (see below). Note that this method does not provide information about low molecular weight species. See summary of assessment of size species below.

Size Variant Analysis - SEC-HPLC with UV detection and MALS

Size Exclusion Chromatography (SEC-HPLC) separates protein components according to size where the smallest protein components are retained longer on the column. SEC-HPLC allows for the separation of protein size variants from a main peak and detected by UV absorbance (Figures 11 and 12). The components of the peaks are further characterized using multi-angle light scattering (MALS), where static light scattering (SLS) directly measures molecular mass and dynamic light scattering (DLS), measures particle size (hydrodynamic radius) and particle size distribution. From these measurements the sponsor identified and labeled the variants as high molecular weight proteins (HMWP) and low molecular weight proteins (LMWP) and compared their levels between MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta. The variants are described in (Table G) and the SEC-HPLC results are summarized in Table 18 and shown in scatter plots in Figure 13. SEC-MALS results are shown in Figure 52, Table 43, and Figure 53. The labeled impurities shown in Figures 11 and 12 were identified by analyses using multiple methods. The presence of the impurities is supported by RP-HPLC-MS. See the DS characterization section 3.2.S.3.2 for additional information.

Table G. Summary of Size Variants Identified by SEC-HPLC/MALS

Impurity	Identity	Modification Details
HMWP-1	Aggregates	Higher Order Oligomers
HMWP-2	Dimers	Two PEG-G-CSF Molecules
HMWP-3	Di-PEGylated G-CSF	PEGylation at both M ₁ and K ₁₇
LMWP	Des-PEG Species N-terminal Truncated Species	No PEG moiety, G-CSF only PEG-M ₁ PEG-M ₁ -TPLGPAS ₈ PEG-M ₁ -TPLGPA ₇

Note: The table was prepared by the CMC reviewer

Figure 11: Representative Overlay for Size Variants in MYL-1401H, US-licensed Neulasta and EU-approved Neulasta Lots

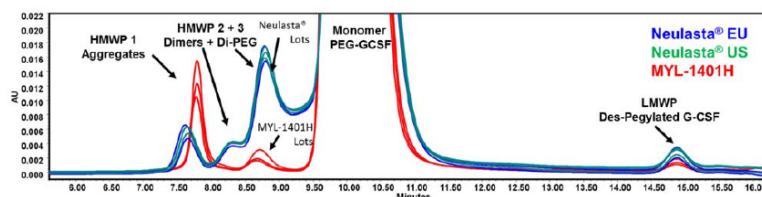


Figure 12: Zoomed in view of representative overlaid SEC-HPLC Chromatograms of MYL-1401H, US-licensed Neulasta and EU-approved Neulasta

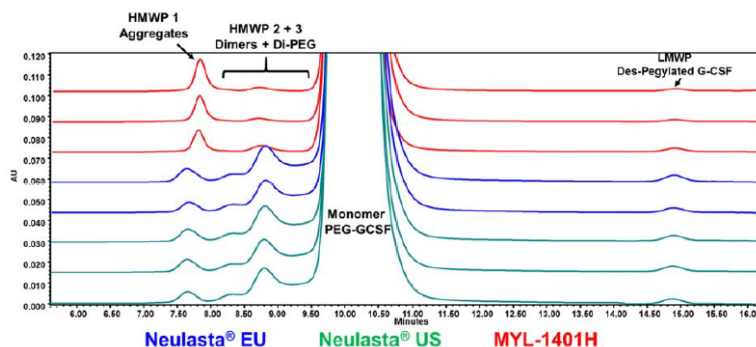
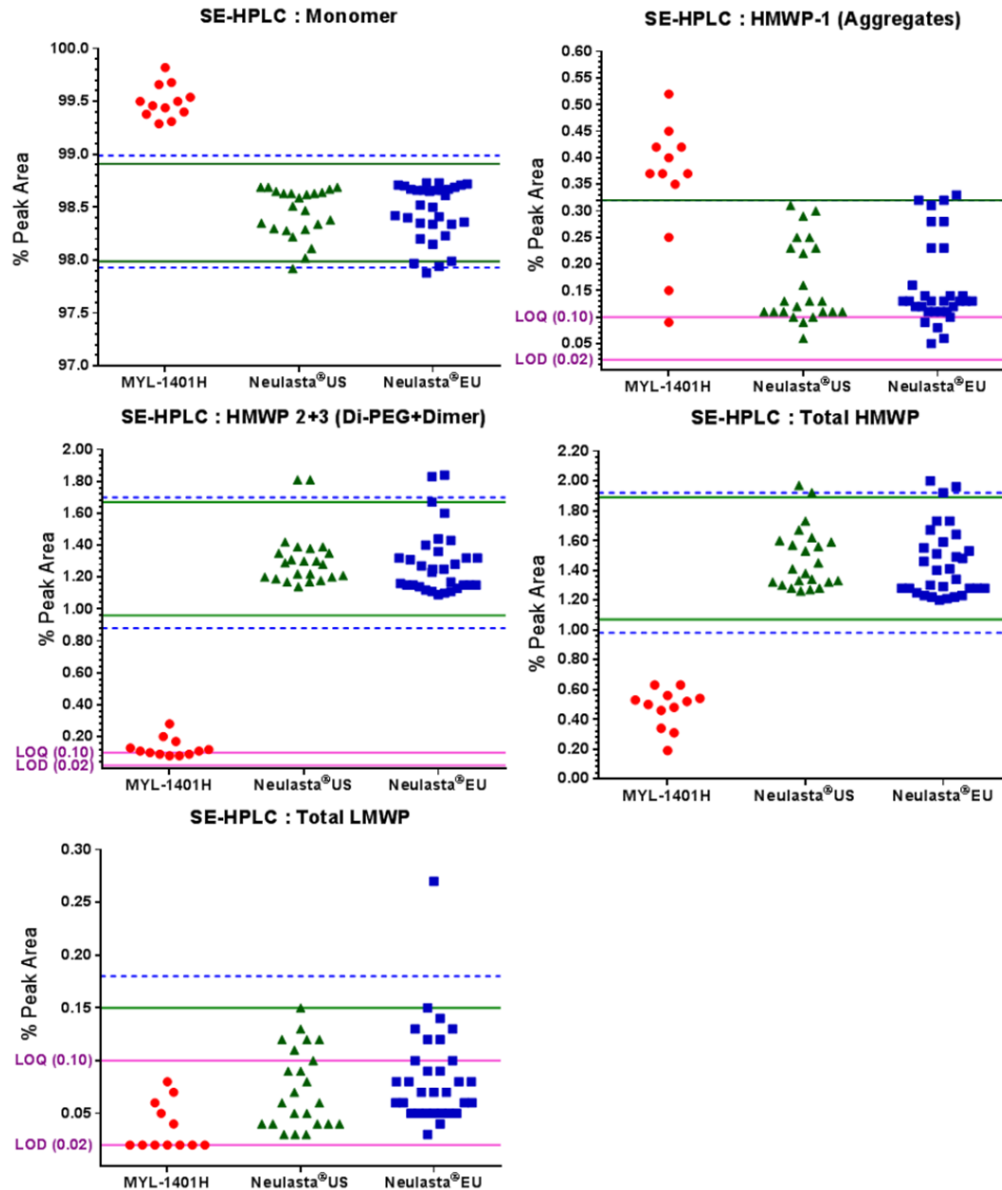


Table 18. Summary Results of Size Variants by SEC-HPLC for MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta

	# Lots Analyzed	Age of Lot (Months)		% HMWP-1 (Aggregates)	% HMWP-2+3 (Di-PEG & Dimer)	% PEG-G-CSF Monomer	% Total HMWPs	% Total LMWPs
MYL-1401H	12	min 2	Mean	0.35	0.13	99.50	0.47	0.05
		max 29	Stand Dev	0.12	0.06	0.16	0.13	0.02
U.S.-Licensed Neulasta	23	min 8	Mean	0.16	1.32	98.45	1.48	0.07
		max 33	Stand Dev	0.08	0.18	0.23	0.21	0.04
E.U.-Approved Neulasta	31	min 14	Mean	0.16	1.29	98.46	1.45	0.08
		max 36	Stand Dev	0.08	0.20	0.26	0.23	0.05

Note: The table was prepared by the CMC reviewer

Figure 13. Scatter Plots of Monomer, Aggregates, Di-PEG+Dimer, Total HMWP, and Total LMWP Levels by SEC-HPLC for MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta



Solid green line: U.S.-licensed Neulasta quality range
Dashed blue line: E.U.-approved Neulasta quality range
Note: The plots were combined into a single figure by the CMC reviewer

Reviewer Comment: Figures 11 and 12 show that compared to U.S.-licensed Neulasta and E.U.-approved Neulasta, MYL-1401H has lower levels of dimers, Di-PEG-G-CSF and Des-PEG-G-CSF by SE-HPLC. Figure 13 shows that HMWP-1 levels are higher in MYL-1401H and the retention times are shifted in the chromatograms (Figures 11 and 12) indicating that HMWP species of a slightly different size comprise at least a fraction of this peak. This is supported by the SEC-MALS results that show a slightly lower molecular weight species eluting from 7.6 – 7.8 min (Figure 52). The difference in HMWP-1 species between MYL-1401H and the comparator products is ~0.19% (Table 18). See summary of assessment of size species below.

