CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

761039Orig1s000

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW(S)

Office of Clinical Pharmacology
351(k) Biosimilar Review

351(k) BLA Number	761039 (57)
Applicant	Coherus Biosciences Inc
Submission Date	May 3, 2018 (Complete Response)
Submission Type	*Standard*
Link to EDR	\\CDSESUB1\evsprod\BLA761039\
Proprietary Name (Proposed) / Nonproprietary Names	CHS-1701/ UDENYCA
Dosage Form and Strength	6 mg/0.6 mL injection in a single-dose prefilled syringe
Route of Administration	*Subcutaneous (SC)*
Proposed Indication(s)	*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	115573
Reference Product Information (U.S.-licsensed)	
Proprietary (Non-Proprietary) 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	*Sriram Subramaniam, Ph.D.* *Olanrewaju Okusanya,PharmD, MS,*
OCP Final Signatory	*Nam Atiqur Rahman, PhD*

In this document, we generally refer to the applicant's proposed product by the applicant-provided descriptor "CHS-1701", which was the name used to refer to this product during development. Subsequently, the nonproprietary name for this proposed product has been conditionally accepted to be "UDENYCA"

Table of Contents

1. EXECUTIVE SUMMARY

On August 9, 2016, the Applicant originally submitted a new Biologic License Application (BLA) for CHS-1701 [pegylated recombinant human granulocyte stimulating factor (peg-G-CSF)), under Section 351(k) of the Public Health Service Act (42 U.S.C. 262(k)], seeking approval of CHS-1701 as a proposed biosimilar to US-licensed Neulasta licensed under BLA 125031 by Amgen Inc.

In the original submission, the Applicant submitted pharmacokinetic (PK) and pharmacodynamics (PD) data from the following studies to support a demonstration of no clinical meaningful difference between CHS-1701 and US-licensed Neulasta:

1) a pivotal PK/PD similarity study, CHS-1701-05: a randomized, single-blind, partial reference-replicated, 3-sequence, 3-period crossover study in 122 healthy subjects to evaluate PK and PD (absolute neutrophil count (ANC)) following a single 6 mg subcutaneous (SC) dose, and
2) an immunogenicity study, CHS-1701-04: randomized, double-blind, 2-period, parallel-arm study in 303 healthy subjects to assess the immunogenicity of two SC doses. Trough concentrations were collected to evaluate the potential impact of immunogenicity on the PK of pegfilgrastim.

The original clinical pharmacology evaluation found that PK and PD similarity was established in Study CHS-1701-05 as the pairwise comparison of CHS-1701 and the US-licensed Neulasta met the pre-specified acceptance criteria for PK and PD (ANC) similarity (90% CIs of the geometric mean ratios for AUCinf and Cmax (PK) and ANC $AUEC_{inf}$, ANC $AUEC_{last}$, and ANCmax (PD), were within 80 - 125%) (DARRTS ID 4094240). However, the impact of immunogenicity on PK and PD could not be adequately assessed in CHS-1701-04, as the study did not meet the pre-specified endpoints for immunogenicity and the neutralizing antibody assay was unacceptable, as outlined in the CRL.

Following evaluation of the BLA, the Agency issued a Complete Response Letter (CRL) on June 9, 2017 as the Applicant did not fully characterize the immunogenicity of CHS-1701 and provide a comparative immunogenicity assessment of the neutralizing antibody formation between CHS-1701 and US-licensed Neulasta (DARRTS ID 4109894).

In addition, the Office of Study Integrity and Surveillance (OSIS) inspection found that the analysis of peg-G-CSF (PK) were inconsistent in Studies CHS-1701-05 and CHS-1701-04 in that the acceptance of several analytical runs were not objective, which may potentially affect the PK data in the studies.

The current submission is a resubmission of the BLA for CHS-1701, providing a complete response to the deficiencies outlined in the Agency's CRL. In addition to addressing the immunogenicity issues outlined in the CRL, the current submission, provided reprocessed PK data for Studies CHS-1701-05 and CHS-1701-04 to address the OSIS inspectional findings.

The current review is limited to evaluation of:

1) Reprocessing of PK data in Studies CHS-1701-05 and CHS-1701-04,
2) Assessment of PK similarity in Study CHS-1701-05 using the reprocessed PK data, and
3) Evaluation of the impact of immunogenicity on PK in Study CHS-1701-04 using the reprocessed PK data.

The current review finds that the pairwise comparison of CHS-1701 and US-licensed Neulasta using the reprocessed PK data met the pre-specified acceptance criteria for PK similarity, thus confirming PK similarity.

There was a difference in the incidence of anti-drug antibodies (ADA) between CHS-1701 and US-lincesed Neulasta in Study CHS-1701-04, and the study did not meet the pre-specified endpoints. The assessment of the impact of ADA on PK and PD is constrained by the limited number of ADA positive subjects and the high intra-subject variability.

Overall, the PK/PD study support a demonstration of no clinically meaningful difference between CHS-1701 and US-licensed Neulasta, and adds to the totality of the evidence to support a demonstration of biosimilarity of CHS-1701 and US-licensed Neulasta.

1.1 Recommendations

Review Issue	Recommendations and Comments
Pivotal evidence of PK similarity	PK similarity was observed between CHS-1701 and US-licensed Neulasta. The 90% CI of the geometric mean ratio of CHS-1701 to US-licensed Neulasta for Cmax and AUC_{inf} fell within the pre-specified margin of 80-125%.
Pivotal evidence of PD similarity, if applicable	PD similarity was observed between CHS-1701 and US-licensed Neulasta. The 90% CI of the geometric mean ratio of CHS-1701 to US-licensed Neulasta for ANC $AUEC_{last}$ and ANC_{max} fell within the pre-specified margin of 80-125%.
Evidence of immunogenicity comparability	Study CHS-1704 for immunogencity did not meet the predefined endpoint. The impact of ADA on PK and PD is constrained by the limited number of ADA positive subjects and the high intra-subject variability
Other (specify)	None

1.2 Post-Marketing Requirements and Commitments

None

2. SUMMARY OF CLINICAL PHARMACOLOGY ASSESSMENT

2.1 Clinical Pharmacology and Pharmacokinetics

Refer to the original clinical pharmacology review for details of CHS-1701, a proposed biosimilar to US-licensed Neulasta (DARRTS ID 4094240).

The results of the PK similarity assessment in Study CHS-1701-05 using reprocessed PK data (see **Section Error! Reference source not found.** for details on reprocessing) is provided in **Table 1**. The 90% confidence intervals (CI) for AUC_{inf} and Cmax after a single dose were within the pre-defined limits

of 80 - 125%. PD data remained the same as the original submission. Therefore the statistical results were not evaluated in the current submission. The original clinical pharmacology review showed that the results of PD similarity assessment in Study CHS-1701-05 were within the 90% CI limits of 80 - 125% for ANC AUEClast and ANCmax after a single dose (DARRTS ID 4094240).

