CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

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CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW(S)

Office of Clinical Pharmacology
351(k) Biosimilar Review

351(k) BLA Number	761075
Applicant	Mylan GmbH
Submission Date	December 9, 2016
Submission Type	Standard
Link to EDR	\\CDSESUB1\evsprod\BLA761075\
Brand (Generic) Name	MYL-1401H
Dosage Form and Strength	6 mg/0.6 mL in a single-dose prefilled syringe (PFS)
Route of Administration	Subcutaneous (SC)
Proposed Indication(s)	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
Associated IND	123389
Reference Product Information (U.S.-licensed)	
Brand (Generic) Name	Neulasta (pegfilgrastim)
Dosage Form and Strength	6 mg/0.6 mL in a single-dose prefilled syringe (PFS)
OCP Review Team Signers	
OCP Review Team	Saeho Chong, PhD, Sarah J. Schrieber, PharmD
OCP Final Signatory	Nam Atiqur Rahman, PhD

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1. EXECUTIVE SUMMARY

This Biologic License Application (BLA) for MYL-1401H (pegylated recombinant human granulocyte stimulating factor (PEG-G-CSF)) has been submitted under Section 351(k) of the Public Health Service Act (42 U.S.C. 262(k)). The applicant is seeking approval for MYL-1401H as a proposed biosimilar to US-licensed Neulasta licensed under BLA 125031 by Amgen Inc. The applicant is seeking licensure only for the neutropenia indication. The applicant submitted pharmacokinetic (PK), pharmacodynamics (PD), immunogenicity data and a comparative clinical study to support a demonstration of no clinically meaningful difference between MYL-1401H and US-licensed Neulasta.

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 $AUC_{0\text{-}inf}$ and PD endpoints of ANC_{max} and ANC $AUEC_{last}$. 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 indicates that there is no increase in immunogenicity risk for MYL-1401H as compared to US-licensed 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 along with the analytical similarity allows for relying on data from the study using EU-approved Neulasta as a comparator product for the overall biosimilarity assessment. In conclusion, 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.

1.1 Recommendations

The Office of Clinical Pharmacology recommends approval of MYL-1401H based on demonstration of PK and PD similarity between MYL-1401H and US-licensed Neulasta.

Review Issue	Recommendations and Comments
Pivotal evidence of PK similarity	PK similarity was demonstrated between MYL-1401H and US-licensed Neulasta, as well as the PK portion of the scientific bridge between MYL-1401H, US-licensed Neulasta, and EU-approved Neulasta. The 90% CI of the geometric mean ratio for each product pairwise comparison for the primary PK endpoints of C_{max} and AUC_{inf} fell within the pre-specified margin of 80-125%.
Pivotal evidence of PD similarity	PD similarity was demonstrated between MYL-1401H and US-licensed Neulasta, as well as the PD portion of the scientific bridge between MYL-1401H, US-licensed Neulasta, and EU-approved Neulasta. The 90% CI of the geometric mean ratio for each product pairwise comparison for the primary PD endpoints of ANC_{max} and ANC $AUEC_{last}$ fell within the pre-specified margin of 80-125%.
Evidence of immunogenicity comparability	The results of Study MYL-1401H-3001 indicate similar incidence of anti-drug antibodies (ADA) for MYL-1401H and EU-approved Neulasta. The results of Study MYL-1401H-1002 indicate similar incidence of ADA for MYL-1401H and US-licensed Neulasta.

1.2 Post-Marketing Requirements and Commitments

None

2. SUMMARY OF CLINICAL PHARMACOLOGY ASSESSMENT

2.1 Clinical Pharmacology and Pharmacokinetics

MYL-1401H is a proposed biosimilar to US-licensed Neulasta. US-licensed Neulasta (pegfilgrastim) is a covalent conjugate of recombinant methionyl human G-CSF (filgrastim) and monomethoxypolyethylene glycol. Pegfilgrastim is obtained by covalently binding a 20kD monomethoxypolyethylene glycol molecule to the N-terminal methionyl residue of filgrastim. Filgrastim is obtained as a result of the bacterial fermentation of a strain of *Escherichia coli* with a genetically engineered plasmin containing the human G-CSF gene. Details on the clinical pharmacology of US-licensed Neulasta can be found in the product label (USPI).

In Study MYL-1401H-1001, the 90% confidence interval (CI) for the geometric mean ratios of the primary PK endpoints of C_{max} and $AUC_{0\text{-}inf}$ and the primary PD endpoints of ANC_{max} and ANC $AUEC_{last}$ were within the pre-specified limits of 80% to 125% in the pairwise comparisons between MYL-1401H, US-licensed Neulasta, and EU-approved Neulasta, as summarized in **Table 1**.