Figure 52. Representative Overlays of SEC-MALS data for MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta

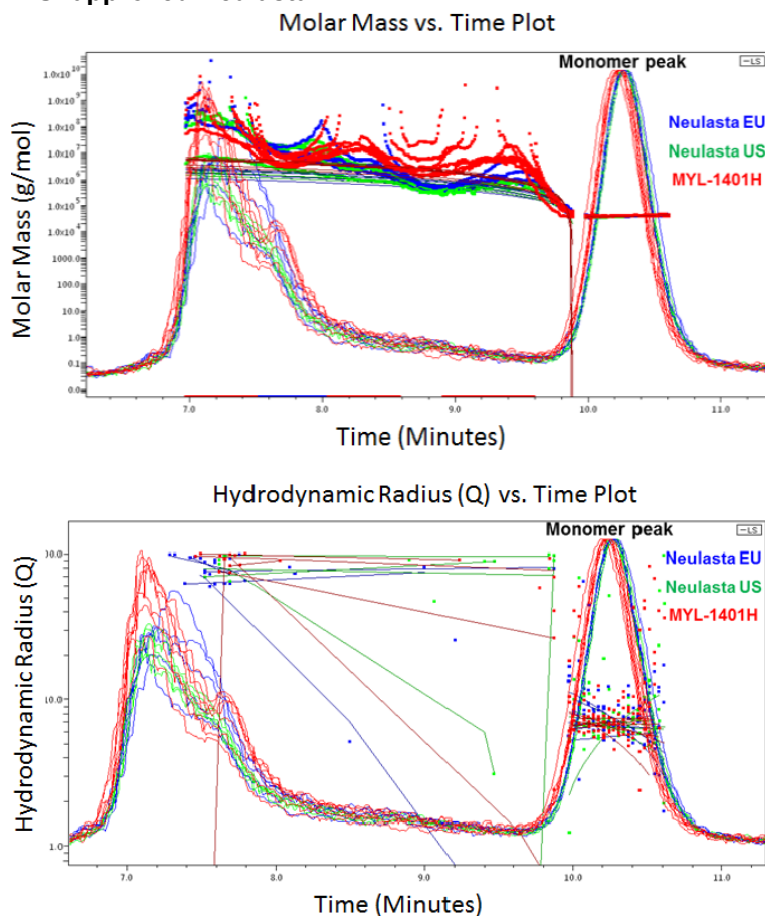
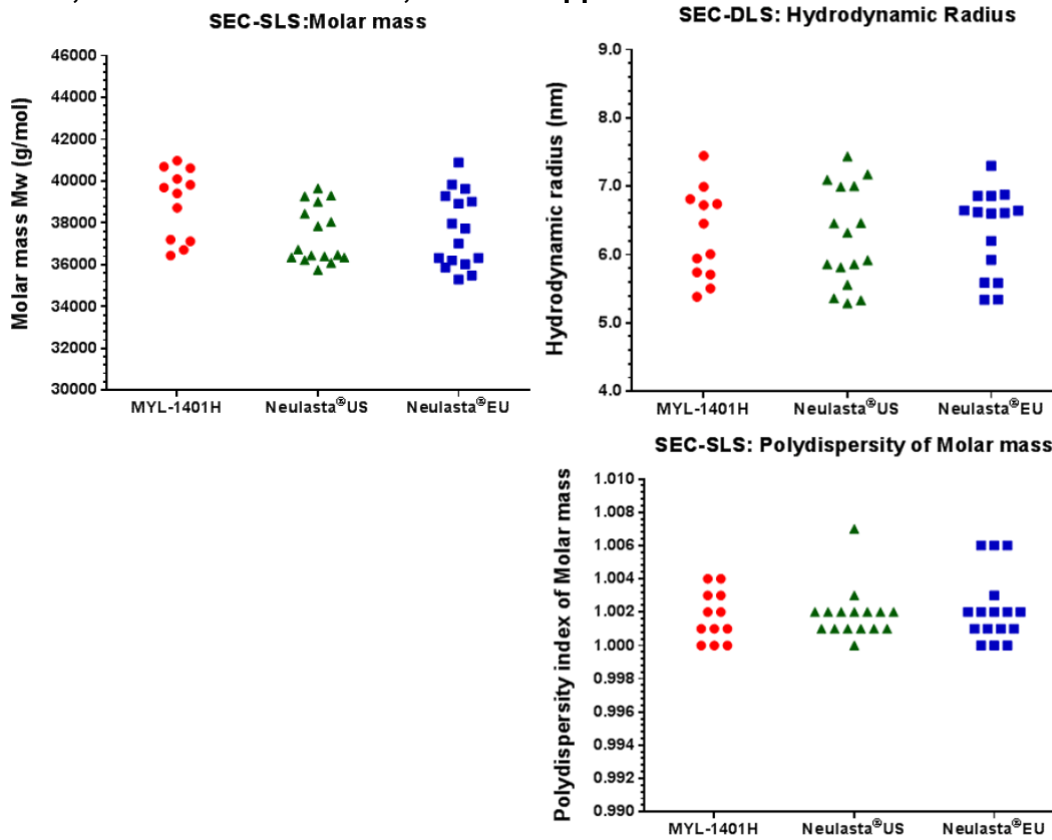


Table 43. Summary Mean Values of SEC-MALS Results for MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta

	# Lots Analyzed	Age of Lot (Months)		PEG-G-CSF Hydrodynamic Radius (nm)	PEG-G-CSF Weight Avg Mol Wt (g/mol)	Polydispersity Index (M_w/M_n)
MYL-1401H	12	min 2	Mean	6.334	38958	1.002
		max 29	Stand Dev	0.625	1667	0.001
U.S.-Licensed Neulasta	16	min 8	Mean	6.246	37399	1.002
		max 28	Stand Dev	0.723	1362	0.002
E.U.-Approved Neulasta	15-16	min 14	Mean	6.289	37614	1.002
		max 35	Stand Dev	0.660	1779	0.002

Note: The table was prepared by the CMC reviewer

Figure 53. Scatter Plots of Molar Mass, Hydrodynamic Radius, and Polydispersity for MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta



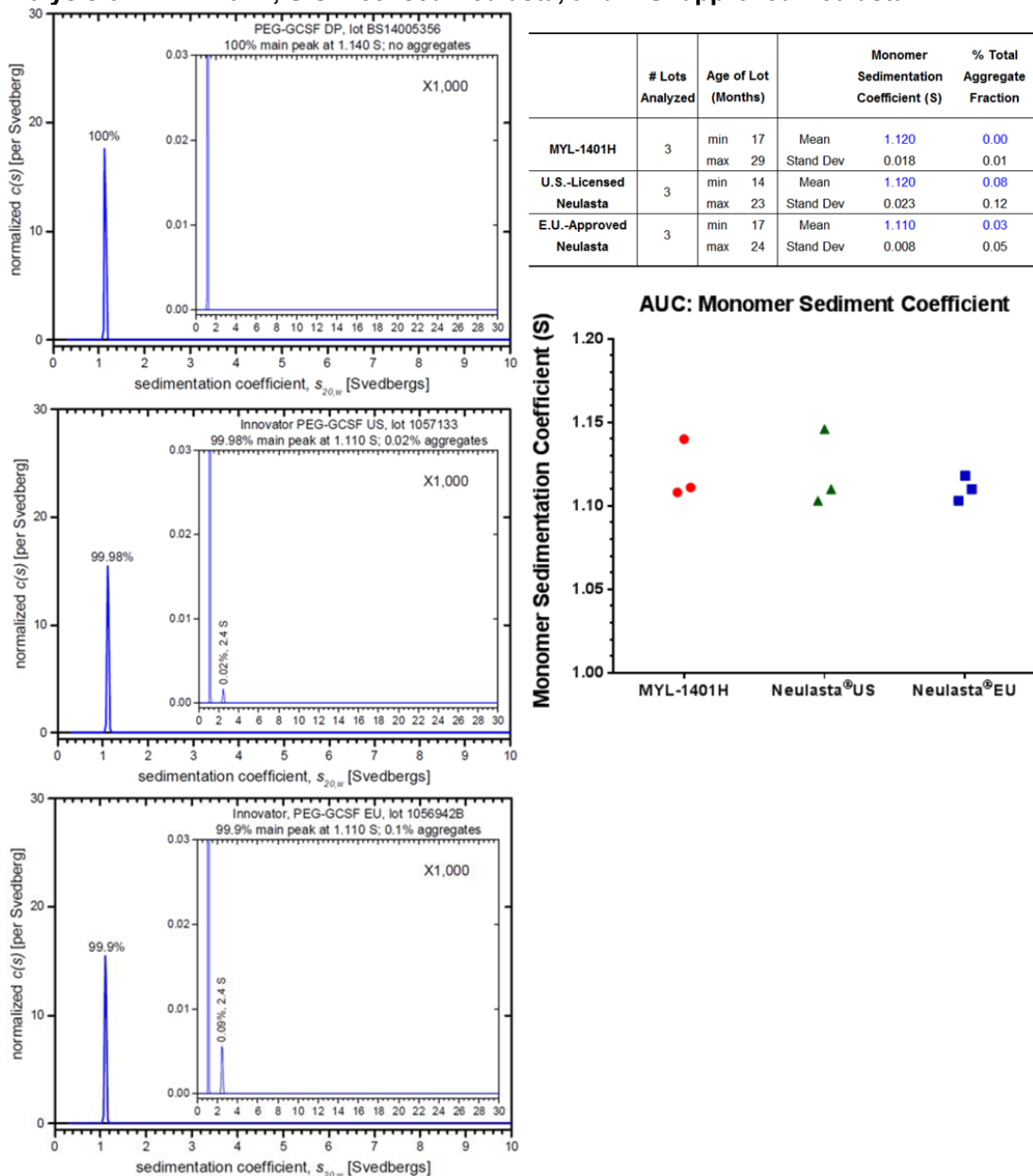
Note: The plots were combined into a single figure by the CMC reviewer

Reviewer Comments: The data in Table 43 and Figure 53 show that the hydrodynamic radius and average molecular weight and polydispersity is similar in the three products.

Aggregate Content and Monomer Sedimentation Coefficient - AUC

Analytical ultracentrifugation provides information about molecular weight, oligomerization, and hydrodynamic radius of protein samples. These molecular properties are measured by spinning samples in solution at high speeds and measuring the opposing sedimentation and diffusion forces within the sample cell, described by a sedimentation coefficient. This method allows for the detection of aggregates and other minor components at levels < 1% by weight and is sensitive to protein conformational changes and overall molecular shape that alter sedimentation coefficients based on hydrodynamic friction. AUC analyses were performed for MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta.

Figure 42. Normalized Sedimentation Coefficient, Scatter Plots, and Tabulated Summary Data for AUC Analysis of MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta



Note: The table was prepared and the plots combined into a single figure by the CMC reviewer

Reviewer Comment: *The normalized sedimentation coefficient distribution for MYL-1401H shows no aggregates, compared to 0.03% for E.U.-approved Neulasta and 0.08% for U.S.-licensed Neulasta (Figure 42). AUC may underestimate size heterogeneity for PEG proteins due to a “parachute effect”⁸ However, any underestimation is expected to be the same for MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta so a comparison of the results is meaningful even if the absolute measurement of size heterogeneity is not accurate. Therefore, the results support that MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta are similar with respect to monomer and aggregate content by AUC.*

Reviewer’s Summary of assessment of size species

SDS-PAGE (Figure 48) and CE-SDS (Figure 49) data show a main band running at ~ 50 kDa (monomer) which is present in the three products. However, one lot of E.U.-approved Neulasta and two lots of U.S.-licensed Neulasta show high molecular weight bands running at ~ 116 kDa, which could be attributed to dimer or dipegylated GCSF. This band is absent in MYL-1401H. Overall, these data show that MYL-1401H has lower levels of HMWP.

On the other hand, the SEC-HPLC-MALS results show higher levels of aggregates (HMWP-1) in MYL-1401H. The difference in HMWP-1 species between MYL-1401H and the comparator products is ~0.19% (Table 18). The sponsor explained the discrepancy in the results between SDS-PAGE and SEC by the difference in method sensitivity. The LOQ and LOD for SEC-HPLC are 0.03% and 0.01%, respectively. The LOQ for SDS-PAGE was not determined because this method was validated for identity only. The sponsor also noted that the total levels of HMWP which includes HMWP-1, dimers and dipeg-GCSF, are lower in MYL-1401H compared to U.S.-licensed Neulasta and E.U.-approved Neulasta. This could explain the HMWP bands shown in SDS-PAGE for U.S.-licensed Neulasta and E.U.-approved Neulasta but not for MYL-1401H (Figure 48). The sponsor’s explanation is reasonable.

