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APPLICATION NUMBER:

761045Orig1s000

CLINICAL PHARMACOLOGY
REVIEW(S)

**Office of Clinical Pharmacology
351(k) Biosimilar Review**

351(k) BLA Number	761045 SDN 49
Applicant	Sandoz, Inc
Submission Date	February 27, 2019
Submission Type	Resubmission
Link to EDR	Application 761045 - Sequence 0048 - 0048 (49) 02/27/2019 ORIG-1 /Multiple Categories/Subcategories
Brand (Generic) Name	LA-EP2006 (Pegfilgrastim, a proposed biosimilar to Neulasta)
Dosage Form and Strength	Injection: 6 mg/0.6 mL solution in a single-dose prefilled syringe with UltraSafe Passive™ Needle Guard for manual use only
Route of Administration	Intravenous infusion
Proposed Indication(s)	ZIEXTENZO is 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.
Associated IND	109743
Reference Product Information (U.S.-licensed)	
Brand (Generic) Name	Neulasta (Pegfilgrastim)
Dosage Form and Strength	Injection: 6 mg/0.6 mL solution in a single-dose prefilled syringe
OCP Review Team Signers	
OCP Review Team	Christy S. John, Ph.D. Salaheldin Hamed, Ph.D.
OCP Final Signatory	Brian Booth, Ph.D.

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1. EXECUTIVE SUMMARY

This Biologic License Application (BLA) for LA-EP2006 has been re-submitted under Section 351(k) of the Public Health Service Act (42 U.S.C. 262(k)). The applicant is seeking approval for LA-EP2006 as a proposed biosimilar to US-licensed Neulasta (Neulasta US) licensed under BLA 125031 by Amgen, Inc. The proposed indication is 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. LA-EP2006, has been developed for subcutaneous use as a 6 mg/0.6 mL prefilled syringe (PFS).

A COMPLETE RESPONSE LETTER (CRL) was issued by the Agency on June 24, 2016 because the PK similarity study LA-EP06-101 failed to demonstrate PK similarity between LA-EP2006 and Neulasta US and failed to establish the PK portion of the of the scientific bridge between LA-EP2006, Neulasta US, and EU-approved Neulasta (Neulasta EU). Study LA-EP06-104 – included in this resubmission – was designed to address the deficiencies raised by the Agency.

Results from three clinical studies (Study LA-EP06-104, Study LA-EP06-301, and Study LA-EP06-302) support the demonstration of no clinically meaningful difference between LA-EP2006 and Neulasta US. Study LA-EP06-104 is a single-dose, 3-period crossover PK/PD similarity study in 577 healthy subjects comparing the LA-EP2006, Neulasta US, and Neulasta EU. The 90% confidence intervals (CI) for all three pairwise comparisons of the PK endpoints (AUC_{0-inf} , AUC_{0-last} , and C_{max}) as well as the PD endpoints ($AUEC_{0-last}$ and E_{max}) were within the specified limits of 80 to 125%. The results of the study established the PK and PD similarity between LA-EP2006 and Neulasta US Neulasta. The study also established the PK portion of the scientific bridge between LA-EP2006, Neulasta US, and Neulasta EU.

The Office of Study Integrity and Surveillance (OSIS) investigated the clinical study site at Celerion Tempe, AZ and found that the blinding codes were not available and accurate dosing of 59 subjects could not be confirmed. However, re-analyses of PK data (after excluding patients from Tempe site) did not change overall conclusion of PK similarity.

Studies LA-EP06-301 and LA-EP-302 – submitted in the original BLA submission – are two randomized, double-blind, parallel-group, multi-center comparative studies investigating efficacy, safety, and immunogenicity of LA-EP2006 and Neulasta EU in 624 female patients with breast cancer treated with myelosuppressive chemotherapy. The overall incidence of immunogenicity was comparable at the end of treatment (18 weeks; 6 cycles of treatment) and study follow-up (6 months for Study LA-EP06-301 and 4 weeks for Study LA-EP-302). Anti-drug antibodies (ADA) were against the PEG moiety, with no neutralizing antibodies (nAb). Moreover, the incidence of immunogenicity for LA-EP2006 and Neulasta US and Neulasta EU was compared in Study LA-EP06-104. The results indicate similar incidence and titers of ADA for both products. No apparent impact of ADA on safety, efficacy, or PK endpoints was observed.

In conclusion, the PK, PD, and immunogenicity results from Study LA-EP06-104, Study LA-EP06-301, and Study LA-EP06-302 support the demonstration of no clinically meaningful differences between LA-EP2006 and US-licensed Neulasta and add to the totality of the

evidence to support a demonstration of biosimilarity between LA-EP2006 and US-licensed Neulasta.