Table 1. Summary of Statistical analyses for assessment of PK and PD (ANC) similarity (Study MYL-1401H-1001)

Comparison	Geometric Mean Ratio* (90% CI)			
	PK Endpoints		PD Endpoints	
	C_{max}	$AUC_{0\text{-}inf}$	ANC_{max}	ANC $AUEC_{last}$
MYL-1401H vs US-licensed Neulasta	98.7 (90.3-107.9)	99.6 (92.8-106.9)	99.7 (97.2-102.3)	100.4 (98.7-102.1)
MYL-1401H vs EU-approved Neulasta	107.0 (98.2-117.4)	104.6 (97.5-112.3)	99.4 (97.3-101.6)	99.1 (97.5-100.1)
EU-approved Neulasta vs US-licensed Neulasta	92.2 (84.9-100.0)	95.6 (89.4-102.2)	100.8 (98.4-103.2)	101.8 (100.2-103.4)

*Presented as percent

Overall, the submitted clinical pharmacology studies adequately demonstrate similarity of PK and PD (ANC) among MYL-1401H, US-licensed Neulasta, and EU-approved Neulasta. The PK and PD results support a demonstration of no clinically meaningful differences between MYL-1401H and US-licensed Neulasta, and add to the totality of the evidence to support a demonstration of biosimilarity of MYL-1401H and US-licensed Neulasta.

The incidence of 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 was also compared in a multiple-dose, parallel-arm study in 50 healthy subjects (MYL-1401H-1002). The results of the studies indicate similar incidence and titers of anti-drug antibodies (ADAs) 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 indicates that there is no increase in immunogenicity risk for MYL-1401H as compared to US-licensed Neulasta, which supports the demonstration that there are no clinically meaningful differences between MYL-1401H and US-licensed Neulasta.

2.2 Outstanding Issues

None

3. COMPREHENSIVE CLINICAL PHARMACOLOGY REVIEW

3.1 Regulatory Background

3.1.1 Describe relevant regulatory history for the review of this 351(k) BLA.

MYL-1401H is a proposed biosimilar to US-licensed Neulasta. The applicant is seeking the 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". The applicant is not seeking the indication "to increase survival in patients acutely exposed to myelosuppressive doses of radiation (Hematopoietic Sub syndrome of Acute Radiation Syndrome)", given that the indication is currently under an exclusivity agreement.

3.2 Clinical Pharmacology Review Questions

3.2.1 What are the design features of the clinical pharmacology and/or clinical studies to support biosimilarity?

The applicant conducted two pivotal clinical pharmacology studies and one comparative clinical study as described in **Table 2**.

Table 2. Summary of Relevant MYL-1401H Clinical Studies

Protocol	Title	Subjects	Objectives	Route/Dose/Duration
PK/PD Similarity Study				
MYL-1401H-1001	Single Center, Randomized, Double-Blind, 3-Period, 3-Treatments, 3-Way Crossover Pharmacokinetics (PK)/Pharmacodynamics (PD) Study To Assess PK, PD, Safety And Tolerability of Myl-1401H After Single Subcutaneous (SC) Injection at One Dose Level (2 mg) Comparing to an US-licensed Neulasta and EU-approved Neulasta in Healthy Subjects	Healthy (N=216)	PK, PD (ANC), safety, tolerability, immunogenicity	2 mg SC single dose of MYL-1401H vs. US-licensed Neulasta vs. EU-approved Neulasta with ≥ 28 day washout
Immunogenicity Study				
MYL-1401H-1002	Single Center, Randomized, Open-Label, Parallel Study to Compare Immunogenicity, Safety, and Tolerability of Myl-1401H and US-licensed Neulasta after Two SC Injections at One Dose Level (6 mg) in Healthy Subjects	Healthy (N=50)	PK, PD, safety, tolerability, immunogenicity	Two 6 mg SC doses of either MYL-1401H or US-licensed Neulasta with ≥ 28 day washout
Comparative Clinical Study				
MYL-1401H-3001	A Multicenter, Double-Blind, Randomized, Comparative Clinical Study of MYL-1401H and EU-approved Neulasta in Stage II/III Breast Cancer Patients Receiving Neoadjuvant or Adjuvant Chemotherapy	Breast Cancer (N=194)	Efficacy, safety at the end of cycle 1	6 mg SC doses of either MYL-1401H or EU-approved Neulasta once per chemotherapy cycle

Study MYL-1401H-1001 was used to support PK and PD (ANC) similarity. This study was a single-dose, randomized, double-blind, 3-period, 3-treatment, 3-way crossover study designed to compare the PK and PD (ANC) profiles of MYL-1401H, US-licensed Neulasta, and EU-approved Neulasta administered as a single 2 mg subcutaneous (SC) dose to healthy subjects (N=216). The pre-specified PK endpoints were C_{max} and $AUC_{0\text{-}inf}$ and the pre-specified PD endpoints were ANC_{max} and ANC $AUEC_{last}$. PK and PD similarities were concluded if the 90% CI of the geometric mean ratios for each pairwise comparison between MYL-1401H, US-licensed Neulasta, and EU-approved Neulasta were within the pre-specified limits of 80% to 125%.

The study design of Study MYL-1401H-1001 is considered adequate due to the following reasons:

1. A cross-over study design is recommended for products with short half-life and the PD (ANC) response is rapid.
2. A study in healthy subjects is considered safe and more sensitive compared with that in patients with potentially confounding factors such as underlying disease, concomitant medications, and other factors.
3. Considering PK assay sensitivity, dose-exposure linearity, and tolerability, a single SC dose of 2 mg pegfilgrastim is considered acceptable.
4. The 4-week washout between each treatment period was adequate. Serum pegfilgrastim concentrations were below the lower limit of quantitation (LLOQ) by Day 8 post-dose after each treatment period, and ANC had returned to baseline by around Day 15 after each treatment as well.