Analytical ultracentrifugation results show a % total aggregate fraction near zero for all three products (Figure 42), but the results can be obscured by the PEG moiety due to a parachute effect.²⁵

Overall, the different orthogonal methods for size species indicate that MYL-140H contains slightly higher levels of aggregates (HMWP-1) than the comparator products. By SEC, the difference is approximately 0.19%. This difference is unlikely to have an impact on safety and efficacy due to the low levels and the low immunogenicity risk of the PEG-G-CSF products.

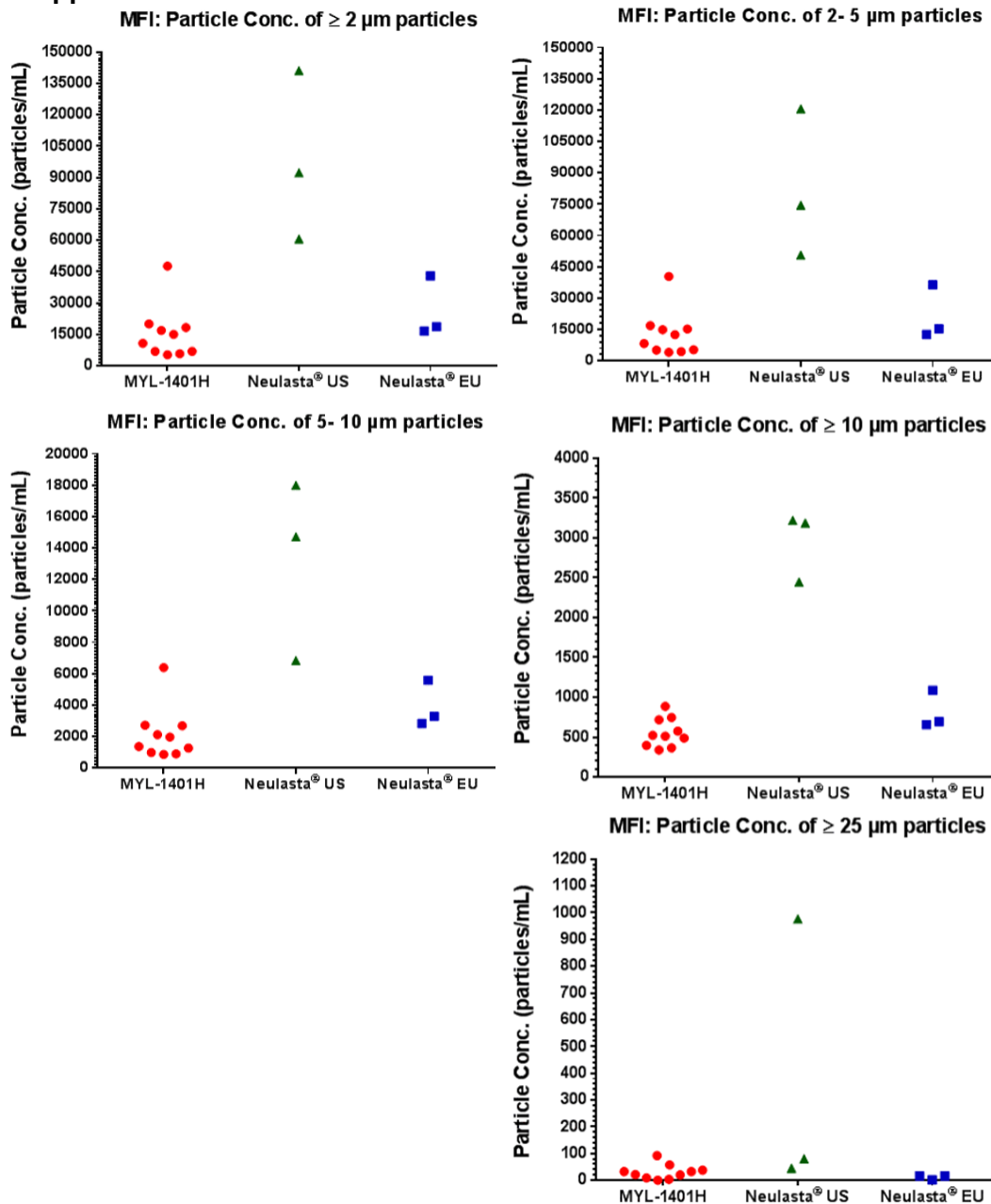
The remaining attributes that are outside the U.S.-licensed Neulasta and E.U.-approved Neulasta quality ranges are not expected to negatively impact product quality because they result in slightly higher purity in MYL-1401H by ~1%.

²⁵ Gokarn et al, 2012, Mol. Pharmaceutics, 9:762–773

Sub-Visible Particles – MFI

Microflow imaging (MFI) utilizes a camera to image particles within a sample flowing through an imaging cell. MFI is an orthogonal particle counting method to light obscuration, which is typically used to measure the number of particles of sizes $\geq 10 \mu\text{m}$ and $\geq 25 \mu\text{m}$ according to USP <788>. MFI is capable of counting particles in the 2-10 μm range and can provide particle morphology information for particle sizes $\geq 5 \mu\text{m}$ (Corvari et al., 2015, Biologicals, 43:457-473). Particles were counted by MFI for MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta with the results shown in Figure 54 and Table 45.

Figure 54. Scatter Plot of Particle Counts by MFI for MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta



Note: The plots were combined into a single figure by the CMC reviewer

Table 45. Summary results of Particle Counts by MFI for MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta

	# Lots Analyzed	Age of Lot (Months)		Number of Particles Per mL						
				Total	≥ 2 μm	2-5 μm	5-10 μm	≥ 10 μm	≥ 25 μm	
MYL-1401H	10	min	2	Mean	42666	15369	12692	2121	556	30
		max	29	Stand Dev	37308	12624	10903	1655	179	28
U.S.-Licensed Neulasta	3	min	8	Mean	265192	98013	81883	13181	2949	367
		max	28	Stand Dev	87544	40521	35613	5739	437	528
E.U.-Approved Neulasta	3	min	14	Mean	84836	26096	21388	3894	813	11
		max	35	Stand Dev	43800	14667	12988	1480	238	8

Note: The table was prepared by the CMC reviewer

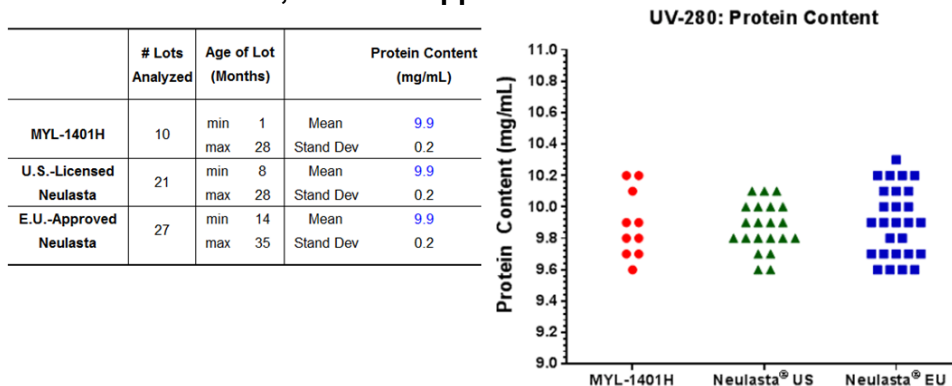
Reviewer Comments: The measured particle counts for MYL-1401H at all sizes are similar to or lower than U.S.-licensed Neulasta and E.U.-approved Neulasta. Four of the ten lots of MYL-1401H analyzed were the same age or older than the three lots analyzed each for U.S.-licensed Neulasta and E.U.-approved Neulasta, therefore, the age of the lots are not factoring into these results. Due to the high variability associated with the MFI method, the standard deviations provided are large as is true for other particle counting methods. Overall, the sub-visible particle results for MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta are similar.

PROTEIN CONTENT, G-CSF RECEPTOR BINDING, AND POTENCY

UV-Visible Spectrophotometry - Protein Content

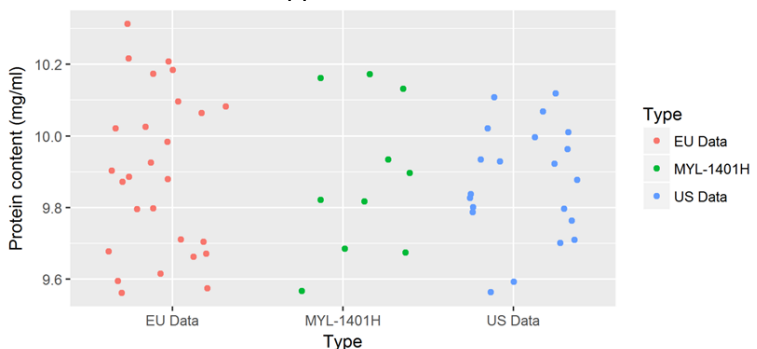
Ultraviolet-visible absorbance (UV-Vis) at 280 nm was used to determine the protein content (concentration) of PEG-G-CSF in MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta by measuring the absorbance exhibited of the test sample at 280nm using ultraviolet (UV) spectrophotometry to calculate the protein concentration.

Figure 1. Summary Data and Scatter Plot Results of Protein Content Analysis for MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta

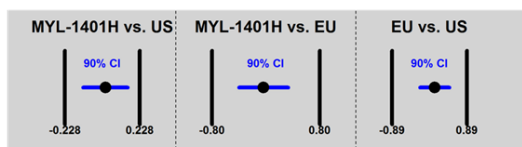


Note: The table was prepared and the figure adapted by the CMC reviewer

Figure A. Tier 1 equivalence testing of protein content for MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta



Comparison	# of lots	Mean difference, %	90% CI for mean difference, %	Equivalence margin, %	Pass equivalence test ?
MYL-1401H vs. US	(10, 21)*	0.019	(-0.118, 0.155)	(-0.228, 0.228)	Yes
MYL-1401H vs. EU	(10, 27)*	-0.014	(-0.165, 0.137)	(-0.329, 0.329)	Yes
EU vs. US	(27, 21)	0.032	(-0.058, 0.122)	(-0.228, 0.228)	Yes



* If $n_b < 1.5n_c$ or $n_c < 1.5n_b$, 90% CI is adjusted by the imbalance of two groups' sample sizes

Note: Figure A was prepared by the CMC statistics reviewer

Reviewer Comment: The sponsor's protein content analysis included the lots of all three products used in the clinical studies (refer to Table F above at the beginning of the review). Protein content was initially calculated based on a theoretical extinction coefficient for PEG-G-CSF ($\epsilon_{280 \text{ nm}} [0.1\% \text{ 1 g/L solution}] = 0.836 \text{ L mol}^{-1} \text{ cm}^{-1}$), estimated from the PEG-G-CSF sequence (e.g. using the ExpASy Bioinformatics Resource Portal ProtParam tool). A scatter plot of the results shows that the data spread is consistent between products and from lot to lot (Figure 1 and Figure A). The sponsor's Tier 1 assessment based on statistical equivalence testing of protein content is adequate (Figure A).