1.1 Recommendations

The Office of Clinical Pharmacology recommends approval of LA-EP2006 based on demonstration of PK similarity between LA-EP2006 and US-licensed Neulasta.

Review Issue	Recommendations and Comments
Pivotal evidence of PK similarity	The results of Study LA-EP06-104 demonstrate PK/PD similarity between LA-EP2006 and US-licensed Neulasta, as well as establish the PK portion of the scientific bridge between LA-EP2006, US-licensed Neulasta, and EU-approved Neulasta. The 90% confidence intervals (CI) for all three pairwise comparisons of the PK endpoints (AUC_{0-inf} , C_{max}) and PD endpoints ($AUEC_{0-last}$ and E_{max}) were within the specified limits of 80 to 125%.
Evidence of immunogenicity comparability	The results of Studies LA-EP06-104, LA-EP06-301 and LA-EP06-302 indicate similar incidence of anti-drug antibodies (ADA) for LAEP2006 to US-approved Neulasta.

1.2 Post-Marketing Requirements and Commitments

None.

2. SUMMARY OF CLINICAL PHARMACOLOGY ASSESSMENT

2.1 Clinical Pharmacology and Pharmacokinetics

LA-EP2006 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. Filgrastim is a water-soluble 175 amino acid protein with a molecular weight of approximately 19 kilodaltons (kD). Filgrastim is obtained from the bacterial fermentation of a strain of E coli transformed with a genetically engineered plasmid containing the human G-CSF gene. To produce pegfilgrastim, a 20 kD monomethoxypolyethylene glycol molecule is covalently bound to the N-terminal methionyl residue of filgrastim. The average molecular weight of pegfilgrastim is approximately 39 kD. Details on the clinical pharmacology of US-licensed Neulasta can be found in the product label (USPI).

In Study LA-EP06-104, the 90% CI for the geometric mean ratios of the primary PK endpoint of AUC_{0-inf} and C_{max} was within the specified limits of 80% to 125% in the pairwise comparisons between LA-EP2006, US-licensed Neulasta, and EU-approved Neulasta, as summarized in **Table 1** for all patients in Study LA-EP2006.

**Table 1. Summary of statistical comparisons of PK parameters (Study LA-EP06-104)
(analyses based on all patients in Study LA-EP06-104)**

Comparison	Geometric Mean Ratio* (90% CI)	
	AUC _{0-inf}	C _{max}
LA-EP2006 vs US-licensed Neulasta	106.54 (101.22, 112.12)	104.99 (99.86, 110.39)
LA-EP2006 vs EU-approved Neulasta	104.51 (99.30, 110.00)	102.67 (97.64, 107.95)
EU-approved Neulasta vs US-licensed Neulasta	98.10 (93.20, 103.26)	97.79 (92.99, 102.83)

*Presented as percent

PD parameters were calculated from the absolute neutrophil counts (ANC) time profiles for all subjects using non compartmental analysis. The primary PD endpoints were area under the effect curve of ANC measured from the time of dosing to the last measurable concentration (AUEC_{0-last}) and the maximum effect measured by ANC attributable to the investigational drug (E_{max}). The primary PD comparisons of LA-EP2006, Neulasta US and Neulasta EU are summarized in Table 2. For each pairwise comparison, the 90% CIs for the ratios of geometric means for AUEC_{0-last} and E_{max} were contained within the pre-defined PD similarity margins of 80% to 125% (Table 2). Therefore, PD similarity between LA-EP2006 and Neulasta US, LA-EP2006 and Neulasta EU and Neulasta US and Neulasta EU was demonstrated.

Table 2. Summary of statistical comparisons of PD parameters (Study LA-EP-06-104)

Comparison	Geometric Mean Ratio* (90% CI)	
	AUEC _{0-last} 10 ⁹ *h/L	E _{max} 10 ⁹ /L
LA-EP2006 vs US-licensed Neulasta	99.9 (99.1, 100.8)	99.7 (98.6, 100.8)
LA-EP2006 vs EU-approved Neulasta	100.1 (99.3, 100.9)	100.6 (99.5, 101.7)
EU-approved Neulasta vs US-licensed Neulasta	100.2 (99.3, 101.02)	100.8 (99.7, 102.0)

*Presented as percent

In studies LA-EP06-301 and LA-EP06-302, serum samples were collected prior to the first administration on day 1 cycle 1, at day 15 of cycle 6, at the end of study (EOS) visit 4 weeks after the last administration, and at the early termination visit in case of early termination.

One patient in the LA-EP2006 treatment group had a positive neutralizing antibody result at cycle 1, day 1, i.e. at pre-dose. The characterization of the ADA response in the binding assay had demonstrated that ADAs were targeted against pegfilgrastim and/or PEG, but not filgrastim. All post-dose sampling time points of this patient were determined to be negative for binding ADA. Neutralizing antibody results of the other patients with confirmed positive results for ADA binding were negative.