Study MYL-1401H-3001 was a comparative clinical study in patients with stage II/III breast cancer receiving neoadjuvant or adjuvant chemotherapy. The incidence of immunogenicity for MYL-1401H and EU-approved Neulasta was compared in 194 patients undergoing chemotherapy. The incidence and titers of ADAs during the first 6 cycles of chemotherapy were similar between the two products. Refer to Clinical review for further details.

Study MYL-1401H-1002 was an open-label, randomized, parallel-arm study evaluating the incidence of immunogenicity of MYL-1401H and US-licensed Neulasta following two doses of 6 mg SC in 50 healthy subjects. The results indicate similar incidence and titers of anti-drug antibodies (ADA) for both products.

The data from these two immunogenicity studies indicates that there is no increase in immunogenicity risk for MYL-1401H as compared to US-licensed Neulasta, which supports the demonstration that there are no clinically meaningful differences between MYL1401H and US-licensed Neulasta.

3.2.2 What are the endpoints in the clinical pharmacology and/or clinical studies to support biosimilarity?

In Study MYL-1401H-1001, the pre-specified PK similarity criteria for C_{max} and $AUC_{0\text{-}inf}$ were that the 90% CI of the geometric mean ratio should lie within 80-125%. This margin proposed by the applicant was acceptable. The pre-specified PD (ANC) similarity criteria for maximum ANC count (ANC_{max}) and area under the effect curve (ANC $AUEC_{last}$) were that the 95% CI of the geometric mean ratio should lie within 80 - 125%. The proposed 95% CI was not acceptable, and FDA applied a 90% CI of the geometric mean ratio with a margin of 80-125% for the PD (ANC) similarity analyses.

- PK serum samples were collected on Day 1 at predose, 2, 4, 6, 8, 10, 12, 16, 20, 24, 48, 72, 96, 120, 144, 168, 192, 264, 336, and 504 hours postdose.
- PD (ANC) serum samples were collected on screening, Day -1, -1, -0.5 hours before dose and on Day 1 at predose, 0.5, 1, 2, 4, 8, 12, 24, 48, 72, 96, 120, 144, 168, 192, 264, 336, and 504 hours postdose.

In Study MYL-1401H-1002,

- PK serum samples were collected in both periods on Day -1 (predose), 168 (Day 8), 336 (Day 15), and 504 (Day 22) hours postdose and at follow-up (Day 29).

3.2.3 Are the pharmacologically active moieties of the proposed biosimilar and the reference product in plasma (or other biological matrix) appropriately identified and measured to assess the PK parameters?

Yes. See **Section 4.1** for details. PEG-G-CSF levels were measured in serum by a validated enzyme-linked immunosorbent assay (ELISA). Absolute neutrophil counts (ANC) were determined using appropriate hematology analyzers.

3.2.4 Is PK similarity met?

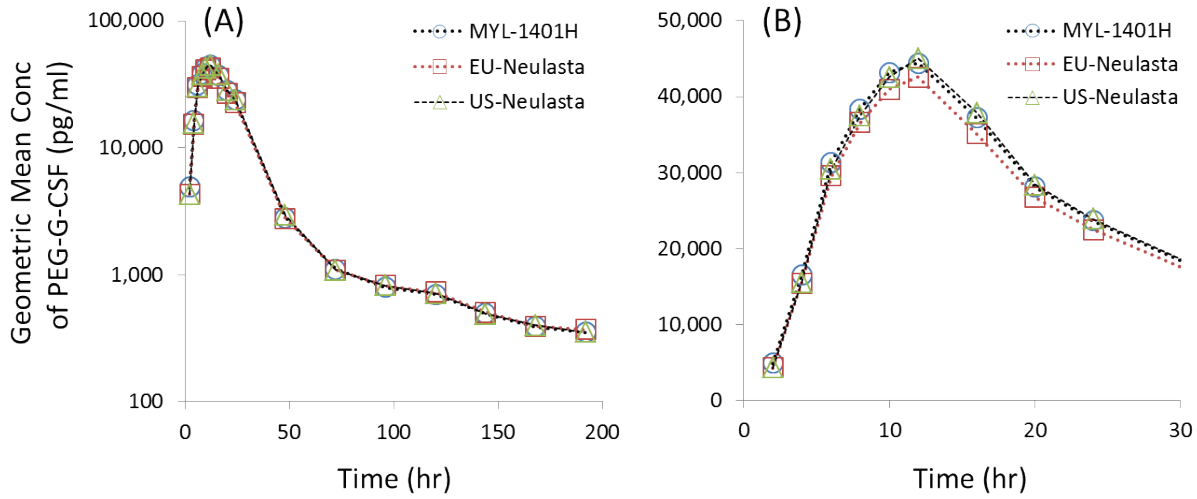
Yes, PK similarity between MYL-1401H, US-licensed Neulasta, and EU-approved Neulasta was demonstrated, where the 90% confidence intervals of geometric mean ratios of PK endpoints for each product pairwise comparison were contained within prospectively defined criteria of 80 to 125% (**Table 3**). Also, as shown in **Figure 1**, the PK profiles of MYL-1401H, US-licensed Neulasta, and EU-approved Neulasta overlay each other. A summary of the 2 mg SC single dose PK parameters from Study MYL-1401H-1001 for each product is shown in **Table 4**.