Table 1. Summary Statistical analyses for assessment of PK and PD (ANC) similarity (Study CHS-1701-05)

Parameter	PK Parameters		PD (ANC) Parameters*	
	Cmax (ng/mL)	AUCinf (ng/mL*hr)	ANCmax (10^9/L)	AUEClast (h•10^9/L)
	(N = 84)	(N = 83)	(N = 85)	(N = 85)
Geometric mean ratio (%)	102	97	99.6	99.8
90% CI	92.4 – 112.0	88.1 – 107	96.2 – 103	97.7 – 102

Ratio (%): CHS-1701/US-Neulasta
*Reproduced from the original clinical pharmacology review (DARRTS ID 4094240)

Overall, the submitted clinical pharmacology study is adequate to demonstrate similarity of PK and PD (ANC) exposure between CHS-1701 and US-licensed Neulasta. Study CHS-1701-05, conducted in healthy subjects, is considered sufficiently sensitive to detect clinically significant differences in PK and PD (ANC) exposure among the products. Single-dose PK and PD (ANC) similarity pre-specified margins were met. The demonstration of similar PK and PD (ANC) exposure supports a finding of no clinically meaningful differences between CHS-1701 and US-licensed Neulasta.

2.2 OSIS status

An OSIS inspection of assays for G-CSF-specific neutralizing antibody (NAb) and titer (Ab titer) of G-CSF-specific binding antibodies at [redacted] (b) (4) was completed on [redacted] (b) (4) and a report was issued on [redacted] (b) (4) (DARRTS ID [redacted] (b) (4)). No significant issues were uncovered during the inspection.

2.3 Outstanding Issues

None.

3. COMPREHENSIVE CLINICAL PHARMACOLOGY REVIEW

3.1 Regulatory Background

3.1.1 If applicable, describe (in tabular format) relevant regulatory history for the review of this 351(k) BLA.

Refer to the earlier clinical pharmacology review (DARRTS ID 4094240).

3.2 Clinical Pharmacology Review Questions

3.2.1 Are the design features of the clinical pharmacology and/or clinical studies to support biosimilarity acceptable?

Refer to the earlier clinical pharmacology review for design, objectives, dose, co-primary PK and PD endpoints, and criteria for PK and PD similarity of Study CHS-1701-05 (DARRTS ID 4094240). The study design of Study CHS-1701-05 was considered adequate. An overview of the two pivotal clinical pharmacology studies is provided in **Table 2**.

Table 2. Summary of Relevant CHS-1701 Clinical Studies

Protocol	Title	Subjects	Objectives	Route/Dose/Duration
PK/PD study to support biosimilarity assessment				
CHS-1701-05	A Randomized, Single-Blind, Crossover Study to Assess the PK and PD Similarity of CHS-1701 with US-Neulasta in Healthy Subjects.	Healthy (n=122)	PK, PD (ANC), safety, tolerability, immunogenicity	6 mg SC single dose of CHS-1701 vs. US-licensed Neulasta with ≥ 28 day washout
Immunogenicity study to support biosimilarity assessment				
CHS-1701-04	A Randomized, Double-Blind, 2-period, Parallel-Arm Study to Assess the Immunogenicity of 2 SC Doses of CHS-1701 Compared with 2 Subcutaneous Doses of US-Neulasta in Healthy Subjects	Healthy (n=303)	PK, PD, safety, tolerability, immunogenicity	Two 6 mg SC doses of either CHS-1701 or US-licensed Neulasta with a 6 week washout between periods

A schematic of the study is shown in **Table 3**. The PK endpoints evaluated in this study were AUC_{inf}, AUC_{last}, and Cmax and the PD (ANC) endpoints were ANC $AUEC_{last}$ and ANCmax. PK and PD similarity is concluded if the 90% CI of the geometric mean ratios for the PK and PD (ANC) endpoints were within 80% to 125% between CHS-1701 to US-licensed Neulasta.

Table 3. Study CHS-1701-05 Schematic

	Period 1	Period 2	Period 3
Sequence A	CHS-1701	Neulasta	Neulasta
Sequence B	Neulasta	CHS-1701	Neulasta
Sequence C	Neulasta	Neulasta	CHS-1701

The PK/PD Study CHS-1701-05 was considered critical in the assessment of no clinically meaningful difference in safety, purity, and potency between CHS-1701 and US-licensed Neulasta.

3.2.2 Are the endpoints in the clinical pharmacology and/or clinical studies to support biosimilarity acceptable?

Refer to the original clinical pharmacology review for PK and PD sampling time points in Studies CHS-1701-05 and CHS-1701-04 and co-primary PK and PD endpoints and the expected criteria for PK and PD similarity for Study CHS-1701-05 (DARRTS ID 4094240).

3.2.3 Are the pharmacogicallyactive 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. Refer to the original clinical pharmacology review (DARRTS ID 4094240) and **Appendix 4**. for details. Peg-G-CSF was measured to characterize the PK and was measured in plasma by a validated enzyme-linked immunosorbant assay (ELISA). Absolute neutrophil counts (ANC) were determined using appropriate hematology analyzers.

3.2.4 Is PK similarity met?

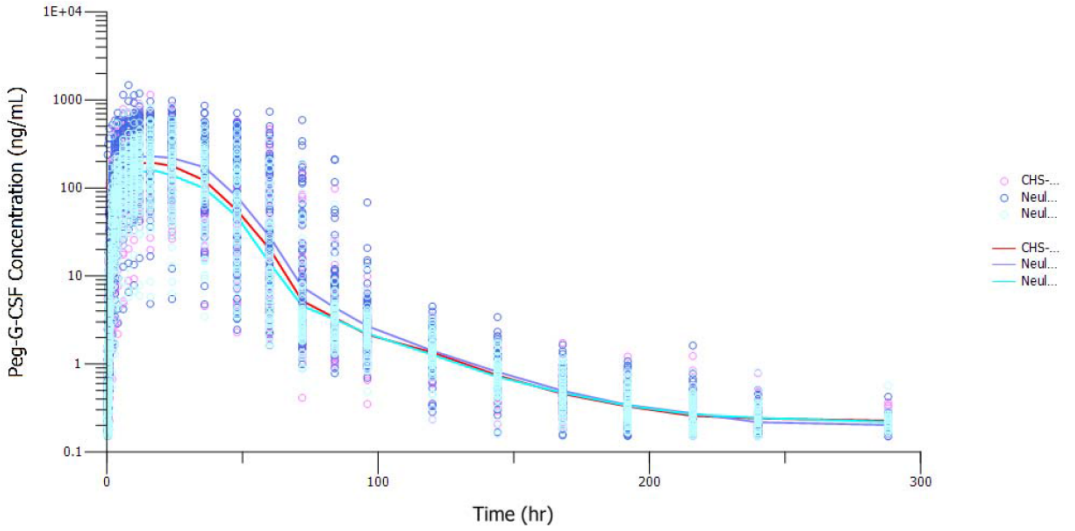
Yes. PK similarity between CHS-1701 and US-licensed Neulasta was demonstrated in that the 90% confidence intervals of geometric mean ratios of the PK endpoints were contained within prospectively defined criteria of 80 to 125% (**Table 4**).

Table 4. Statisitical analyses for assessment of PK similarity (Study CHS-1701-05)

PK Parameters	Geometric Mean Ratio (90% CI)
Cmax (ng/L)	102 (92.4 – 112.0)
AUClast (ng/L•hr)	97.1 (88.2 – 106.9)
AUCinf (ng/L•hr)	97.1 (88.1 – 107.0)
Ratio (%): CHS-1701/US-Neulasta	

As shown in **Figure 1**, the PK profiles of CHS-1701 and US-licensed Neulasta overlay each other.