Mylan was asked to calculate an experimentally determined extinction coefficient to confirm the theoretical value or provide a scientific justification for their use of a theoretical extinction coefficient in the analytical similarity assessment. Mylan emphasized that the amino acid sequence and PEGylation site for MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta were thoroughly characterized with ample data to support that they are the same. Therefore, it is reasonable to use the same theoretical extinction coefficient to determine protein content for all three products. With respect to the analytical similarity assessment, the reviewer agrees with the sponsor's justification for the use of the theoretical extinction coefficient to determine protein content because the data provided support that the three products have the same sequence and similar high order structure as determined by multiple orthogonal methods.

In addition, the same extinction coefficient was used to calculate protein concentration for the three products.

Overall, the results support that the protein content is similar between MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta and support an analytical bridge. Refer to DS section 3.2.S.3 for additional information regarding the use of the extinction coefficient for the determination of protein content as part of the overall control strategy for MYL-1401H.

G-CSF Receptor Binding - SPR

The G-CSF receptor binding assay utilizes surface plasmon resonance (SPR) to quantitate the kinetics of binding (rate of association, k_a and rate of dissociation, k_d) between PEG-G-CSF and the GCSF receptor. The comparison of these rate constants across different concentrations yields the equilibrium dissociation constant, K_D , or receptor binding affinity. Receptor binding kinetics and affinities are compared between MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta for the analytical similarity assessment.

Figure 6. Representative Sensograms of G-CSF Receptor Binding for MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta

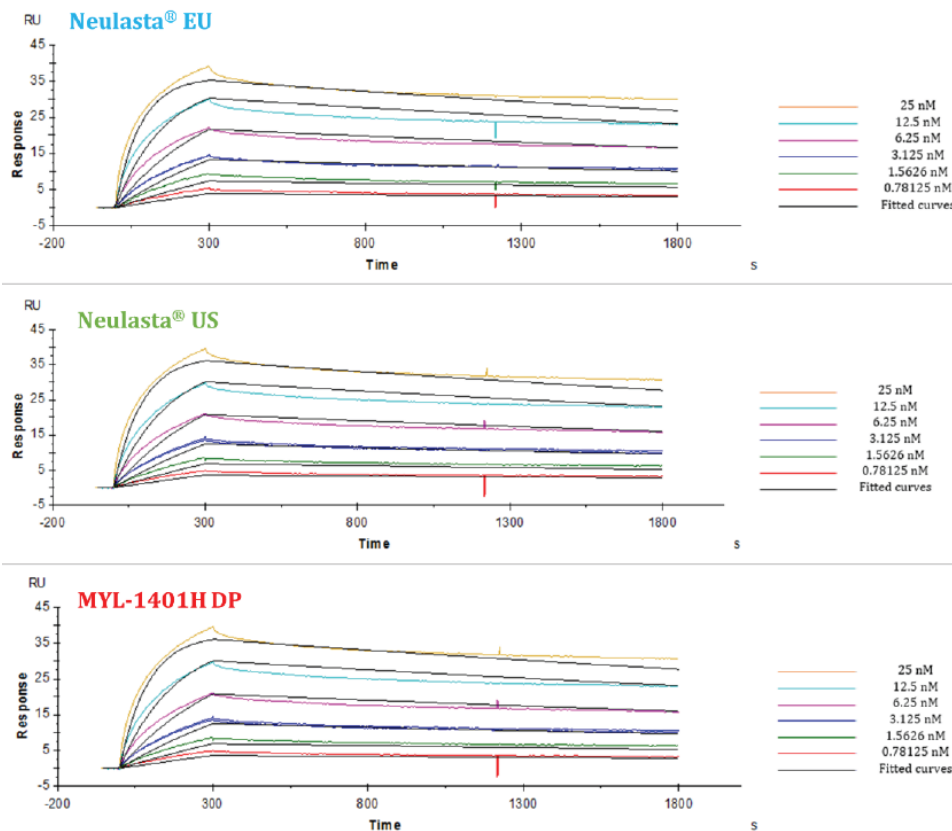


Table 15. Summary Results for G-CSF Receptor Binding Affinity and Kinetic Parameters for MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta

	# Lots Analyzed	Age of Lot (Months)			k_a (1/Ms)	k_d (1/s)	K_D (pM)
					Average (N=3)	Average (N=3)	Average (N=3)
MYL-1401H	10	min	2	Mean	4.33E+05	1.69E-04	394
		max	29	Stand Dev	2.90E+04	1.38E-05	40
U.S.-Licensed Neulasta	16	min	8	Mean	4.50E+05	1.70E-04	380
		max	21	Stand Dev	3.95E+04	2.62E-05	49
E.U.-Approved Neulasta	20	min	14	Mean	4.57E+05	1.79E-04	397
		max	30	Stand Dev	4.35E+04	1.62E-05	51

Note: The table was prepared by the CMC reviewer

Reviewer Comments: The representative binding curves shown in Figure 6 are individual sensograms (colored curves) generated for the product samples and concentrations indicated, with response factors < 50 RU. The curves were fit to a 1:1 Langmuir interaction model (black lines overlaying colored lines). The reported (k_a), (k_d), and (K_D) shown in Table 15 are the averaged results of three replicates. The provided method qualification data was adequate. Collectively, these parameters are consistent with a well-designed biosensor analysis.²⁶ Overall, the results support similar G-CSF receptor binding by MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta.

Mouse NSF-60 Cell-Based Assay – Potency

The sponsor determined potency (biological activity) for MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta using an MNFS-60 (murine) cell based assay. The MNFS-60 cell line of murine origin, is derived from myelogenous leukemia, and depends on the presence of G-CSF growth factor. Proliferation of these cells correlates with the concentration of added growth factor and is measured by a change in absorbance associated with the reduction of a tetrazolium compound (Formazan) included in the assay. The assay results are reported as mean relative potency with respect to an internal reference standard as shown in the dose-response curves in Figure 3 below (left panels), with each analyzed lot measured in triplicate. The internal reference standard is calibrated against an international reference standard for G-CSF. Figure 3 also shows a four-parameter logistic fit (4-PL) of the dose-response curves and parallel line analysis (right panels). The 4-PL fit assumes that the upper and lower regions of the curves are symmetrical. Figure 13 shows a tabulated summary of the mean potency results for each product as well as a scatter plot of the potency results for each lot tested.

²⁶ Rich, R.L. and D.G. Myszkka, J. Mol. Recognit., 2009, 23:1-64

Figure 3: Representative Dose Response Curves for MYL-1401H, US-licensed Neulasta and EU-approved Neulasta

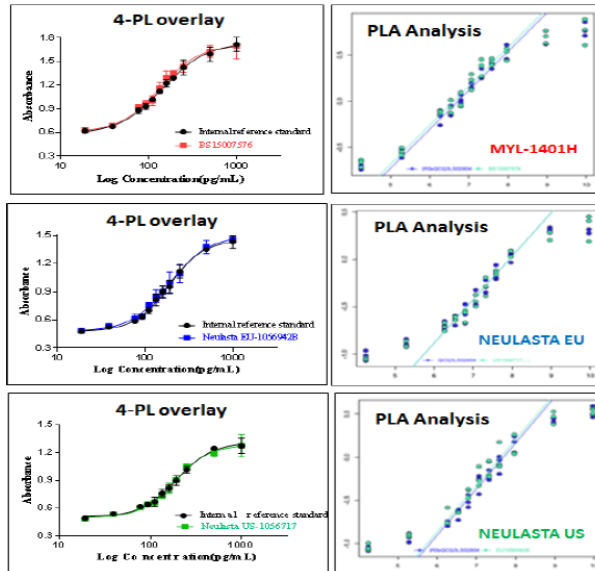
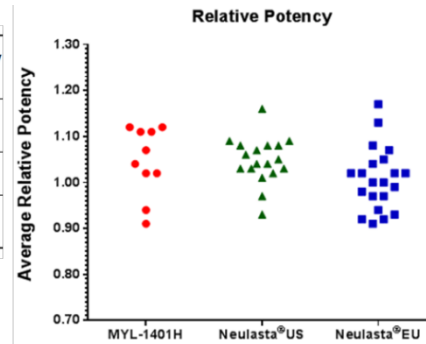


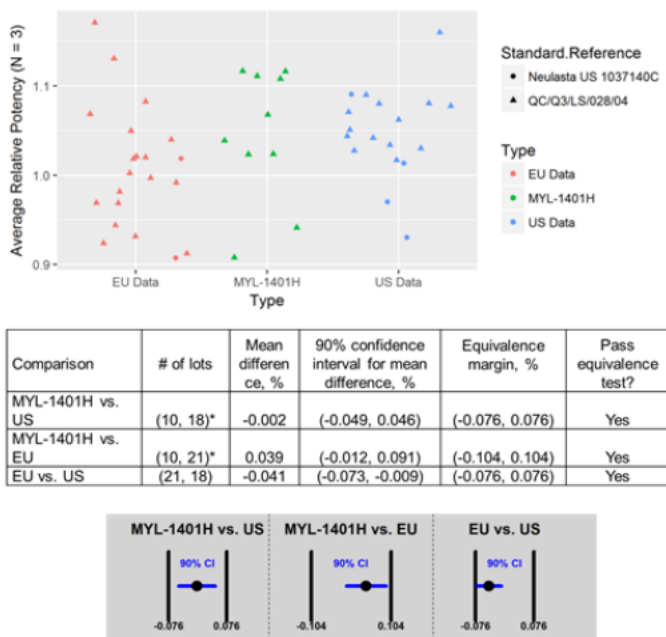
Figure 13. Summary of Relative Potency Results for MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta

	# Lots Analyzed	Age of Lot (Months)	Relative Potency Average (N=3)
MYL-1401H	10	min 1	Mean 1.05
		max 28	Stand Dev 0.08
U.S.-Licensed Neulasta	18	min 8	Mean 1.05
		max 26	Stand Dev 0.05
E.U.-Approved Neulasta	21	min 13	Mean 1.01
		max 35	Stand Dev 0.07



Note: The table was prepared and the figure adapted by the CMC reviewer

Figure B. Tier 1 equivalence testing of potency for MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta



Note: The Figure was prepared by the CMC statistics reviewer

Reviewer Comments: The tabulated summary data shown in Figure 13 show mean relative potency values and standard deviations that compare closely between MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta. Figure B shows that the confidence interval for MYL-1401H falls entirely within the equivalence margins for both U.S.-licensed Neulasta and E.U.-approved Neulasta, and that the confidence interval for E.U.-approved Neulasta falls entirely within the equivalence margin for U.S.-licensed Neulasta. The lots of MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta used in the clinical studies were included in the potency assay testing, with the exception of E.U.-approved Neulasta lot 1047632A (see Table F above).