Table 3. Summary statistical analyses for PK similarity (Study MYL-1401H-1001)

Comparison	Geometric Mean Ratio* (90% CI)		
	$AUC_{0\text{-}inf}$	$AUC_{0\text{-}t}$	C_{max}
MYL-1401H vs US-licensed Neulasta	99.6 (92.8-106.9)	100.0 (92.8-107.8)	98.7 (90.3-107.9)
MYL-1401H vs EU-approved Neulasta	104.6 (97.5-112.3)	105.4 (97.8-113.5)	107.4 (98.2-117.4)
EU-approved Neulasta vs US-licensed Neulasta	95.6 (89.4-102.2)	95.3 (88.8-102.2)	92.2 (84.9-100.0)

*Presented as percent

Figure 1. Geometric mean serum PEG-G-CSF concentration vs. time profile (Study MYL-1401H-1001)



(A) concentrations in logarithmic scale, (B) concentrations in linear scale: time from 0 to 30 hours postdose only is presented for clarity

Table 4. Summary of PK parameters (Study MYL-1401H-1001)

PK parameters	Geometric Mean (% CV)		
	MYL-1401H (n=204)	US-licensed Neulasta (n=207)	EU-approved Neulasta (n=203)
C_{max} (ng/mL)	36.7 (101)	37.3 (99)	34.2 (110)
AUC_{0-t} (ng/mL*hr)	827.0 (85)	832.4 (85)	783.0 (90)
AUC_{0-inf} (ng/mL*hr)	861.5 (80)	870.3 (79)	822.1 (83)
V_z/F (L)	126.2 (123)	137.3 (116)	137.6 (128)
CL/F (L/day)	55.7 (80)	55.2 (79)	58.4 (83)

% CV: coefficient of variation, V_z/F: apparent volume of distribution, CL/F: clearance

3.2.5 Is PD similarity met?

Yes, PD (ANC) similarity between MYL-1401H, US-licensed Neulasta, and EU-approved Neulasta was demonstrated, where the 90% confidence intervals of geometric mean ratios of PD endpoints for each product pairwise comparison were contained within prospectively defined criteria of 80 to 125%. (**Table 5**). Also, as shown in **Figure 2**, the PD profiles of MYL-1401H, US-licensed Neulasta, and EU-approved Neulasta overlay each other. A summary of PD parameters from Study MYL-1401H-1001 for each product is shown in **Table 6**.

Table 5. Summary statistical analyses for PD similarity (Study MYL-1401H-1001)

Comparison	Geometric Mean Ratio* (90% CI)	
	ANC_{max}	ANC $AUEC_{last}$
MYL-1401H vs US-licensed Neulasta	99.7 (97.2-102.3)	100.4 (98.7-102.1)
MYL-1401H vs EU-approved Neulasta	99.4 (97.3-101.6)	99.0 (97.5-100.1)
EU-approved Neulasta vs US-licensed Neulasta	100.8 (98.4-103.2)	101.8 (100.2-103.4)

*Presented as percent

Figure 2. Geometric mean ANC vs. time profile (Study MYL-1401H-1001)

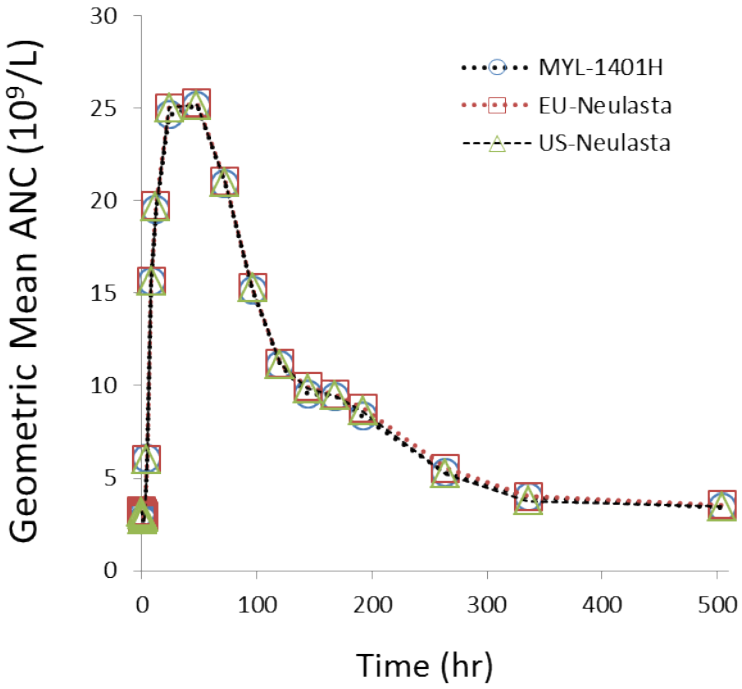


Table 6. Summary of PD parameters (Study MYL-1401H-1001)

PK parameters	Geometric Mean (%CV)		
	MYL-1401H (n=204)	US-licensed Neulasta (n=207)	EU-approved Neulasta (n=203)
ANC_{max} (10^9/L)	25.6 (25)	25.7 (28)	25.9 (25)
ANC $AUEC_{last}$ (h*10^9/L)	4308.3 (22)	4300.0 (25)	4380.7 (22)

% CV: coefficient of variation

Immunogenicity

3.2.6 Is the immunogenicity assay capable of detecting the antidrug antibodies (ADA) in the presence of concentration of product in the study samples?