In Study LA-EP06-104, the immunogenicity of pegfilgrastim as determined by the formation of antibodies against the drug was evaluated using validated immunoassays. The incidences of ADAs were similar across all treatment groups (LA-EP2006: 31/512 subjects, 6.1%; Neulasta US: 36/511 subjects, 7.0%; and Neulasta EU: 39/501 subjects, 7.8%). Most of the confirmed positive ADAs were PEG specific.

Samples of subjects with confirmed specificity to pegfilgrastim alone or to filgrastim were analyzed for the neutralizing potential of ADAs. Among these subjects, 3 subjects developed nAbs: 1 in the LA-EP2006 group (1/512, 0.2%) and 2 in the Neulasta EU group (2/501, 0.4%).

Three subjects developed nAbs at baseline, 1 subject in the LA-EP2006 group and 2 subjects in the Neulasta EU group. On Day 15, 1 subject in the LA-2006 group and 1 subject in Neulasta EU group developed nAbs. On Day 28, 1 subject in Neulasta EU developed nAb. All nAbs had low titer. The nAbs did not have a noticeable effect on the safety profile in these subjects. A summary of immunogenicity results (confirmed ADA positive) by treatment Safety Analysis Set in Study LA-EP06-104 is given in **Table 3**.

Table 3. Summary of immunogenicity results (confirmed ADA positive) by treatment Safety Analysis Set in Study LA-EP06-104

	LA-EP2006 (N=512) n(%)	Neulasta US (N=511) n(%)	Neulasta EU (N=510) n(%)
Baseline			
Total number of subjects with at least one positive ADA	31 (6.1)	36 (7.0)	39 (7.8)
Total number of subjects with at least one positive nAb	1 (.2)	0	2 (0.4)
Day 1			
Total number of subjects with at least one positive ADA	4 (0.8)	9 (1.6)	13 (2.6)
Total number of subjects with at least one positive nAb	0	0	0
Day 15			
Total number of subjects with at least one positive ADA	22 (4.5)	22 (4.5)	28 (5.6)
Total number of subjects with at least one positive nAb	1 (0.2)	0	1 (0.2)
Day 28			
Total number of subjects with at least one positive ADA	9 (1.6)	16 (3.1)	

			7 (1.4)
Total number of subjects with at least one positive nAb	0	0	1 (0.2)

ADA=Anti-drug-antibody
nAb=Neutralizing antibody

The incidence of treatment emergent adverse events (TEAEs) in ADA positive subjects was similar across all treatment groups (LA-EP2006: 41/74 subjects, 55.4%; Neulasta US: 45/78 subjects, 57.7%; Neulasta EU: 40/76 subjects, 52.6%).

The incidences of TEAEs and of study drug-related TEAEs were lower in subjects with ADAs than in the overall study population, given some limitations of the comparison. In ADA positive subjects, there were no SAEs, no TEAE resulted in premature discontinuation, and all TEAEs were either mild or moderate.

The Agency’s Office of Study Integrity and Surveillance (OSIS) investigated the clinical study site at Celerion Tempe, AZ and found that the blinding codes were not available and accurate dosing of 59 subjects could not be confirmed (see review by Dr. Xingfang Li, DARRTS date: 7/9/2019). Consequently, clinical data from these 59 subjects were considered unreliable and were removed from PK re-analysis, which relied on data obtained from additional 5 clinical sites. The re-analyses of remaining PK data from the 429 subjects who completed all three treatment periods, demonstrated the of PK and PD similarity among LA-EP2006, US-licensed Neulasta, and EU-licensed Neulasta as shown in **Table 4**.

Table 4. Summary statistical analyses for PK similarity (Study LA-EP-06-104) (Analyses performed after excluding patient data from Celerion, Tempe site)

Comparison	Geometric Mean Ratio* (90% CI)	
	AUC _{0-inf}	C _{max}
LA-EP2006 vs US-licensed Neulasta	94.2 (89.29, 99.38)	95.53 (90.66, 100.65)
LA-EP2006 vs EU-approved Neulasta	99.56 (94.40, 105.08)	101.1 (95.95, 106.52)
EU-approved Neulasta vs US-licensed Neulasta	105.73 (100.22, 111.53)	105.83 (100.44, 111.51)

*Presented as percent

The OSIS also investigated ^{(b) (4)} for Absolute Neutrophil Count (ANC) data for study LA-EP06-104. No objectionable conditions were observed and Form FDA 483 was not issued at the inspection close-out. However, ORA investigator identified discrepancies in how ANC data were reported to the FDA. It was concluded by the OSIS that ANC data for subjects at PRA in Lenexa for study LA-EP06-104 were verified. The clinical

pharmacology review team did not deem this error impactful to the overall approvability of LA-EP2006.