Following the inspection of manufacturing site for MYL-1401H at (b) (4), it was discovered that two reference standards (b) (4) and (b) (4) were used for the potency assays performed for comparison of MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta (Appendix III). Since the potency results are relative to a reference standard, Mylan was asked to provide data to bridge the two reference standards. The sponsor’s data to support a bridge between the reference standards included qualification reports containing full characterization of the RSs, dose response curves of RSs (b) (4) and (b) (4) compared to an “anchor” RS (QC/Q3/LS/028/03) and relative potency values for the RSs. The dose-response curves for the reference standard QC/Q3/LS/028/03 vs (b) (4) and (b) (4) and the fitted slopes of the curve inflexions overlay closely. The relative potency data for the RS were also similar (Figure 7, Appendix B) and the RSs were also adequately characterized. These data support a bridge between the RS, therefore the potency data can be pooled for analysis.

The overall results support that MYL-1401H is similar to U.S.-licensed Neulasta with respect to potency in addition these data support an analytical bridge between the three products. Refer to section 3.2.S.4.3 for the review of potency method validation.

COMPARATIVE STABILITY STUDIES

Mylan based their comparative stability study on the following attributes (Table 48) under the listed accelerated and stressed stability conditions:

Table 48. Quality attribute and methodology employed for comparative forced degradation studies of MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta

Category	Quality Attribute	Methodology employed during FD studies	Risk Rank
Product Variants	HMWP	SEC	High
	LMWP	SEC-MALLS	High
	Charge variants	CIEX-HPLC	Moderate
	Deamidation	RP-HPLC	Moderate
	Related Protein variants		High
	Methionine oxidation		High
Product Attributes	Primary sequence	PMF	Very High
	Secondary structure	Far UV CD	Very High
	Tertiary structure	Fluorescence (extrinsic and intrinsic)	Very High
Biological activity	Potency	MNFS-60 assay	Very High

Comparative stability conditions:

- Temperature Stress at 25°C and 40°C
- Mechanical Stress – Agitation at 230 rpm at 25°C
- Acidic pH 2 and Basic pH 9 Stress
- Oxidative Stress – 0.01% and 0.10% Peroxide at 25°C and 60% RH
- Photostability

Reviewer Comment: the selection of the quality attributes and stability conditions are acceptable.

The lots used for comparative stability are shown in Table 49.

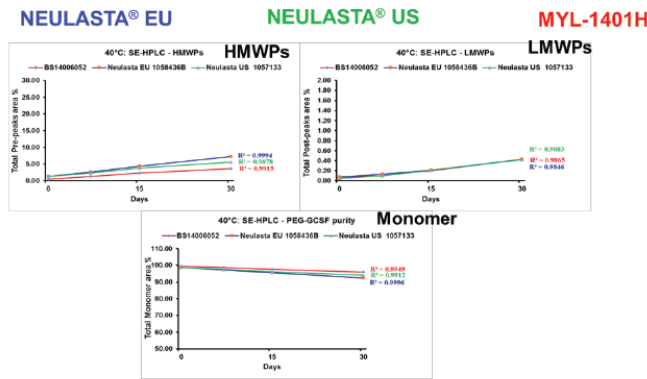
Table 49: MYL-1401H and Neulasta batches used for comparative FD studies

S. No.	Sample	Batch Number	Manufacturing Date	Expiry Date	Product Age at Start of Study
1.	NEULASTA® EU - approved	1058436B	August 2014 ^b	August 2017	14 months
2.	NEULASTA® US - licensed	1057133	October 2014 ^b	October 2017	12 months
3.	MYL-1401H Drug product (Test sample)	BS14006052 ^a	July 2014	July 2016	15 months
^a - BS14006052 was manufactured using drug substance batch DE-B14-03-000018. ^b - Manufacturing dates were estimated based on the expiry date stated on the reference samples. Shelf life of reference product is (b) (4) months.					

Temperature Stress (Accelerated Stability) at 25°C and 40°C

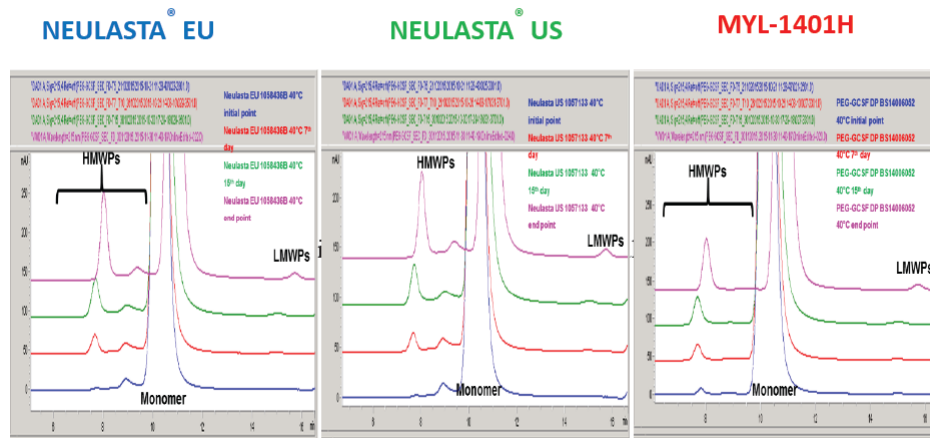
The degradation profiles and statistics for size variants in MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta are shown in Figure 55. Figure 56 shows the chromatographic profiles.

Figure 55. Graphical presentation and respective degradation statistics of Size variants evaluated for 40°C condition - SE-HPLC analysis



Sample details	2-8°C	25°C	40°C	2-8°C	25°C	40°C	2-8°C	25°C	40°C
	PEG-GCSF Monomer			Total HMWPs			Total LMWPs		
Degradation rate - statistics									
Neulasta EU 1058436B Slope (% per day)	0.007	-0.002	-0.212	-0.006	0.000	0.200	0.000	0.001	0.012
Neulasta US 1057133 Slope (% per day)	0.004	-0.002	-0.156	-0.003	0.001	0.144	0.000	0.001	0.013
MYL-1401H BS14006052 Slope (% per day)	0.003	-0.002	-0.118	-0.002	0.001	0.105	0.000	0.002	0.013

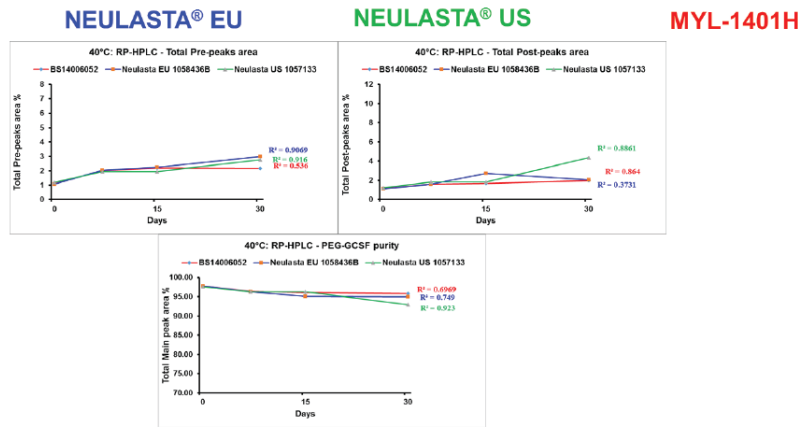
Figure 56. Comparison of SE-HPLC UV chromatograms of EU –approved 1058436B and US -licensed 1057133 batches with MYL-1401H drug product BS14006052 for conditions 40°C ±2°C, 75%+- 5% RH at different time point 0th, 7th, 15th and 30th day.



Reviewer Comment: Figures 55 and 56 show a similar profile of HMWP and LMWP that increase over time in a similar manner with exposure to 40°C for MYL-1401H, U.S.-licensed Neulasta and E.U.-approved Neulasta. A comparison of the slopes for the changes in the levels of HMWP, LMWP, and PEG-G-CSF monomer over time shows that MYL-1401H degrades

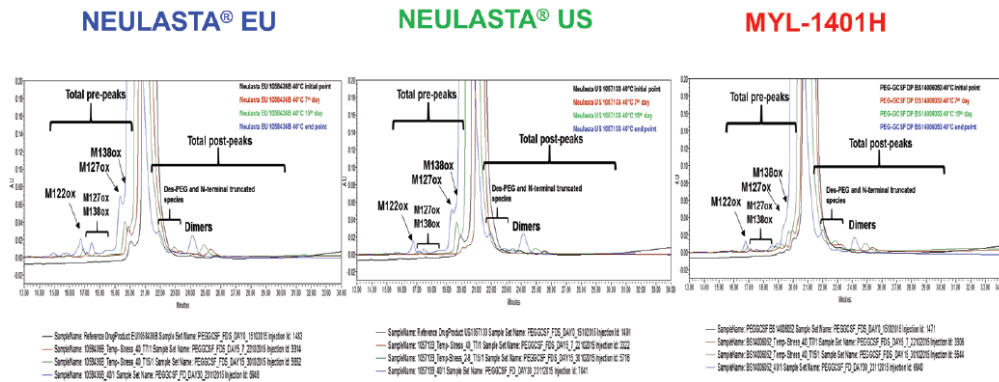
similarly to U.S.-licensed Neulasta and E.U.-approved Neulasta. The estimated ages of the lots tested are within 2 months with MYL-1401H as the oldest, but it is unlikely that this difference significantly affects the comparison of the size variants because the degradation rate for this attribute under these conditions show minimal degradation (Figure 55). The degradation profiles and statistics for hydrophobic variants (Figure 57 and 58) by RP-HPLC also indicated that MYL-1401H degrades similarly to U.S.-licensed Neulasta and E.U.-approved Neulasta.

Figure 57. Graphical presentation and respective degradation statistics of quality attributes such as purity (main peak) and impurities (total pre-peaks and total post-peaks) evaluated for 40°C condition - RP-HPLC analysis



Sample details	2-8°C			25°C			40°C		
	PEG-GCSF main peak purity			Total pre-peaks			Total post-peaks		
Degradation rate - statistics									
Neulasta EU 1058436B - Slope (% per day)	-0.016	-0.018	-0.089	0.010	0.014	0.058	0.006	0.004	0.032
Neulasta US 1057133 - Slope (% per day)	-0.009	-0.009	-0.150	0.005	0.009	0.048	0.003	0.00	0.102
PEG-GCSF DP BS14006052 - Slope (% per day)	-0.002	-0.018	-0.055	0.005	0.015	0.03	-0.004	0.003	0.025

Figure 58. Comparison of RP-HPLC UV chromatograms of EU –approved Neulasta 1058436B and US -licensed Neulasta 1057133 batches with MYL-1401H drug product BS14006052 for conditions 40°C ±2°C, 75%+/- 5% RH, at the initial and end time point.