The sensitivities of the ADA assay were 7.9 ng/ml for anti-PEG-G-CSF and 6.1 ng/ml for anti-PEG. The low positive control was 30 ng/ml and 150 ng/ml for anti-PEG-G-CSF and anti-PEG, respectively. The high positive control was 2000 ng/ml for both anti-PEG-G-CSF and anti-PEG. The drug tolerance of the ADA assay was 31.25 ng/ml for both anti-PEG-G-CSF and anti-PEG at 1 µg/mL of MYL-1401H.

Refer to the immunogenicity assay review by the Office of Biological Products review team for details regarding the assays.

3.2.7 Is the sampling plan adequate to capture baseline, early onset, and dynamic profile (transient or persistent) of anti-drug antibodies (ADA) formation?

The sampling schedules in the studies were appropriate to minimize interference from the presence of the product in the samples, if the ADA assay is not drug-tolerant. The sampling schedules for the studies were as follows:

- Study MYL-1401H-3001: Day 1 of Cycle 1 (baseline), and Day 21 of Cycles 2, 4, 6, and at follow-up (168 days from Day 2 Cycle 1).
- Study MYL-1401H-1002 (both periods): Day -1 (baseline), Days 8, 15, and 22 post-dose and at follow-up (Day 29).

3.2.8 What is the incidence of anti-drug antibodies (ADA)? (Provide the incidence of pre-existing antibodies at baseline and the incidence of ADA throughout the study.)

In Study MYL-1401H-3001, similar titers (data not shown) and incidences of ADAs during the first 6 cycles of chemotherapy were observed for MYL-1401H and EU-approved Neulasta when utilizing a confirmatory cut-point with a 1.0% false-positive error rate (**Table 7**).

Table 7. Immunogenicity results for binding ADA in Study MYL-1401H-3001

	N*	Anti-PEG-G-CSF		nAb
		Baseline	Treatment-Induced	
MYL-1401H	126	21/126 (16.7%)	0/105 (0%)	0/126 (0%)
EU-approved Neulasta	67	14/67 (20.9%)	1/53** (1.9%)	0/67 (0%)

*Number of subjects

**Patient tested positive for anti-PEG-G-CSF and anti-PEG at Cycle 2 Day 21 only

In Study MYL-1401H-1002, similar titers (data not shown) and incidences of ADAs were observed for MYL-1401H and US-licensed Neulasta when utilizing a confirmatory cut-point with a 1.0% false-positive error rate (**Table 8**).

Table 8. Immunogenicity results for binding ADA in Study MYL-1401H-1002

	N*	Anti-PEG-G-CSF			Anti-G-CSF***	Anti-PEG***	nAb
		Baseline	Treatment-Induced	Treatment-Induced, Persistent**			
MYL-1401H	25	3/25 (12.0%)	6/22 (27.3%)	3/22 (13.6%)	1/22 (4.5%)	5/22 (22.7%)	1/22 (4.5%)
US-licensed Neulasta	25	1/25 (4.0%)	7/24 (29.2%)	2/24 (8.3%)	2/24 (8.3%)	6/24 (25.0%)	0/24 (0%)

*Number of subjects
**Persistent is defined as at least 2 positive time points, with at least 1 positive time point after the second dose (Period 2).
***Subjects with treatment-induced ADAs included only.

The data from the two studies indicate that there is no increase in immunogenicity risk for MYL-1401H as compared to EU-approved Neulasta and US-licensed Neulasta, respectively, and supports the demonstration that there are no clinically meaningful differences between MYL-1401H and US-licensed Neulasta. Of note, a scientific bridge was established between MYL-1401H, US-licensed Neulasta, and EU-approved Neulasta, supporting the relevance of comparative data, including immunogenicity data, generated using EU-approved Neulasta to support a demonstration of no clinically meaningful differences between MYL-1401H and US-licensed Neulasta.

3.2.9 Do the anti-drug antibodies (ADA) have neutralizing activity?
In Study MYL-1401H-3001, no patients in either group (MYL-1401H or EU-approved Neulasta) tested positive for neutralizing antibodies.

In Study MYL-1401H-1002, 1 of 22 subjects (4.5%) and 0 of 24 subjects (0%) in MYL-1401H and US-licensed Neulasta groups, respectively, tested positive for neutralizing antibodies.

3.2.10 What is the impact of anti-drug antibodies (ADA) on the PK, PD, efficacy, and safety of the therapeutic protein?
In Study MYL-1401H-3001, the impact of ADA on PK cannot be assessed as PK samples were only collected on Day 21 post-dose during Cycles 2, 4, and 6; pegfilgrastim serum concentrations in patients on Day 21 post-dose were below the lower limit of quantification (300 pg/ml). Furthermore, the impact of ADA on PD (ANC), efficacy, or safety endpoints cannot be concluded due to the small number of patients with treatment-induced ADAs (e.g., no patient sample was ADA positive in the MYL-1401H treatment group and only 1 patient sample was ADA positive in EU-approved Neulasta treatment group (see **Table 7**)).

In Study MYL-1401H-1002, PK samples were collected weekly (e.g., Day 8, 15, and 22 in both periods) after administration of either MYL-1401H or US-licensed Neulasta. The Cmax of pegfilgrastim is generally reached 10 to 12 hours postdose, and the concentrations on Day 8 in most subjects were variable and close to LLOQ (300 pg/ml). Therefore, the assessment of the impact of ADA on PK is

limited. No remarkable differences in PD (ANC) were observed between ADA-negative and ADA-positive subjects within MYL-1401H and US-licensed Neulasta groups.