Figure 1. Time vs. Geometric mean peg-G-CSF concentration profile (Study CHS-1701-05)



A summary of the PK parameters by study drug is shown in **Error! Reference source not found.**.

Table 5: PK Parameters of CHS-1701 and US-licensed Neulasta (Study CHS-1701-05)

PK Parameters	CHS-1701 (n = 84)	US-licensed Neulasta		Intra-subject CV (%)
		Dose 1 (n = 83)	Dose 2 (n = 68)	
	Mean (%CV)	Mean (%CV)	Mean (% CV)	
Cmax (ng/L)	221 (101)	247 (105)	175 (114)	34%
$AUC_{0\text{-last}}$ (ng/L•hr)	7495 (110)	8940 (122)	5996 (116)	40%
$AUC_{0\text{-inf}}$ (ng/L•hr)	7534 (109)[a]	9003 (120)[a]	6081 (113)[c]	40%

Sensitivity Analyses was performed to assess the impact of subject PK profiles with insufficient PK data points and to assess the period effect (refer to **Appendix 4.2.1** for details)

3.2.5 Is PD similarity met?

Yes, PD (ANC) similarity between CHS-1701 and US-licensed Neulasta was demonstrated. Refer to the original clinical pharmacology review for PD statisrical results and PD parameters for Study CHS-1701-05 (DARRTS ID 4094240). The PD data was not reprocessed.

Immunogenicity

3.2.6 What is the ability of the immunogenicity assay to detect the antidrug antibodies (ADA) in the presence of concentration of product in the study samples?

The sensitivities of the ADA assay were 200 ng/ml for anti-G-CSF and anti-PEG. The low positive control (LPC) was 300 ng/ml for anti -G-CSF and anti-PEG, and the high positive control (HPC) was 20,000 ng/ml for anti- G-CSF and anti-PEG. The drug tolerance of the ADA assay was 1 µg/mL at both the lower limit positive control (LLPC: 200 ng/mL) and the LPC, and was 10 µg/mL at the HPC. The LLPC generates a titer of 4-8.

Anti-G-CSF antibodies using an electrochemiluminescent bridging assay was validated and samples from Study CHS-1701-04 confirmed to have G-CSF-reactive anti-pegfilgrastim ADA were analyzed at (b) (4) The assay sensitivity was 4.7 ng/mL. Minimum titer measured in the anti-G-CSF antibody titer assay is 2, corresponding to ~16.3 ng/ml concentration of anti-G-CSF rabbit polyclonal antibody positive control. Also, 200 ng/mL medium positive control (MPC) generates a titer of 16-32; in comparison, 200 ng/mL of the same positive control generates a titer of 4-8 in the ADA assays.

A new NAb assay was developed and validated a (b) (4) and all ADA-positive samples from all clinical studies were tested in this assay. The method is based on a 3-tiered analysis scheme for the detection of NAb against pegfilgrastim: screening (for ≤ cutpoint), specificity (for > cutpoint, and confirmatory (for ≥cutpoint). All samples positive for NAb against pegfilgrastim were tested in a cross-reactivity assay to assess neutralizing capacity against G-CSF.

The sensitivity of the NAb assay was 137 ng/mL using the in-study cut point (0.760). The LPC at the assay sensitivity of 137 ng/mL,was estimated at 264 ng/mL. The validation results showed that 1.1% (1 of 88) of the 250 ng/mL LPC were negative, above the in-study cut point. Per Applicant, peg-G-CSF is not expected to interfere with detection of NAb, as peg-G-CSF levels in ADA samples in the study were <1 µg/mL and the assay detected 150 ng/mL of anti-G-CSF positive control antibody in presence of ≤2.4 ng/mL of CHS-1701 or US-licensed Neulasta.

Refer to the immunogenicity assay review by the OBP 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?

Yes. Refer to the earlier clinical pharmacology review (DARRTS ID 4094240) for details of ADA sampling time points in the Studies CHS-1701-05 and CHS-1701-04.

3.2.8 What is the incidence of anti-drug antibodies (ADA) and neutralizing antibody (Nab)and how do the findings compare between products?

The ADA assay cut points were redetermined and a confirmed ADA result was defined based on only the CHS-1701 confirmatory assay. The revision increased the number of treatment-emergent, confirmed-positive, titer ≥2, and persistent subjects in CHS-1701-04. The immunogenicity results from Study CHS-1701-04 are provided in **Table 6.**

Table 6. Immunogenicity results for binding ADA in Study CHS-1701-04

	N†	Anti-PEG-G-CSF			Anti-G-CSF†	Anti-PEG**	NAb*
		Baseline	Treatment-Induced*	Treatment-Induced, Persistent**			
CHS-1701	134	12/134 (9.0%)	39/121 (32.2%)	12/122 (9.8%)	5/134 (4.1%)	9/122 (9.0%)	0/121 (0%)
US-licensed Neulasta	134	13/134 (9.7%)	28/117 (23.9%)	6/120 (5.0%)	1/134 (0.8%)	6/120 (5.0%)	0/117 (0%)
Source: Tables 11-3, 11-5, 11-7, 16.2.6.8, Study CHS-1701-104, Module 5.3.5.4, SDN 57							
†safety population: who received one or more doses of CHS-1701 or US-licensed Neulasta * subjects with an ADA assessment post dose excluding subjects with pre-existing ADA **Persistent is defined as at least 2 positive time points, with at least 1 positive time point after the second dose (Period 2).							

In the immunogencity Study CHS-1701-04, the pre-specified endpoints were the number of subjects positive for neutralizing antibodies (NABs) to peg-G-CSF, and the percentage of treatment-emergent, confirmed-positive, titer ≥1, and persistent ADA. In order to demonstrate similarity in immunogenicity rates, the 1-sided 95% upper bound of the rate difference for ADA must have been ≤10% between treatment groups. The prespecified criteria for NAB were zero occurrences and a 1-sided 95% upper bound of CI ≤3.7% for each treatment group.

Table 7. Comparison of Incidence of treatment-emergent, persistent anti-drug antibody

	Treatment emerging, persistent w/ titer ≥2	1-sided Upper Limit (Wald Asymptotic)
CHS-1701	12/122 (9.8%)	10.3%
US-Neulasta	6/120 (5.0%)	

As shown in **Table 7.** , there is a difference in the incidence of ADA between CHS-1701 and US-lincesed Neulasta. In addition, the study did not meet the pre-specified endpoints, described above.

3.2.9 What is the impact of ADA and nAb on the PK, PD, safety, and efficacy of the therapeutic protein and how do the findings compare between products?

Individual PK profiles of the ADA positive subjects demonstrate high intra-subject variability and lack a consistent pattern. Due to the small number of ADA positive subjects and high intra-subject variability it cannot be concluded that the differences in Cmax and AUC0-last is due to the presence of ADA.

4. APPENDICES

4.1 Summary of Bioanalytical Method Validation and Performance

4.1.1 Pharmacokinetics

The current submission did not involve reassay of PK samples from Studies CHS-1701-05 and CHS-1701-04, and therefore, does not involve any additional PK bioanalysis. Refer to the original clinical pharmacology review (DARRTS ID 4094240) for details of PK assay that was used to assay peg-G-CSF in human plasma, and the summary of validation results.