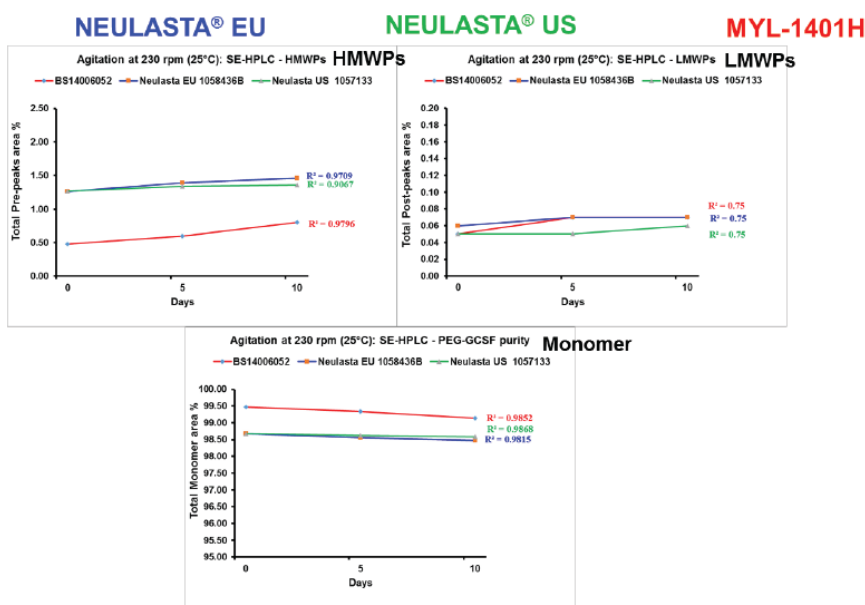
Reviewer Comment: Figure 58 shows RP-HPLC overlaid chromatograms of MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta with similar profiles of hydrophobic variants

over time upon exposure to 40°C. The hydrophobic variants include Met122, Met127, and Met138 oxidized species, as well as Des-PEG, N-terminal truncated species, and dimers. Although the methionine oxidized variants are impurities with the potential to affect PEG-G-CSF potency²³, the results in Figure 59 below shows some variability in potency but no clear trend is observed at 25°C and 40°C. The results remained within the proposed specification ((b) (4) relative potency) for all the conditions tested. Overall the data support similar stability profiles under the accelerated conditions tested.

Mechanical Stress- Agitation at 230 rpm at 25°C

Mylan’s assessment of MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta under the condition of agitation at 230 rpm, 25°C for 0 -10 days is as follows:

Figure 60. Graphical presentation and respective degradation statistics of Size variants evaluated for agitation at 230 rpm, 25°C condition - SE-HPLC analysis

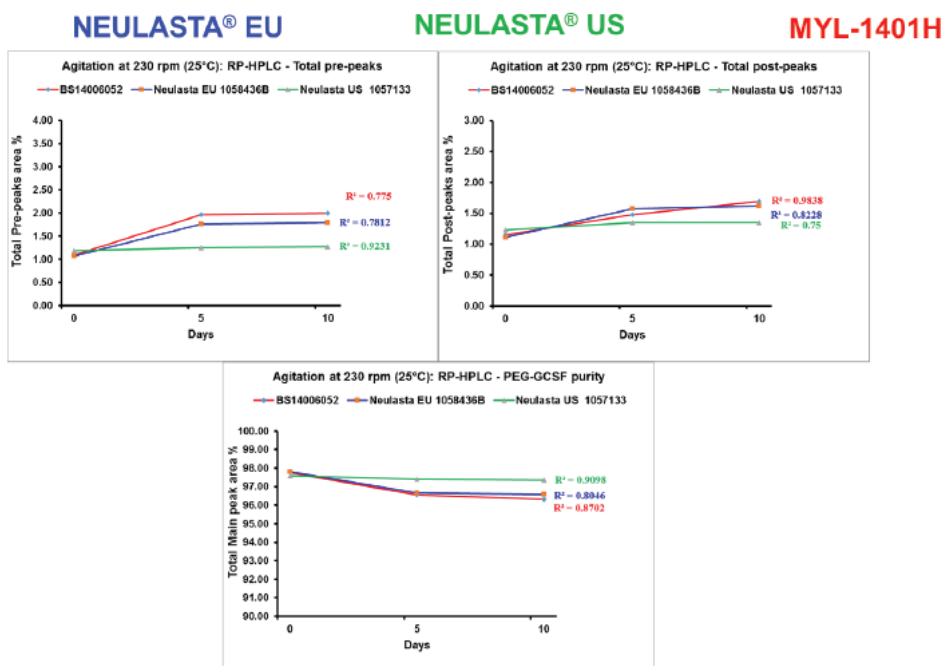


Sample details	PEG-GCSF Monomer purity	Total HMWPs	Total LMWPs
Degradation rate - statistics			
Neulasta EU 1058436B Slope (% per day)	-0.016	0.015	0.000
Neulasta US 1057133 Slope (% per day)	-0.011	0.010	0.001
MYL-1401H - Slope (% per day)	-0.033	0.032	0.002

Reviewer Comment: Figure 60 shows the levels of HMWP (total pre-peaks) and LMWP (total post-peaks) size variants and PEG-G-CSF monomer over time with exposure to agitation as measured by SEC-HPLC. At the beginning of the study, MYL-1401H exhibits levels of HMWP and LMWP that are lower than or compare closely to U.S.-licensed Neulasta and E.U.-approved Neulasta. Over the period of 5-10 days of agitation there is a slightly higher rate of HMWP formation in MYL-1401H versus U.S.-licensed Neulasta and E.U.-approved Neulasta. MYL-1401H also shows a slightly increased rate of PEG-G-CSF monomer degradation compared to

U.S.-licensed Neulasta and E.U.-approved Neulasta, however, the initial monomer level is ~ 1% higher in MYL-1401H and remains higher throughout the 10 day agitation study.

Figure 61. Graphical presentation and respective degradation statistics of quality attributes such as purity (main peak) and impurities (total pre-peaks and total post-peaks) evaluated for agitation at 230 rpm, 25°C condition - RP-HPLC analysis



Sample details	PEG-GCSF main peak purity	Total Pre-peaks	Total Post-peaks
Degradation rate - statistics			
Neulasta EU 1058436B Slope (% per day)	-0.123	0.072	0.051
Neulasta US 1057133 Slope (% per day)	-0.022	0.008	0.012
MYL-1401H BS14006052 Slope (% per day)	-0.145	0.09	0.054

Reviewer Comment: Figure 61 shows the profile of hydrophobic impurities by RP-HPLC following the agitation study described above. Total pre-peaks increase at a faster rate from 0-5 days and are higher overall in MYL-1401H compared to U.S.-licensed Neulasta and E.U.-approved Neulasta following agitation, but the slopes flatten in the same manner from 5-10 days in all three products. The absolute level of pre-peaks for MYL-1401H is ~2% after agitation. This compares with ~1.2% pre-peaks for U.S.-licensed Neulasta and ~1.7% for E.U.-approved Neulasta. Total post-peaks are present in similar levels for all three products after 10 days of agitation, with the rate of increase slightly higher in MYL-1401H. PEG-G-CSF monomer degradation is similar in all three products.

Pre-peaks are comprised of M122 and M127 oxidation species and post-peaks correspond to Q108 deamidation. Methionine oxidation can potentially affect potency Figure 59 (below) shows potency results for the agitation study. The potency data show some variability but no clear trend

is observed under mechanical conditions. The results remained within the proposed specification ((b) (4) relative potency) for all the conditions tested. Overall the data support similar stability profiles under the mechanical stress conditions tested.

Figure 59. Relative potency Scatter plot for temperature and mechanical stress between MYL-1401H, US-licensed Neulasta, and EU-approved Neulasta



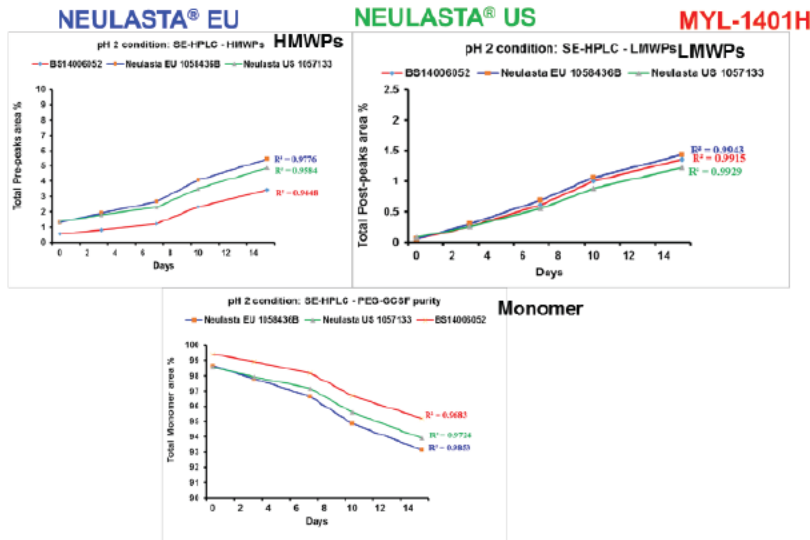
The dotted line in black represents the pass range for the relative potency

Note: The sponsor used a different color scheme for Figure 59 with an MYL-1401H lot shown in green

pH Stress (pH 2 at 40°C and pH 9 at 25°C)

Mylan’s assessment of MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta under pH stress at pH 2 at 40°C and pH 9 at 25°C over 15 days is as follows:

Figure 62. Graphical presentation and respective degradation statistics of Size variants evaluated for pH 2 at 40°C treatment conditions - SE-HPLC analysis

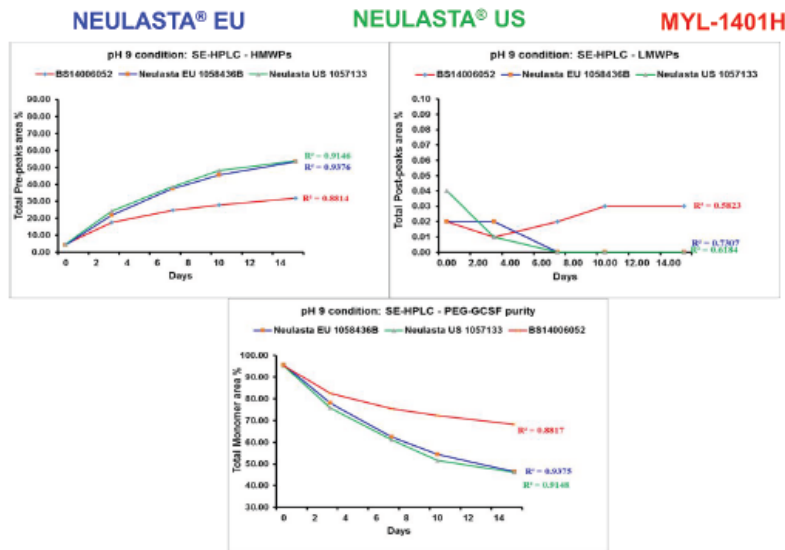


Acidic condition, pH 2: Evaluation of size variants by SE-HPLC			
Sample details	PEG-GC9F Monomer purity	Total HMWPs	Total LMWPs
Degradation rate - statistics			
Neulasta EU 1058436B Slope (% per day)	-0.377	0.282	0.095
Neulasta US 1057133 Slope (% per day)	-0.317	0.238	0.078
MYL-1401HDP BS14006052 Slope (% per day)	-0.289	0.199	0.091

Reviewer Comment: Figure 62 shows the change in size variants by SEC-HPLC in MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta upon exposure to pH 2 and 40°C. The rates of increase of HMWP and LMWP over time compare closely in all three products.