4. APPENDICES

4.1 Summary of Bioanalytical Method Validation and Performance

4.1.1 Pharmacokinetics

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

Total serum PEG-G-CSF concentrations were determined using a validated Enzyme-Linked Immunosorbent Assay (ELISA). The method for the quantification of MYL-1401H, US-licensed Neulasta and EU-approved Neulasta in human serum was validated at (b) (4) (Report # 8308905). The validation report (8308905) was reviewed and deemed sufficient to support the quantitation of PEG-G-CSF in Study MYL-1401H-1001. A summary of the ELISA assay validation for MYL-1401H, US-licensed Neulasta, and EU-approved Neulasta is show in **Table 9.**

Briefly, a monoclonal antibody specific for G-CSF (Granulocyte Colony Stimulating Factor) as a capture antibody is pre-coated onto a microtiter plate. This antibody binds to pegylated G-CSF (MYL-1401H, US-licensed Neulasta, and EU-approved Neulasta). The bound PEG-G-CSF is detected by the addition of HRP conjugated polyclonal antibody specific for G-CSF recognizing the G-CSF part of PEG-G-CSF followed by addition of the chromogenic substrate TMB. The color development is stopped by addition of acid and the product of this reaction is detected with a spectrophotometer at 450 nm, with a background subtraction at 630 nm. The concentrations of PEG-G-CSF in samples are back-calculated from the calibration curve using Soft Max Pro 6.3 GXP software.

Table 9. Summary of MYL-1041H, US-licensed Neulasta and EU-approved Neulasta Assay Validation Reports

Analyte	MYL-1401H	US-licensed Neulasta	EU-approved Neulasta
Matrix	Human Serum		
Reference or analytical standard	Batch BS14005356, (b) (4) mg/ml	Batch 1044948, 10.0 mg/ml	Batch 1047632A, 10.(b) (4) mg/ml
Minimum dilution	1 in 10		
Standard curve concentrations (pg/mL)	100 (anchor), 300 (LLOQ), 600, 1100, 1600, 2200, 3000, 3800, 4600 (ULOQ), and 6400 (anchor) pg/ml		
Limit of detection	100 pg/ml		
LLOQ	300 pg/ml		
ULOQ	4600 pg/ml		
Standard curve accuracy (%Bias) from 300 to 4600 pg/ml	-1.4 to 3.4		

Inter-assay Precision: %CV	LLOQ (300 pg/ml): 8.5 Low (900 pg/ml): 10.5 Mid (2300 pg/ml): 5.5 High (3450 pg/ml): 7.5 ULOQ (4600 pg/ml): 7.0	LLOQ (300 pg/ml): 12.1 Low (900 pg/ml): 5.5 Mid (2300 pg/ml): 7.6 High (3450 pg/ml): 8.3 ULOQ (4600 pg/ml): 9.5	LLOQ (300 pg/ml): 10.2 Low (900 pg/ml): 6.0 Mid (2300 pg/ml): 6.5 High (3450 pg/ml): 5.3 ULOQ (4600 pg/ml): 7.1
Inter-assay Accuracy: %Bias	LLOQ (300 pg/ml): 7.8 Low (900 pg/ml): 10.4 Mid (2300 pg/ml): 2.0 High (3450 pg/ml): 2.7 ULOQ (4600 pg/ml): 1.0	LLOQ (300 pg/ml): 11.8 Low (900 pg/ml): 2.7 Mid (2300 pg/ml): 2.3 High (3450 pg/ml): 5.2 ULOQ (4600 pg/ml): 0.7	LLOQ (300 pg/ml): 1.5 Low (900 pg/ml): -3.9 Mid (2300 pg/ml): -3.2 High (3450 pg/ml): -3.9 ULOQ (4600 pg/ml): -5.2
Intra-assay Precision: %CV	LLOQ (300 pg/ml): <13.1 Low (900 pg/ml): <15.7 Mid (2300 pg/ml): <7.6 High (3450 pg/ml): <10.0 ULOQ (4600 pg/ml): <10.9	LLOQ (300 pg/ml): <11.7 Low (900 pg/ml): <5.2 Mid (2300 pg/ml): <4.6 High (3450 pg/ml): <5.9 ULOQ (4600 pg/ml): <5.3	LLOQ (300 pg/ml): <20.7 Low (900 pg/ml): <5.1 Mid (2300 pg/ml): <5.4 High (3450 pg/ml): <8.1 ULOQ (4600 pg/ml): <9.8
Intra-assay Accuracy: %Bias	LLOQ (300 pg/ml):-14.2 to 16.9 Low (900 pg/ml):-8.9 to 29.6 Mid (2300 pg/ml):-11.7 to 13.3 High (3450 pg/ml):-14.2 to 16.9 ULOQ (4600 pg/ml):-11.9 to 18.1	LLOQ (300 pg/ml):-6.4 to 41.2 Low (900 pg/ml):-4.6 to 16.9 Mid (2300 pg/ml):-7.3 to 16.3 High (3450 pg/ml):-8.4 to 19.6 ULOQ (4600 pg/ml):-13.1 to 17.5	LLOQ (300 pg/ml): -15.3 to 23.1 Low (900 pg/ml): -10.2 to 12.6 Mid (2300 pg/ml): -10.5 to 13.9 High (3450 pg/ml): -13.4 to 12.1 ULOQ (4600 pg/ml): -12.1 to 17.3
Hook effect	No hook effect observed at the ULOQ		
Linearity in normal human serum sample (% difference from preceding dilution)	From 1:2500 through 1:25000, 80 to 100% samples were within 20% Bias. %CV ≤4.9	From 1:2500 through 1:25000, 80 to 100% samples were within 20% Bias. %CV ≤13.4	From 1:2500 through 1:25000, 100% samples within 20% Bias. %CV ≤5.5
Interference	No matrix effect		
Analyte Room Temperature:	Up to 27 hours	Up to 27 hours	Up to 27 hours
Analyte Freeze/Thaw Stability:	6 cycles	6 cycles	6 cycles
Long-term Stability	Up to 364 days at -60 to -80°C and -15 to -30°C	Up to 364 days at -60 to -80°C and -15 to -30°C	Up to 364 days at -60 to -80°C and -15 to -30°C