The original OSIS inspection (DARRTS ID [redacted] (b) (4)) of the analytical site ([redacted] (b) (4)) found that the specificity and selectivity in hemolyzed plasma was not validated at the lower range (200 pg/mL). Further, study samples exceeded the accumulated benchtop stability of 13 hours and 52 minutes at room temperature and four freeze/thaw cycles when stored at -80 °C. In response, the analytical site repeated the hemolysis experiment and conducted benchtop and free-thaw (F-T) validation to extend benchtop stability to 24 hours at -20 to -80C and F-T stability to 6 F-T cycles. (**Table 8**). Hemolysis experiment confirmed hemolyis interference at 200 pg/mL. Consequently, 34 hemolyzed samples in Study CHS-1701-05 and 22 hemolyzed samples in CHS-1701-04 were excluded in the PK dataset for the respective studies.

Table 8. Revised Assay Validation of Hemolysis, and Benctop and Free-thaw stability

	Neulasta	
	Normal	**Hemolyzed**
Hemolysis	Unspiked:15/15 (100%) BLOQ QC1: 6/10 (60%) ≥20% RE QC3: 6/9 (67%) ≥20% RE	Unspiked:4/4 (100%) BLOQ QC1: 1/4 (25%) ≥20% RE QC3: 2/2 (100%) ≥20% RE
	CHS-1701	**Neulasta**
Bench-top Stability for 24 hours at -20 to -80°C, accuracy (precision)	QC1: 97% (3%) QC3: 92% (4%)	QC1: 92% (3%) QC3: 92% (3%)
Freeze-thaw stability for 6 cycles, accuracy (precision)	QC1: 99% (4%) QC3: 95% (4%)	QC1: 96% (4%) QC3: 92% (4%)
Accuracy=%mean values/nominal, Precision=%CV Source:		

The original OSIS inspection (DARRTS ID [redacted] (b) (4)) also found that the estimation of calibration curve response in 19 analytical runs in Study CHS-1701-05 and 16 analytical runs in Study CHS-1701-04 were not objective, potentially impacting the acceptability of the analytical runs in question. In response, the analytical site reprocessed the calibration curve response in the analytical runs using an objective criteria, and backcalculated the subject concentrations in the runs. The reprocessing resulted in 8 analytical runs failing to meet the quality control (QC) acceptance criteria, 4 run failed to meet the calibration acceptance criteria, and 4 analytical runs failed to meet the dilution QC acceptance criteria in Study CHS-1701-05. Four analytical runs remained unsucceeful following reprocessing. As a result, 5% (396 of 7634) of the subject concentrations were excluded from the PK dataset due to failure of analytical runs to meet QC or dilution QC acceptance criteria, and the 97 peg-G-CSF subject concentrations were revised in Study CHS-1701-05 following data reprocessing. Similarly, reprocessing of questionable analytical runs resulted in exclusion of 6% (284 of 4557) of subject concentrations from the PK dataset and revision of an additional 39 subject concentrations in Study CHS-1701-04.

In addition, to understand whether the PK assay is measuring pegfilgrastim bound to ADA or not bound to ADA, interference testing with ADA was requested. ADA interference (including ADAs against G-CSF and PEG) with the PK assay was investigated Mutiple monoclonal anti-PEG antibodies with different affinities, isotype and binding domains, and a neutralizing anti-G-CSF antibody were tested at multiple concentrations (20 to 2000 ng/mL). In general, interference with pegfilgrastim measurement was observed at high concentrations (2000 ng/mL) of the anti-PEG and anti-G-CSF antibodies (data not shown). Among the antibodies tested, the neutralizing anti-G-CSF antibody exhibited the most interference with the quantitation of pegfilgrastim in human plasma (pegfilrastim recovery of 12% at 2.5 ng/mL, 37% at 25 ng/mL and 70% at 250 ng/mL at 2000 ng/mL of the antibody). The results confirm that pegfilgrastin unbound to ADA is measured by the PK assay. Further, ADA titers in the study were

generally low (16: ~200 ng/mL) to interfere with pegfilgrastim measurement in human plasma, the number of ADA positive samples were low, and pegfilgrastin Cmax levels in the study was in the 250 ng/mL range.

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?

Refer to the original clinical pharmacology review (DARRTS ID 4094240) for details of PD assay used to measure ANC.

4.2 Sensivity Analyses

4.2.1 Study CHS-1701-05

A sensitivity analysis was performed after deleting PK profiles for Subjects (b) (6) (Trt 1), (b) (6) (Trt 3), (b) (6) (Trt 1) and (b) (6) (Trt 1) as there were insufficient PK time points to accurately capture the PK profiles (**Table 9**) . The PK biosimilarity was not affected with the analysis.

Table 9. Statisitical analyses for assessment of PK similarity (Study CHS-1701-05)

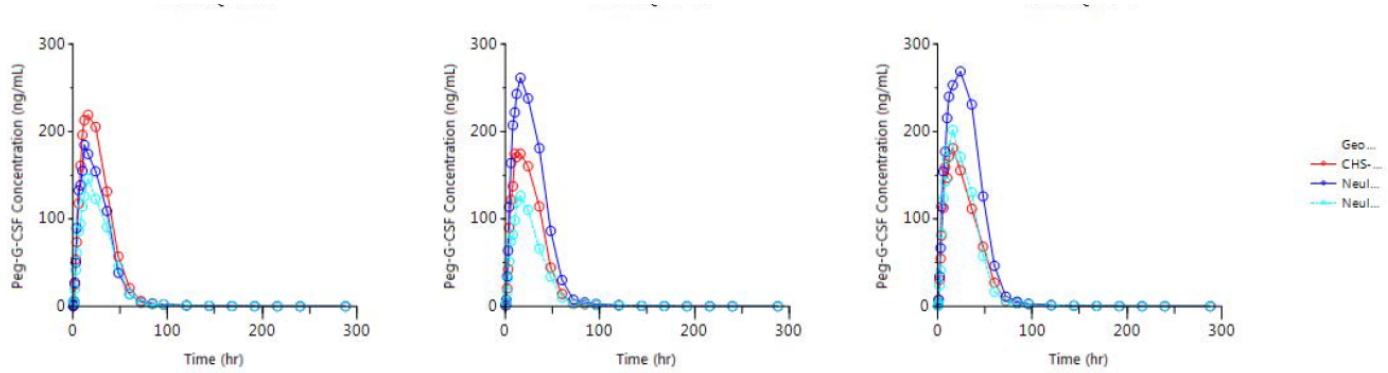
PK Parameters*	**Geometric Mean Ratio (90% CI)**
Cmax (ng/L)	104 (94.4 – 114.5)
AUClast (ng/L•hr)	98.0 (88.8 – 108.1)
AUCinf (ng/L•hr)	98.2 (88.9 – 108.4)

Ratio (%): CHS-1701/US-Neulasta.
*Deletion of AUCinf for Subjects (b) (6) (Period 3), and PK profiles for (b) (6) (Period 3), (b) (6) (Period 1) and (b) (6) (Period 1)

As noticed in the original review, a period effect was observed between Period 1 and Period 2 and between Period 2 and Period 3 (**Figure 2**), possibily due to the short washout period of 28 days used in this study. Refer to the original clinical pharmacology review (DARRTS ID 4094240) for details of evaluation of the impact of the period effect on the results of the PK similarity assessment.