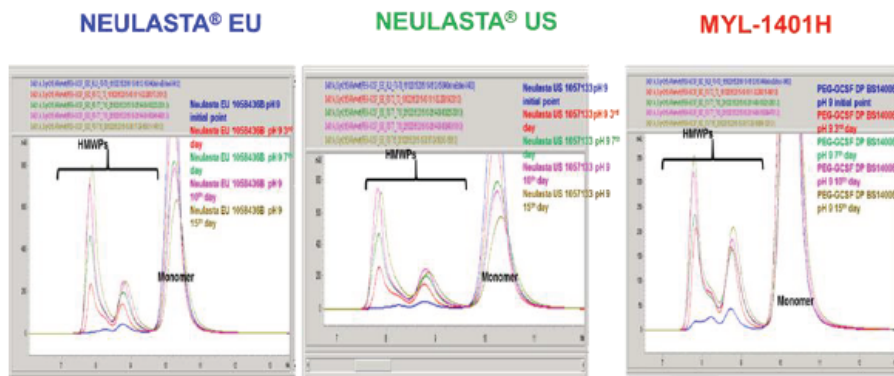
At pH 9 and 25°C HMWP are shown to increase over time for MYL-1401H after 4 days exposure to pH 9 conditions with a subsequent decrease in monomer (Figure 63). MYL-1401H degradation rates for these attributes appear to be slower compared to the comparator products.

Figure 63. Graphical presentation and respective degradation statistics of Size variants evaluated for pH 9 at 25°C treatment conditions - SE-HPLC analysis



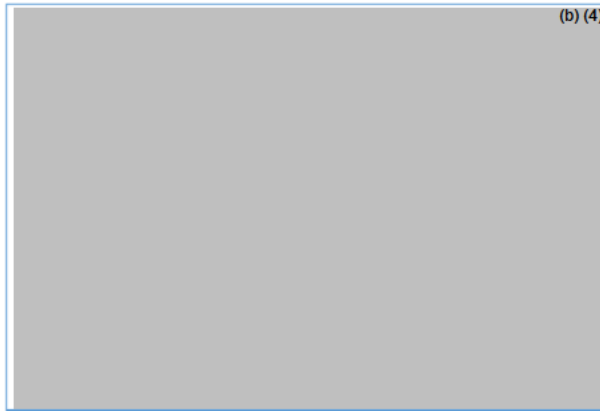
Basic condition, pH 9: Evaluation of size variants by SE-HPLC			
Sample details	PEG-GCSF Monomer purity	Total HMWPs	Total LMWPs
Degradation rate - statistics			
Neulasta EU 1058436B Slope (% per day)	-3.238	3.240	-0.002
Neulasta US 1057133 Slope (% per day)	-3.235	3.237	-0.002
MYL-1401H DP BS14006052 Slope (% per day)	-1.700	1.699	0.001

Figure 64. Comparison of SE-HPLC UV chromatograms of EU – approved Neulasta 1058436B and US - licensed Neulasta 1057133 batches with MYL-1401H drug product BS14006052 for pH 9 at 25°C treatment condition for the initial and end time point.



The effect of acidic and basic conditions on potency is shown in Figure 69.

Figure 69. Relative potency Scatter plot for acidic and basic condition between MYL-1401H, US-licensed Neulasta, and EU-approved Neulasta



The dotted line in black represents the pass range for the relative potency

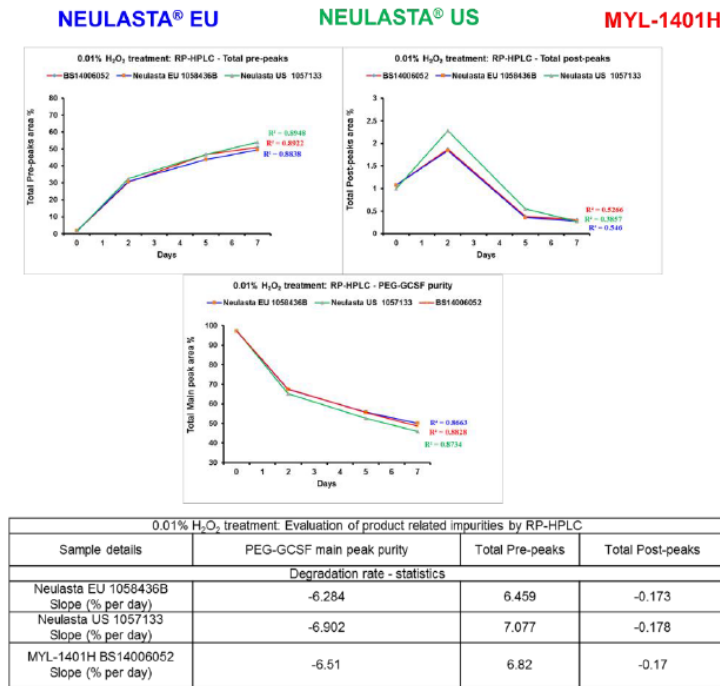
Reviewer Comment: *The effect of acidic and basic conditions on potency is similar between MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta. Potency results are within the proposed specification for up to 15 days pH 2 up and less than 7 days at pH 9. The decrease in potency as exposure time increases follows the same trend in all three products.*

Data were also submitted and reviewed (Figures 65-68) for the assessment of hydrophobic impurities in MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta using RP-HPLC following exposure to pH stress at pH 2 at 40°C and pH 9 at 25°C over 15 days (data not shown). The results showed no differences in the stability profiles for the three products and support that hydrophobic impurity profiles are similar for MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta. Overall, the data support a similar stability profile for all three products under these conditions.

Oxidative Stress

Mylan’s assessment of MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta under oxidative stress at 0.01% and 0.1% peroxide for 5 days is as follows:

Figure 74. Graphical presentation and respective degradation statistics of quality attributes such as purity (main peak) and impurities (total pre-peaks and total post-peaks) evaluated for 0.01% H₂O₂ treatment - RP-HPLC analysis



Reviewer Comment: Figure 74 shows representative stability profiles for MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta that are similar between all three products, as measured by RP-HPLC. The reviewer notes that oxidizing conditions are controlled in DP manufacturing.

Data were also submitted and reviewed (Figures 70-76) for the assessment of size variants and hydrophobic impurities in MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta using SEC-HPLC and RP-HPLC following exposure to oxidative stress at 0.01% and 0.1% over 7 days (data not shown). The results showed no differences in the stability profiles for the three products. In addition, potency decreased similarly over time for all three products with exposure to these conditions.

Photo Stress

MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta were exposed to light with a minimum of 1.2 million lux hours according to ICH Q1B.

The effects of photoexposure on the SE-HPLC profile for MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta are shown below.

Figure 79. Graphical presentation and respective degradation statistics of Size variants evaluated for Photo exposure condition - SE-HPLC analysis

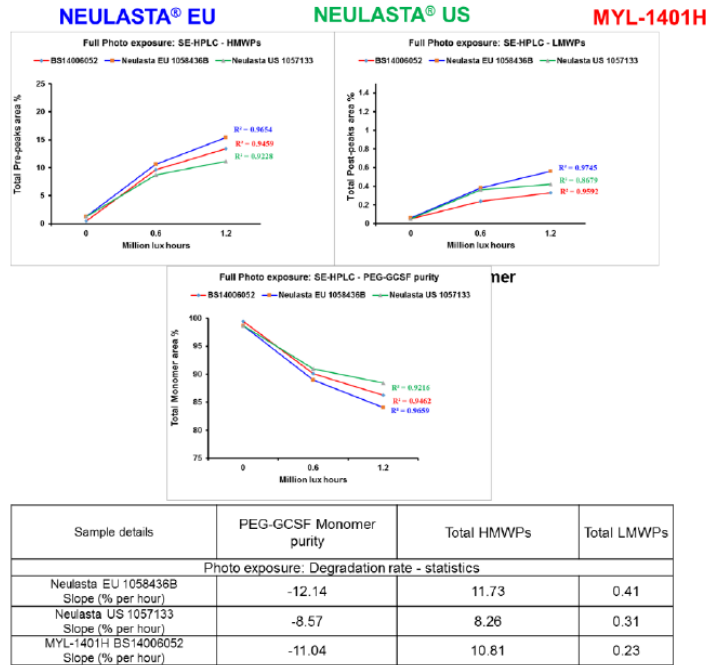
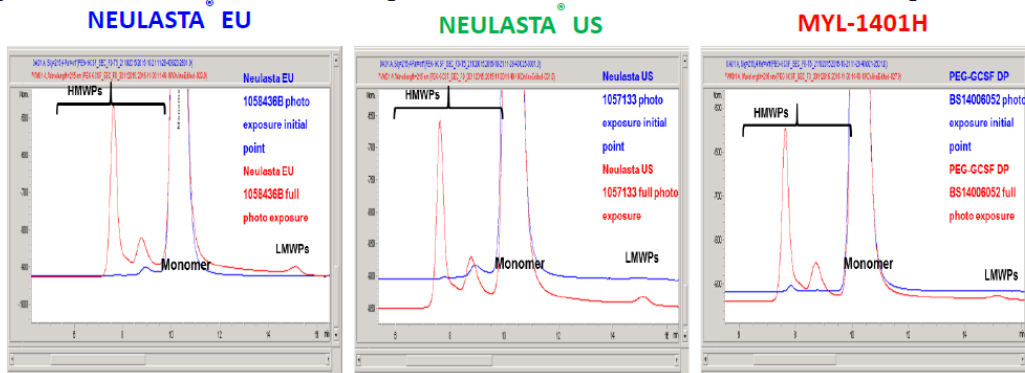


Figure 80. Representative comparison of SE-HPLC UV chromatograms of EU approved Neulasta 1058436B and US licensed Neulasta 1057133 batches with MYL-1401H drug product BS1400652 for Photo exposure condition for the initial and end time point.