In Study MYL-1401H-1001, total serum PEG-G-CSF concentrations were determined by the same ELISA assay as described above and analyzed by (b) (4). The data generated using the ELISA method described above demonstrated that US-licensed Neulasta and EU-approved Neulasta QC recoveries were comparable to MYL-1401H. Therefore, it was acceptable for MYL-1401H standards

and controls to be used for analysis of samples for subjects dosed with any of those three products. A total of 547 sample analysis batches were performed. Of the 547 analysis batches, 23 runs failed due to either the QC (20 out of 23) or calibration curve (3 out of 23) not meeting the pre-specified analysis criteria, and 4 runs failed due to improper documentation. Incurred sample reproducibility was performed on 773 samples and 91.6% of samples met the pre-specified criteria. Samples were stored at -60 to -80°C until time of analysis. The maximum sample storage duration between collection and analysis for all samples was 346 days, which was within the established stability interval of 364 days. A summary of the ELISA assay results are show in **Table 10**.

Table 10. Summary of ELISA assay parameters from Study MYL-1401H-1001

Analyte	MYL-1401H
Matrix	Human Serum
Reference or analytical standard	Batch BS14005356, (b) (4) mg/ml
Minimum dilution	1 in 10
Standard curve concentrations (pg/ml)	100 (anchor), 300 (LLOQ), 600, 1100, 1600, 2200, 3000, 3800, 4600 (ULOQ), and 6400 (anchor) pg/ml
Limit of detection	100 pg/ml
LLOQ	300 pg/ml
ULOQ	4600 pg/ml
Standard curve accuracy (%Bias) from 300 to 4600 pg/ml	-1.3 to 2.9%
Inter-assay Precision: %CV	Low (900 pg/ml): 8.1 Mid (2300 pg/ml): 7.3 High (3450 pg/ml): 7.5
Inter-assay Accuracy: %Bias	Low (900 pg/ml): -6.1 Mid (2300 pg/ml): -9.3 High (3450 pg/ml): -9.9

4.1.2 Pharmacodynamics

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

Absolute neutrophil count (ANC) was determined with an Advia 2120i Hematology analyzer by the (b) (4) using a validated hematology method as detailed in (b) (4) Standard Operating Procedures (SOPs). All assays were validated and reports were submitted.

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/s/

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I concur.

Date: 10 April 2017

From: James L Weaver, PhD, Kristina Howard, PhD; DARS/OCP

Through: David Strauss, MD, PhD; Director DARS/OCP

To: Saeho Chong, Sarah Schrieber; DCP V/OCP

Subject: Evaluation of ELISA assay used for PK/PD in BLA761075 for MYL-1401H

Executive Summary

Original Question: *"Is the assay used to assess the PK and PD of MYL-1401H fit for purpose."*

The sponsor performed an extensive series of validation experiments on the performance of the ELISA assay kit to measure PGE-G-CSF. The data showed that the assay performs well when measuring the plasma concentration of MYL-140H. In addition, the performance was highly similar when measuring MYL-1401H in comparison with either US sourced innovator product or EU-sourced innovator product.

Conclusion: The assay is fit for purpose.

Background

Mylan has submitted a BLA for a proposed biosimilar to the innovator product Neulasta (PEG conjugated G-CSF). The measurement of drug concentration in clinical samples was performed by an ELISA method. Therefore the performance of this assay is critical to the evaluation of the BLA. The question to be addressed is: "Is the assay used to assess the PK and PD of MYL-1401H fit for purpose."

Evaluation

The assay used in this study was performed at (b) (4). A full validation study of the assay was performed and is included in the EDR as Report: 5.3.1.4 Validation of an ELISA Assay for the Quantitation of MYL-1401H/Neulasta in Human Serum. This report includes comparison of measurement of US Neulasta, EU Neulasta and the proposed biosimilar MYL1401H.

Assay Design:
The assay used to measure the reagents was a standard ELISA assay with the capture anti-G-CSF antibody precoated on the plate, the test reagent, and a polyclonal anti-G-CSF conjugated to horseradish peroxidase (HRP) as the detection antibody. Detection was by standard colorimetric assay using 3,3',5,5' - tetramethylbenzidine (TMB) as the HRP substrate. Color is read at 450 nm with a reference background subtraction at 630 nm. All calibration standards, QC samples and validation samples were run in duplicate.