Figure 2. Geometric mean peg-G-CSF time vs. concentration profile for each period by sequence (Study CHS-1701-05)

Sequence A Sequence B Sequence C



In addition to assess the impact of period effect, a sensitivity analysis was performed with a 2-way cross-over statistical analysis using only Period 1 and Period 2 PK profiles of subjects from Sequences A and B (

Table 10). The PK biosimilarity was not affected with either analyses.

Table 10. Statisitical analyses for assessment of PK similarity using 2x2 cross-over (Study CHS-1701-05)

PK Parameters*	**Geometric Mean Ratio (90% CI)**
Cmax (ng/L)	102 (88.8 – 117.4)
AUClast (ng/L•hr)	98 (85.2 – 111.9)
AUCinf (ng/L•hr)	98 (85.2 – 111.8)

Ratio (%): CHS-1701/US-Neulasta.
*Deletion of PK profiles for Sequence C, Period 3, (b) (6) (Period 1), and (b) (6) (Period 1)

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

/s/

RUBY LEONG on behalf of SRIRAM SUBRAMANIAM
10/17/2018
Signing on behalf of Dr. Sriram Subramaniam.

OLANREWAJU OKUSANYA
10/17/2018

NAM ATIQUR RAHMAN
10/17/2018

Office of Clinical Pharmacology
351(k) Biosimilar Review

351(k) BLA Number	761039
Applicant	Coherus Biosciences Inc
Submission Date	August 9, 2016
Submission Type	*Standard*
Link to EDR	\\CDSESUB1\evsprod\BLA761039\
Brand (Generic) Name	CHS-1701
Dosage Form and Strength	6 mg/0.6 mL injection in a single-dose prefilled syringe
Route of Administration	*Subcutaneous (SC)*
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Associated IND	*115573*
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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	*Olanrewaju Okusanya,PharmD, MS,* *Sarah J. Schrieber, PharmD*
OCP Final Signatory	*NamAtiqur Rahman, PhD*

Table of Contents

1. EXECUTIVE SUMMARY

This Biologic License Application (BLA) for CHS-1701 (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 CHS-1701 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 for which US-licensed Neulasta is currently approved. The applicant submitted pharmacokinetic (PK) and pharmacodynamics (PD) data from two studies to support a demonstration of no clinical meaningful difference between CHS-1701 and US-licenced Neulasta. The pivotal PK/PD similarity study, CHS-1701-05, was conducted in healthy subjects comparing CHS-1701 and US-licensed Neulasta. The immunogenicity study, CHS-1701-04, was conducted in healthy subjects to compare the immunogenicity of CHS-1701 and US-licensed Neulasta. Trough concentrations were collected in this study in order to evaluate the potential impact of immunogenicity on the PK of peg-G-CSF concentrations.

Study CHS-1701-05 was a randomized, single-blind, partial reference-replicated, 3-sequence, 3-period crossover study in 122 healthy subjects to evaluate PK and PD (absolute neutrophil count (ANC)) similarity of CHS-1701 to US-licensed Neulasta. The study compared the PK, PD (ANC) safety, tolerability, and immunogenicity of single 6 mg subcutaneous (SC) dose of either CHS-1701 or US-licensed Neulasta. In this study, the pairwise comparison of CHS-1701 and US-licensed Neulasta met the pre-specified acceptance criteria for PK similarity (90% CIs for the ratios of geometric mean of AUCinf and Cmax, within the interval of 80 - 125%), thus establishing the PK similarity. Furthermore, the pairwise comparison of CHS-1701 and US-licensed Neulasta met the pre-specified acceptance criteria for PD (ANC) similarity (90% CIs for the ratios of geometric mean of ANC AUECinf, ANC AUEClast, and ANCmax, within the interval of 80-125%), thus establishing the PD similarity.

Study CHS-1701-04 was a randomized, double-blind, 2-period, parallel-arm study in 303 healthy subjects to assess the immunogenicity of two SC doses of CHS-1701 compared with two SC doses of US-licensed Neulasta. There was a difference in the incidence of ADA between CHS-1701 and US-lincesed Neulasta, and the study did not meet the pre-specified endpoints. The neutralizing antibody assay was found to be unacceptable, and thereafore a comparative immunogenicity assessment of the neutralizing antibody formation between CHS-1701 and US-licesned Neulasta could not be determined.

Overall, the PK/PD study supports a demonstration of PK and PD (ANC) similarity between CHS-1701 and US-licensed Neulasta. The PK/PD study results add to the totality of the evidence to support a demonstration of biosimilarity of CHS-1701 and US-licensed Neulasta. However, the applicant did not fully characterize the immunogenicity of CHS-1701 and provide a comparative immunogenicity assessment of the neutralizing antibody formation between CHS-1701 and US-licensed Neulasta as recommended by the Office of Biotechnology Product (OBP) immunogenicity reviewers. In the absence of these data, the impact of immunogenicity on PK and PD cannot be adequately assessed and the submission is not approvable at this time.

1.1 Recommendations

Review Issue	Recommendations and Comments
Pivotal evidence of PK similarity	PK similarity was observed between CHS-1701 and US-licensed Neulasta. The 90% CI of the geometric mean ratio of CHS-1701 to US-licensed Neulasta for Cmax and AUCinf fell within the pre-specified margin of 80-125%.
Pivotal evidence of PD similarity, if applicable	PD similarity was observed between CHS-1701 and US-licensed Neulasta. The 90% CI of the geometric mean ratio of CHS-1701 to US-licensed Neulasta for ANC AUEClast and ANCmax fell within the pre-specified margin of 80-125%.
Evidence of immunogenicity comparability	Study CHS-1704 for immunogencity did not met the predefined endpoint. The neutralizing assay is unacceptable. Refer to the OBP the immunogenicity assay review for more details.

1.2 Post-Marketing Requirements and Commitments

None

2. SUMMARY OF CLINICAL PHARMACOLOGY ASSESSMENT

2.1 Clinical Pharmacology and Pharmacokinetics

CHS-1701 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).

The results of the PK and PD similarity assessment in Study CHS-1701-05 is provided in **Table 1**. The 90% CI for AUCinf and Cmax after a single dose were within the pre-defined limits of 80 - 125%. The 90% CI for ANC AUEClast and ANCmax after a single dose were within the pre-defined limits of 80 - 125%.

Table 1. Summary Statistical analyses for assessment of PK and PD (ANC) similarity (Study CHS-1701-05)

Parameter	PK Parameters		PD (ANC) Parameters	
	Cmax (ng/mL) (N = 85)	AUCinf (ng/mL*hr) (N = 84)	ANCmax (10^9/L) (N = 85)	AUEClast (h•10^9/L) (N = 85)
Geometric mean ratio	104	97.5	99.6	99.8
90% CI	94.6 – 114	88.6 – 107	96.2 – 103	97.7 – 102

Ratio (%): CHS-1701/US-Neulasta

Overall, the submitted clinical pharmacology study is adequate to demonstrate similarity of PK and PD (ANC) exposure between CHS-1701 and US-licensed Neulasta. Study CHS-1701-05, conducted in healthy subjects, is considered sufficiently sensitive to detect clinically significant differences in PK and PD (ANC) exposure among the products. Single-dose PK and PD (ANC) similarity pre-specified margins were met. The demonstration of similar PK and PD (ANC) exposure supports a finding of no clinically meaningful differences between CHS-1701 and US-licensed Neulasta.