The effects of photoexposure on the RP-HPLC profile for MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta are shown below.

Figure 81. Graphical presentation and respective degradation statistics of quality attributes such as purity (main peak) and impurities (total pre-peaks and total post-peaks) evaluated for Photo exposure condition - RP-HPLC analysis

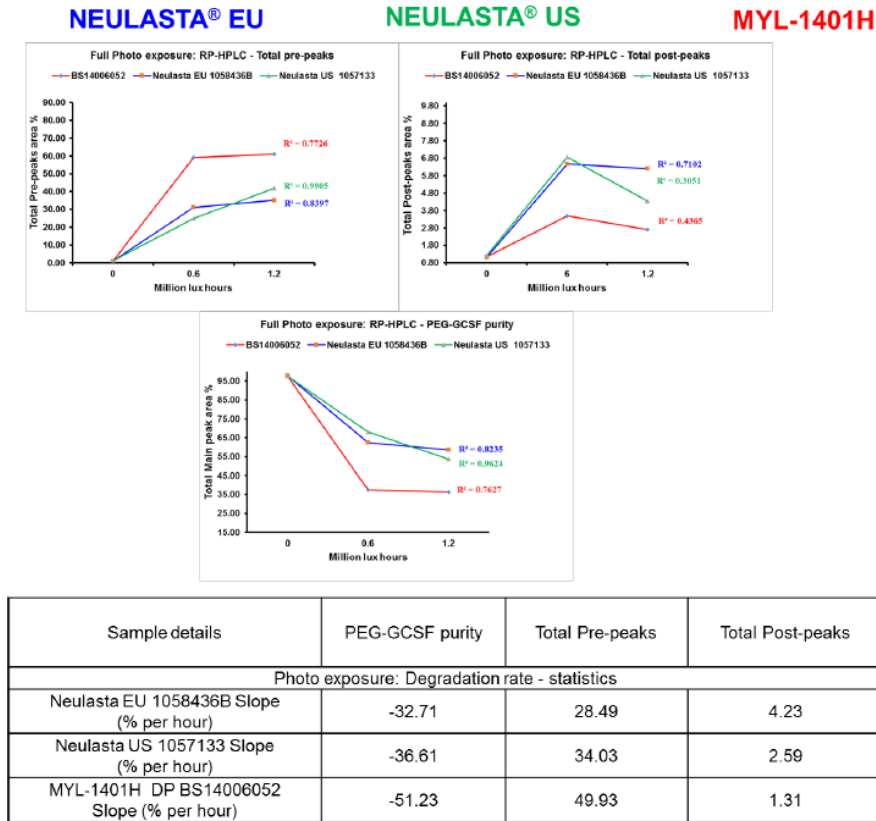
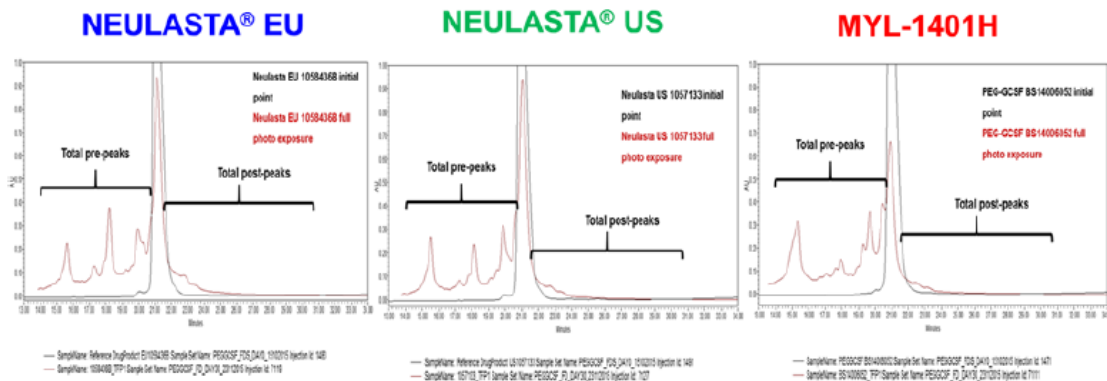


Figure 82. Comparison of RP-HPLC UV chromatograms of EU approved Neulasta 1058436B and US licensed Neulasta 1057133 batches with MYL-1401H drug product BS14006052 for Photo exposure condition for the initial and end time point.



The effects of photoexposure on the relative potency of MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta are shown below.

Figure 83. Relative potency Scatter plot for photo-treatment between MYL-1401H, U.S.-licensed Neulasta, and EU-approved Neulasta



The dotted line in black represents the pass range for the relative potency

Reviewer Comment: After photoexposure, the stability profiles of size variants by SEC-HPLC for all three products compare closely (Figures 79-80). Similar increasing trends in levels of HMWP and LMWP are shown, along with similar decreasing trends in monomer levels, for MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta.

The photostability profiles of hydrophobic variants by RP-HPLC (Figures 81-82) show higher levels of pre-peak oxidation species in MYL-1401H compared to U.S.-licensed Neulasta and E.U.-approved Neulasta. However, relative potency values (Figure 83) show that photoexposure has a similar effect on potency for all three products. Full photoexposure showed no differences in the effect on potency between the products. Slight differences between the three products shown for half photoexposure can be attributed to the variability of the potency assay.

The results from the photostability studies also indicate that MYL-1401H DP is photolabile and should be protected from light during storage, which is consistent with the approved label for U.S.-licensed Neulasta.

Summary - Analytical Similarity:

Table H. Summary of the analytical similarity assessment for MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta.

Quality Attribute	Method/Test	Supports a Conclusion of Highly Similar	Supports an Analytical Bridge	Residual Differences	Notes
Protein Content	UV Abs 280 nm	Yes	Yes		Met equivalence testing; Theoretical Extinction Coefficient
Potency	Mouse NFS-60 cell-based proliferation assay	Yes	Yes		Met equivalence testing Ref Stand Lots bridged
G-CSF Receptor Binding	SPR	Yes	Yes		
Primary Sequence	Peptide Mass Fingerprinting Glu-C and Trypsin	Yes	Yes		
	MALDI-TOF MS	Yes	Yes		
Secondary and Higher Order Structure	Non-reduced Peptide Mass Fingerprint, Glu-C	Yes	Not Tested		Far UV-CD & 1D-NMR supports analytical bridge
	Far-UV-CD	Yes	Yes		$\Theta_{208}/\Theta_{222}$ ratio supports similarity & analytical bridge
	FT-IR	Yes	Yes		
Free Cysteine	Ellman's Assay	Yes	Yes	Minor differences due to method variability	peptide mapping supports Cys similarity
	Extrinsic Fluorescence	Yes	Yes		
	Near-UV-CD	---	---	Spectrum deconvolution inconsistent with literature	1D-NMR supports similar tertiary structure & analytical bridge
	DSC	Yes	Yes		
	1D-NMR (Fingerprint)	Yes	Yes		
	Intrinsic Fluorescence	Yes	Yes		
HMWP - Aggregates	SEC-MALS	Yes	Yes	MYL-1401H lots outside quality ranges 0.55% vs 0.33%	AUC (aggregates) & MFI (particles) support similarity & analytical bridge; HMWP levels are low; Immunogenicity risk historically low
Molecular Weight	AUC	Yes	Yes		
	SEC-MALS	Yes	Yes		
PEG Polydispersity	SEC-MALS	Yes	Yes		
Hydrodynamic radius	SEC-DLS	Yes	Yes		
Dimer	RP-HPLC	Yes	Yes		
N-term PEG confirmation	Peptide Mass Fingerprinting Glu-C and Trypsin	Yes	Yes		
	CNBr, MALDI, MS/MS	Yes	Not Tested		Peptide Mapping Glu-C and Trypsin supports analytical bridge
Di-PEG-G-CSF	CIEX-HPLC	Yes	Yes		
Deamidation Q108	RP-HPLC	Yes	Yes		
LMWP	SEC-MALS	Yes	Yes		
DES-PEG-G-CSF	RP-HPLC	Yes	Yes		
Methionine Oxidation	RP-HPLC	Yes	Yes		Met122, 127, 138
Free PEG	RP-HPLC-CAD	Yes	Yes		
pI	cIEF	Yes	Yes		
Sub-Visible Particles	MFI	Yes	Yes		
Size Variants	CE-SDS	Yes	Yes		
Stability Profile	Multiple Methods	Yes	Yes		

Reviewer's Conclusion for Analytical Similarity

The analytical characterization of MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta is extensive and robust and implemented multiple orthogonal methods that adequately evaluated the necessary attributes for complete characterization of the products. The characterization of the products is consistent with "FDA Guidance for Industry - Quality Considerations in Demonstrating Biosimilarity of a Therapeutic Protein Product to a Reference Product, April 2015."

The analytical similarity studies included a sufficient number of lots of MYL-1401H, including the lots used for clinical studies. All of the MYL-1401H lots studied for analytical similarity were manufactured according to the proposed commercial process.

Differences between MYL-1401H and U.S.-licensed Neulasta were observed and addressed:

- *Data submitted for near-UV-CD analyses to compare the tertiary structure of MYL-1401H and U.S.-licensed Neulasta were inadequate to establish similarity because the experiments were not conducted appropriately. However, the sponsor provided 1D-NMR, intrinsic and extrinsic fluorescence, and differential scanning calorimetry data to support a similar tertiary structure. These data support a similar tertiary structure between the products.*
- *Levels of HMWP in MYL-1401H detected by SE-HPLC and identified as aggregates by the sponsor were slightly higher than the quality range for both U.S.-licensed Neulasta and E.U.-approved Neulasta. The difference in HMWP species between MYL-1401H and the comparator products is ~0.19%. On the other hand, AUC and MFI results show similar low levels of aggregates and particles, respectively. The difference in HMWP species identified by SEC is justified by their low levels of aggregates and a risk assessment of potential impact on product quality.*
- *A photostability study showed higher levels of pre-peak oxidation species by RP-HPLC in MYL-1401H as compared to U.S.-licensed Neulasta and E.U.-approved Neulasta. This difference was addressed because the photoexposed samples showed similar relative potency values for MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta.*

In conclusion, the totality of the analytical similarity data support that MYL-1401H is highly similar to U.S.-licensed Neulasta notwithstanding minor differences in clinically inactive components.

The pair-wise analytical comparisons of MYL-1401H, U.S.-licensed Neulasta, and E.U.-approved Neulasta, support an analytical bridge based on the extensive pair-wise analytical characterization. The analytical similarity data do not raise residual uncertainties about the similarity of MYL-1401H and U.S.-licensed Neulasta.

5.3.1.4 Reports of Bioanalytical and Analytical Methods for Human Studies

Deferred to the immunogenicity reviewer



James (Paul)
Kirwan

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