Components:

The CRO purchased the ELISA kit as the Human G-CSF Quantikine kit from R&D Systems including plates pre-coated with the detection antibody as well as the HRP-conjugated detection antibody. The Quantikine kit includes all reagents needed to perform the assay.

Specificity:
The specificity of the capture and detection antibodies was evaluated by the manufacturer of the kit. These were tested against four human cytokines related to G-CSF and no interference or cross-reactivity was observed.

Controls:
For this study, the proposed biosimilar MYL-1401H was used as the calibration standard. Pooled normal serum was used as the matrix. A matrix blank was included on each plate but was not included in the calibration calculations.

Calibrators:
The calibration curve was constructed from data from 10 concentrations of MYL-1401H ranging from 100 pg/ml up to 6,400 pg/ml. The two end concentrations were used as anchor points and the calibration curve was calculated from the middle concentrations. The LLOQ was set at 300 pg/ml and the ULOQ was set at 4,600 pg/ml.

The LLOQ is well above the reported reference value for G-CSF in blood of 14.7 pg/ml (Kim et al, 2011). By comparison, the linear range reported in the R&D Systems product literature ranges from 40 to 2,500 pg/ml using G-CSF without added PEG as the calibration standard. (b) (4) did not report any comparison between unconjugated G-CSF and PEG-G-CSF in their validation report.

In addition to the calibration curve, three QC standards were included at concentrations of 900, 2,300 and 3,450 pg/ml.

Assay Validation

Validation design:
The sponsor reported on an extensive series of experiments to evaluate assay performance. Comparisons were made among MYL-1410H, US sourced Neulasta and EU sourced Neulasta. Specific studies included:

- Inter-assay Precision and Bias
- Comparison at all QC standard concentrations
- Recovery from human serum
- Recovery from hemolysed or lipemic serum
- Dilutional linearity
- Freeze/thaw stability
- Stability at RT and frozen at -20 and -70 C

Validation Performance:

The results of this extensive series of experiments can be summarized by stating that the performance of the assay was well within the standard performance cutoff values. An example of the performance has been extracted from the Validation Study Report. The data are from six runs with three replicates per run. The specific target criteria are <20 for % Coefficient of Variation (CV) and <30 for % Total Error (TE).

From Tables 4a, 4b & 4c						
		Observed				
	QC Sample	**pg/ml**				
Source:	**pg/ml**	**Mean**	**St Dev**	**% CV**	**% Bias**	**% TE**
MYL-1401H	900	993.6	104.1	10.5	10.4	20.8
US Neulasta	900	923.9	50.8	5.5	2.7	8.2
EU Neulasta	900	864.9	52.0	6.0	-3.9	9.9
MYL-1401H	2300	2346.9	130.2	5.5	2.0	4.1
US Neulasta	2300	2352.7	179.9	7.6	2.3	9.9
EU Neulasta	2300	2226.3	143.7	6.5	-3.2	9.7
MYL-1401H	3450	3544.4	267.5	7.5	2.7	5.5
US Neulasta	3450	3628.1	299.7	8.3	5.2	13.5
EU Neulasta	3450	3316.9	175.6	5.3	-3.9	9.2

Assay performance on study:
Results for on-study use of this assay are reported in file: myl-1401h-1001-report-body-3.pdf which reports on the analysis of a total of 12,614 samples from a phase 1 clinical study ((MYL-1401H-1001). Performance of the QC Samples across 520 accepted runs is included as a basis for comparison.

From Clin Study (MYL-1401H-1001)					
	QC Sample				
Source:	**pg/ml**	**Mean**	**St Dev**	**% CV**	**% Bias**
MYL-1401H	900	844.7	68.5	8.1	-6.1
MYL-1401H	2300	2085.2	152.3	7.3	-9.3
MYL-1401H	3450	3109.1	234.0	7.5	-9.9

A total of 23 runs were failed for not meeting system suitability criteria and four runs were rejected due to documentation errors. Incurred sample reproducibility was performed and performance met generally accepted standards.

Summary and Conclusions

The sponsor has reported a detailed and comprehensive assay validation study on the use of the R&D Systems Quantikine G-CSF kit for measurement of PEG-G-CSF in human plasma. Assay performance was comparable in measuring the proposed biosimilar MYL-1410H as compared to US-Neulasta and to EU-Neulasta. No comparison was made between US-Neulasta and EU-Neulasta in this study report. Assay performance on study was acceptable and comparable to the performance observed in the validation study.

(b) (4)

Is the assay used to assess the PK and PD of MYL-1401H fit for purpose?
Yes.

References and Supporting Documents

BLA location in EDR: \\CDSESUB1\evsprod\BLA761075\0000

Report: 5.3.3.1 Application 761075 - Sequence 0000 - 16.1.13.1 Quantitation of MYL-1401H and Neulasta in Human Serum Samples from Phase 1 Clinial Study (MYL-1401H-1001) using ELISA

Report: 5.3.1.4 Validation of an ELISA Assay for the Quantitation of MYL-1401H/Neulasta in Human Serum, file: \\cdsesub1\evsprod\bla761075\0000\m5\53-clin-stud-rep\531-rep-biopharm-stud\5314-bioanalyt-analyt-met\validation\8308-905-report-body.pdf

Kim, HO, Kim –S, Youn J-C, Park S. (2011) Serum cytokine profiles in healthy young and elderly population assessed using multiplexed bead-based immunoassays. *J Translational Med* **9**, 113.

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/s/

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