2.2 Outstanding Issues

The neutralizing antibody assay is unacceptable. Therefore, an comprehensive assessment of the impact of immunogenicity on the PK of the two products cannot be assessed. Refer to the OBP review for details.

3. COMPREHENSIVE CLINICAL PHARMACOLOGY REVIEW

3.1 Regulatory Background

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

CHS-1701 is a proposed biosimilar to US-licensed Neulasta. The applicant is seeking the following indication, specifically, "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 indiciation "to increase survival in patients acutely exposed to myelosuppressive doses of radiation (Hematopoietic Subsyndrome of Acute Radiation Syndrome)", given that the indication is currently under an exclusivitiy 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 as described in **Table 2**.

Table 2. Summary of Relevant CHS-1701 Clinical Studies

Protocol	Title	Subjects	Objectives	Route/Dose/Duration
PK/PD study to support biosimilarity assessment				
CHS-1701-05	A Randomized, Single-Blind, Crossover Study to Assess the PK and PD Similarity of CHS-1701 with US-Neulasta in Healthy Subjects.	Healthy (n=122)	PK, PD (ANC), safety, tolerability, immunogenicity	6 mg SC single dose of CHS-1701 vs. US-licensed Neulasta with ≥ 28 day washout
Immunogenicity study to support biosimilarity assessment				
CHS-1701-04	A Randomized, Double-Blind, 2-period, Parallel-Arm Study to Assess the Immunogenicity of 2 SC Doses of CHS-1701 Compared with 2 Subcutaneous Doses of US-Neulasta in Healthy Subjects	Healthy (n=303)	PK, PD, safety, tolerability, immunogenicity	Two 6 mg SC doses of either CHS-1701 or US-licensed Neulasta with a 6 week washout between periods

Study CHS-1701-05 was the pivotal study used to support PK and PD (ANC) similarity. This study was a randomized, single-blind, partial reference-replicated, 3-sequence, 3-period crossover study following a single 6 mg/0.6 mL SC injection to compare the PK, PD (ANC), safety, tolerability, and immunogenicity of CHS-1701 and US-licensed Neulasta in healthy subjects (N=122). A schematic of the study is shown in **Table 3**. The PK endpoints evaluated in this study were AUCinf, AUClast, and Cmax and the PD (ANC) endpoints were ANC AUEClast and ANCmax. PK and PD similarity were concluded if the 90% CI of the geometric mean ratio of CHS-1701 to US-licensed Neulasta for the PK and PD (ANC) endpoints were between 80 and 125%.

Table 3. Study CHS-1701-05 Schematic

	Period 1	**Period 2**	**Period 3**
Sequence A	CHS-1701	Neulasta	Neulasta
Sequence B	Neulasta	CHS-1701	Neulasta
Sequence C	Neulasta	Neulasta	CHS-1701

The study design of Study CHS-1701-05 is considered adequate due to the following reasons:

1. A cross-over study design is recommended for products with short half-life (e.g., less than 5 days) and the PD (ANC) response is rapid.
2. Conducting the study in healthy subjects is acceptable as it is safe and more sensitive in evaluating the product similarity due to lack of potentially confounding factors such as underlying and/or concomitant disease and concomitant medications.
3. Considering PK assay sensitivity, dose-exposure linearity, and tolerability, the 6 mg SC dose tested is acceptable and relevant as it is the approved adult dose of US-licensed Neulasta.

The PK/PD Study CHS-1701-05 was considered critical in the assessment of no clinically meaningful difference in safety, purity, and potency between CHS-1701 and US-licensed Neulasta.

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

In the PK/PD Study CHS-1701-05,

- PK plasma samples were collected in each period at: Day 1 within 30 minutes predose and postdose at 15, 30 and 45 minutes and 1, 2, 3, 4, 6, 8, 10, 12, 16, 24, 36, 48, 60, 72, 84, 96, 120, 144, 168, 192, 216, 240, and 288 hours. The predefined PK similarity criteria for AUCinf and Cmax were that the 90% CI of the geometric mean ratio should lie within 80-125%. This margin proposed by the applicant was acceptable.
- The PD sampling times co-incided with the PK sampling times listed above. The predefined PD (ANC) similarity criteria for area under the effect curve (ANC AUEC) and maximum ANC count (ANCmax) were that the 95% CI for the ratio of the geometric means 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.

In the immunogenicity Study CHS-1701-04,

- PK plasma samples were collected in both periods at: Day 1 within 30 minutes pre-dose and postdose at 8, 16, 36, and 72 hours, and on Days 13, 27 and 41. For subjects who are confirmed positive for ADA at their last visit, a PK sample will be collected every 3 months until ADA returns to baseline or 12 months, whichever occurs first.
- PD (ANC) samples were collected in both periods at: Day 1 within 30 minutes pre-dose, and postdose at 8, 16, 36, 72, 96, 120, 288 hours, and on Days 27 and 41.

3.2.3 Are the pharmacogicallyactive 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. G-CSF was measured to characterize the PK and was measured in plasma by a validated enzyme-linked immunosorbant assay (ELISA). Absolute neutrophil counts (ANC) were determined using appropriate hematology analyzers.

3.2.4 Is PK similarity met?

Yes, PK similarity between CHS-1701 and US-licensed Neulasta was demonstrated—90% confidence intervals of geometric mean ratios of PK endpoints were contained within prospectively defined criteria of 80 to 125% (**Table 4**).

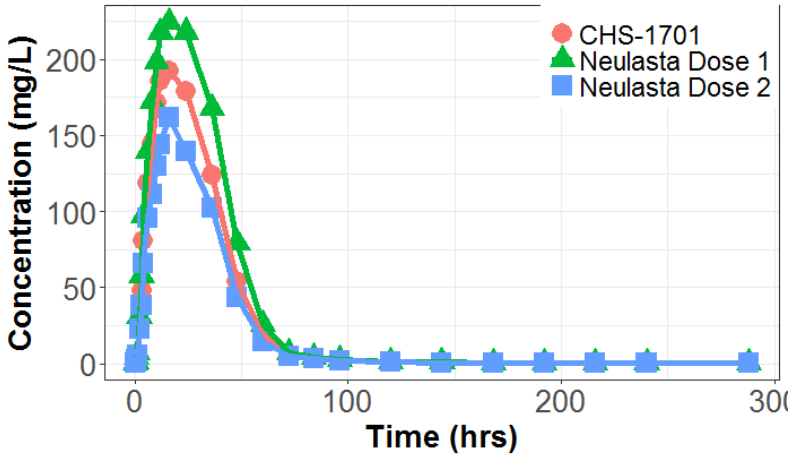
Table 4. Statisitical analyses for assessment of PK similarity (Study CHS-1701-05)

PK Parameters	Geometric Mean Ratio (90% CI)
Cmax (mg/L)	104 (94.6 – 114)
AUClast (mg/L•hr)	98.1 (89.4 – 108)
AUCinf (mg/L•hr)	97.5 (88.6 – 107)

Ratio (%): CHS-1701/US-Neulasta

As shown in **Figure 1**, the PK profiles of CHS-1701 and US-licensed Neulasta overlay each other.

Figure 1. Geometric mean peg-G-CSF time vs. concentration profile (Study CHS-1701-05)



A summary of the PK parameters by study drug is shown in **Table 5**.

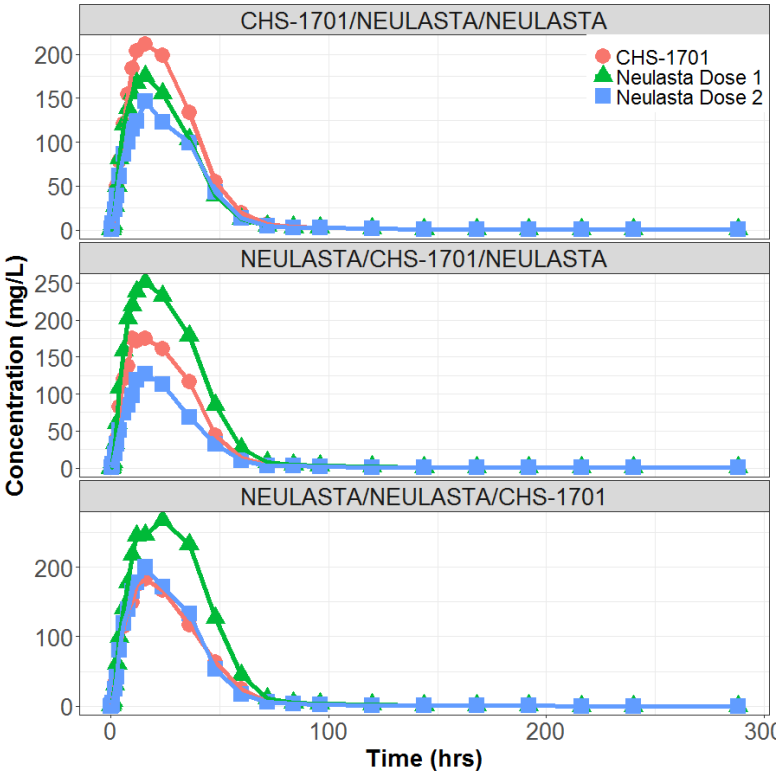
Table 5. Summary of PK Parameters (Study CHS1701-05)

PK Parameters	CHS-1701 (n = 85) Mean (%CV)	US-licensed Neulasta		Intrasubject variability (%)*
		Dose 1 (n = 85) Mean (%CV)	Dose 2 (n = 69) Mean (% CV)	
Cmax (mg/L)	229 (93.5)	246 (104)	177 (112)	37.2
$AUC_{0\text{-last}}$ (mg/L•hr)	7658 (108)	8912 (120)	6024 (114)	36.7
$AUC_{0\text{-288}}$(mg/L•hr)	7657 (108)[a]	9074 (119)[a]	6151 (113)[b]	34.5
$AUC_{0\text{-inf}}$ (mg/L•hr)	7672 (108)[a]	9093 (118)[a]	6131 (114)[c]	34.3

a. n = 84
b. n = 68
c. n = 66
*Intrasubject variability for subjects receiving US-licensed Neulasta doses

As shown in **Figure 2**, a period effect was observed between Period 1 and Period 2 and between Period 2 and Period 3. It is possible that this observed period effect is due to the short washout period of 28 days used in this study.

Figure 2. Geometric mean peg-G-CSF time vs. concentration profile for each sequence and period (Study CHS-1701-05)



Because of the imbalance caused by missing data, the treatment effect and period effect cannot be separated in the sponsor's PK analysis. To evaluate the impact of the period effect on the results of the PK similarity assessment, a completer analysis was conducted to estimate the period effect under the missing at random assumption. To accomplish this, PK similarity for Cmax and AUCinf was conducted for subjects that completed all three periods. The resultant period effect estimate for Period 2 and Period 3 was then used to adjust the PK parameters in the respective periods for the PK population to determine the impact of the period effect on PK similarity. The 90% CI for AUCinf and Cmax from this sensitivity analysis fell between 80-125% (data not shown), supporting the conclusion of PK similarity in two products despite the existence of period effect.

3.2.5 Is PD similarity met?

Yes, PD (ANC) similarity between CHS-1701 and US-licensed Neulasta was demonstrated. The 90% confidence intervals of geometric mean ratios of PD (ANC) endpoints were contained within prospectively defined criteria of 80 to 125% (**Table 6**).

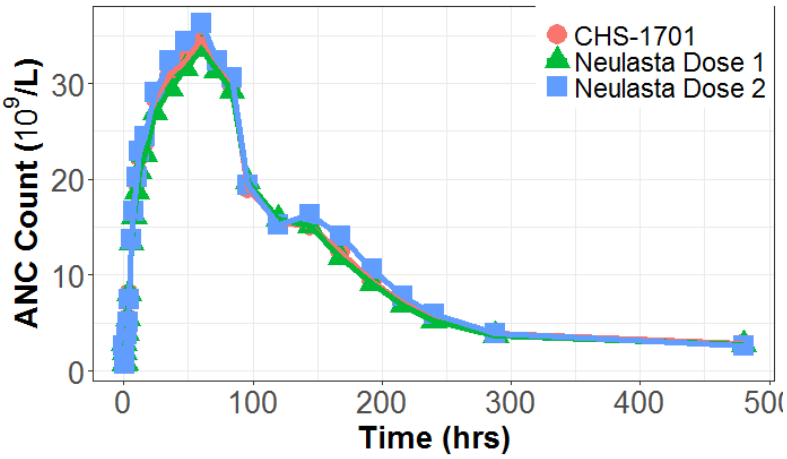
Table 6. Statistical analyses for assessment of PD (ANC) similarity (Study CHS-1701-05)

PD Parameter	Geometric Mean Ratio (90% CI)
ANC AUEClast ($h \cdot 10^9/L$)	96.7 (92.2 – 101)
ANCmax ($10^9/L$)	99.6 (96.2 – 103)

Ratio (%): CHS-1701/US-Neulasta

As shown in **Figure 3**, the PD profiles of CHS-1701 and US-licensed Neulasta overlay each other.

Figure 3. Geometric mean ANC time vs. concentration profile (Study CHS-1701-05)



A summary of the PD (ANC) parameters by study drug is shown in **Table 7**.

Table 7. Summary of PD (ANC) Parameters (Study CHS1701-05)

PK Parameters	CHS-1701 (n = 85) Mean (% CV)	US-licensed Neulasta Dose 1 (n=85) Mean (% CV)	US-licensed Neulasta Dose 2 (n=69) Mean (% CV)	Intrasubject variability (%)*
ANC $AUC_{0\text{-last}}$ (h•10^9/L)	5497 (30.9)	5473 (26.9)	5928 (23.4)	10.7
ANC $AUC_{0\text{-480}}$ (h•10^9/L)	5423 (25.4)[a]	5359 (26.1)[a]	5682 (22.9)[b]	11
ANCmax (10^9/L)	37.3 (28)	36.7 (29.4)	38.7 (25.8)	13

a. n = 84
b. n = 68
* Intrasubject variability for subjects receiving US-licensed Neulasta doses

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 sensitivity of the ADA assay was ~100 ng/mL. The drug tolerance of the ADA assay was 1 µg/mL at both the lower limit positive control (LLPC) and the low positive control (LPC), and was 10 µg/mL at the high positive control (HPC). The neutralizing assay is unacceptable. Refer to the immunogenicity assay review by the OBP 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:

- In the PK/PD Study CHS-1701-05, ADA samples were collected in each period at predose on Day 1, and on Days 11 and 28 post-dose.
- In the immunogenicity Study CHS01701-04, ADA samples were collected in both periods on Days 1 (pre-dose), 13, 27, and 41. Subjects who are confirmed positive for ADA at their last visit

were to be followed every 3 months until ADA returned to baseline or for 12 months, whichever occured first

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 the immunogencity Study CHS-1701-04, the pre-specified endpoints were the number of subjects positive for neutralizing antibodies (NABs) to peg-G-CSF, and the percentage of treatment-emergent, confirmed-positive, titer ≥1, and persistent ADA. Persistent is defined as at least 2 positive timepoints, with at least 1 positive timepoint after the second dose (Period 2). In order to demonstrate similarity in immunogenicity rates, the 1-sided 95% upper bound of the rate difference for ADA must have been ≤10% between treatment groups. The prespecified criteria for NAB were zero occurrences and a 1-sided 95% upper bound of CI ≤3.7% for each treatment group. The prevalence of the anti-drug antibody is provided in **Table 8.**

Table 8. Incidence of treatment-emergent, persistent anti-drug antibody

	Treatment emerging, persistent	1-sided Upper Limit (Exact)	Treatment emerging, persistent w/ titer ≥2	1-sided Upper Limit (Exact)
CHS-1701	16/122 (13.1%)	12.6%	12/122 (9.8%)	10.16%
US-Neulasta	9/120 (7.5%)		7/120 (5.8%)	

As shown in **Table 8.** , there is a difference in the incidence of ADA between CHS-1701 and US-lincesed Neulasta. In addition, the study did not meet the pre-specified endpoints, described above.

3.2.9 Do the anti-drug antibodies (ADA) have neutralizing activity?

The neutralizing assay is unacceptable. Refer to section 3.2.6 above.

3.2.10 What is the impact of anti-drug antibodies (ADA) on the PK, PD and efficacy of the therapeutic protein?

Individual PK profiles of the ADA positive subjects demonstrate high intra-subject variability and lack a consistent pattern. Due to the small number of ADA subjects and high intra-subject variability it cannot be concluded that the differences in Cmax and AUC0-last is due to the presence of ADA.

3.2.11 What is the impact of anti-drug antibodies (ADA) on clinical safety? (Provide information on the incidence of infusion-related reactions, hypersensitivity reactions, and cross-reactivity to endogenous counterparts.)

No remarkable differences in safety was observed between ADA-negative and ADA-positive subjects. In general, it did not appear that the incidence of antibody positivity had an impact on the safety profile.

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?

The assay is a modification of the Quantikine Human GCSF ELISA kit and uses anti-granulocyte-colony stimulating factor (G-CSF) antibodies as capture and detection reagents. The 96-well microtiter plate is coated with a monoclonal antibody specific for human G-CSF. Blanks, Neulasta standards, and quality control (QC) samples containing Neulasta and/or CHS-1701 are added to the anti-GCSF coated microtite plate at various concentrations and are incubated. After washing, an enzyme-linked polyclonal antibody specific for G-CSF is added and incubated. The plate is washed and substrate is added. After addition of the stopping solution, absorbances at 450 nm and 555 nm are measured. The 555 nm absorbance value is subtracted from the 450 nm absorbance value, and Neulasta and/or CHS-1701 concentrations were calculated using standard 4-parameter calibration curves. A summary of the assay validation is provided in **Table 9**.

Table 9. Summary of Neulasta and CHS-1701 Assay Validation Report

Analyte	Neulasta	and CHS-1701
Matrix	Qualified in human plasma (K_2EDTA)	
Reference or analytical standard	Neulasta, Lot 1033257, 9.63 mg/mL	CHS-1701, Lot 1-FIN-1501, 10 mg/mL
Minimum dilution	1/2	
Limit of detection	75 pg/mL	
LLOQ	75 pg/mL	
ULOQ	3000 pg/mL	
Accuracy (%Bias)	Low (93 – 134%)	
Inter-assay Precision (%CV)	LLOQ (75 pg/mL): 14% Low (200 pg/mL): 9% Mid (625 pg/mL): 8% High (1875 pg/mL): 6% ULOQ (3000 pg/mL): 9%	LLOQ (75 pg/mL): 17% Low (200 pg/mL): 11% Mid (625 pg/mL): 7% High (1875 pg/mL): 7% ULOQ (3000 pg/mL):12%
Inter-assay Accuracy	LLOQ (75 pg/mL): – 15% Low (200 pg/mL): -4% Mid (625 pg/mL): -4% High (1875 pg/mL): -4% ULOQ (3000 pg/mL):-8%	LLOQ (75 pg/mL): – 4% Low (200 pg/mL): 2% Mid (625 pg/mL): 0% High (1875 pg/mL): 0% ULOQ (3000 pg/mL):-1%
Intra-assay Precision (%CV)	LLOQ (75 pg/mL): 7 - 20% Low (200 pg/mL): 1 - 11% Mid (625 pg/mL): 5 - 8% High (1875 pg/mL): 2 - 8%	LLOQ (75 pg/mL): 9 - 17% Low (200 pg/mL): 8 - 10% Mid (625 pg/mL): 4 - 8% High (1875 pg/mL): 2 - 6%

	ULOQ (3000 pg/mL): 4 – 11%	ULOQ (3000 pg/mL): 4 – 21%
Intra-assay Accuracy	LLOQ (75 pg/mL): -24 – -5% Low (200 pg/mL): -16 - 4% Mid (625 pg/mL): -14 - 4% High (1875 pg/mL): -10 - 0% ULOQ (3000 pg/mL): -14 – 3%	LLOQ (75 pg/mL): -20 – 3% Low (200 pg/mL): -5 - 13% Mid (625 pg/mL): -11 - 6% High (1875 pg/mL): -8 - 8% ULOQ (3000 pg/mL):-13 – 11%
Hook effect	No hook effect was observed	
Linearity in normal human serum sample (% difference from preceding dilution)	-14% to 10% for up to 1/10000 dilution	
Interference	Possible interference with hemolyzed samples	
Analyte stability	Room temperature: 24 h Neulasta® and CHS-1701 were shown to be stable in normal human plasma after a combined 4 freeze thaw cycles for samples stored at both -20°C and -80°C for ~ 13 hours.	
Long-term Stability	-20ºC and -80º: up to 6 months	

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 was performed on the Coulter LH750 Hematology Analyzer. The Coulter LH750 counts and sizes cells by detecting and measuring changes in electrical resistance when a cell in a conductive liquid passes through a small aperture. As each cell travels through the aperture, it momentarily increases the resistance of the electrical path between two submerged electrodes, one located on each side of the aperture. This causes an electrical pulse that can be counted and sized. The number of pulses indicates particle count and the size of the electrical pulse is proportional to the cell volume. The Coulter LH750 further differentiates white blood cells into 5 different populations: basophils, eosinophils, lymphocytes, monocytes, and neutrophils. The analyzer identifies and separates these cell types in the flow cell where a low frequency current measures the volume, a high frequency current senses cellular internal content through measuring changes in conductivity, and light from the laser bouncing off the individual white blood cells characterizes cellular surface, shape, and reflectivity. Absolute neutrophil count is a calculated parameter performed by the analyzer based on the absolute count (cells/µL of blood) of white blood cells multiplied by the percentage of cells classified as neutrophils.

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