Overall, the submitted clinical pharmacology data adequately demonstrated similarity of PK and PD among LA-EP2006, US-licensed Neulasta, and EU-licensed Neulasta. The submitted data indicates that there is no increase in immunogenicity risk for LA-EP2006. These results support a demonstration of no clinically meaningful differences between LA-EP2006 and US-licensed Neulasta, and add to the totality of the evidence to support a demonstration of biosimilarity of LA-EP2006 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.

This Biologic License Application (BLA) for LA-EP-2006 was submitted under Section 351(k) of the Public Health Service Act (42 U.S.C. 262(k)) in August, 2015 to seek approval for LA-EP-2006 as a proposed biosimilar to US-licensed Neulasta.

The original BLA 761045 was issued CRL on June 24, 2016. In the CRL, the Agency stated that PK similarity between LA-EP2006 and Neulasta US was not established and that root cause analyses should be conducted to identify factors that may have led to the inability to demonstrate PK similarity.

In a BPD Type 1 meeting on September 16, 2016, the applicant attributed the failure of Study LA-EP-101 (the initial PK/PD similarity study) to an underestimation of the intersubject variability in PK and the utilization of a parallel study design along with the non-linearity in pegfilgrastim PK.

In a BPD Type 2 meeting on October 25, 2018, the Agency agreed that Study LA-EP06-104 would be adequate to address the PK/PD similarity issues raised in the CRL.

The BLA was resubmitted on February 27, 2019.

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 human PK/PD studies conducted in healthy volunteers in the original submission and resubmission are listed in **Table 5**. Comparative efficacy, safety, and immunogenicity studies in patients are listed in **Table 6**.

Table 5. Human PK and PD studies in Healthy Subjects

Protocol number & Study dates	Design, goal & population Objectives & evaluations	Total no. & race (w, b, a, o) Age range (mean (SD)) Group no. & sex (m, f)	Test product(s); Dosage regimen; Route of administration Duration of therapy	Study status Type of report
[Module 5.3.4.1 LA-EP06-101] Study initiation date: 24-Jun-2010 Study completion date: 28-Dec-2010 Report date: 05-Jul-2012 Addendum: 26-Nov-2014 Amendment 1: 13-Aug-2015 Amendment 2: 14-Sep-2016 Amendment 3: 17-May-2018	Design, goal & population: Single-dose, randomized, double-blind, 3-arm, parallel-group PK/PD study to demonstrate PK and PD similarity of LA-EP2006, Neulasta US and Neulasta EU after a single 6 mg s.c. injection in healthy subjects Objectives & evaluations: The primary objectives were to determine similarity of LA-EP2006, Neulasta US and Neulasta EU following a single 6 mg s.c. injection in terms of the PK parameter AUC_{0-last} of pegfilgrastim as well as in terms of the PD parameter AUEC_{0-last} of the ANC in healthy subjects. Secondary objectives were to further compare LA-EP2006, Neulasta US and Neulasta EU with respect to: - C_{max}, AUC_{0-inf}, t_{max}, K_{el}, and t_{1/2} of pegfilgrastim - E_{max} and t_{max,E} of the ANC following administration of the 3 pegfilgrastim products - Development of the CD34+ cell count following the administration of LA-EP2006, Neulasta US and Neulasta EU - Safety, immunogenicity, and local tolerability of the 3 pegfilgrastim products Evaluation of possible influences of the 3	total: 279 (279w) age: 18–55 (37.3 (9.40)) 3 groups (156m, 123f): LA-EP2006: 93 (51m, 42f) Neulasta EU: 93 (53m, 40f) Neulasta US: 93 (52m, 41f)	LA-EP2006: 6 mg (10 mg/1 mL, glass vial); single s.c. injection, batch [30114715] Neulasta US: 6 mg (6 mg/0.6 mL, PFS); single s.c. injection, batches [1012807, 1017404] Neulasta EU: 6 mg (6 mg/0.6 mL, PFS); single s.c. injection, batch [1016759] Duration of therapy: Up to 49 days (including screening, single dosing, PK, PD and safety assessments and follow-up)	Complete; Final report
Protocol number & Study dates	Design, goal & population Objectives & evaluations	Total no. & race (w, b, a, o) Age range (mean (SD)) Group no. & sex (m, f)	Test product(s); Dosage regimen; Route of administration Duration of therapy	Study status Type of report
	pegfilgrastim products on electrocardiographic parameters (per Amendment 2)			
[Module 5.3.4.1 LA-EP06-103] Study initiation date: 18-Feb-2016 Study completion date: 16-Jan-2017 Report date: 08-Aug-2017	Design, goal & population: Single-dose, randomized, double-blind, 2-period crossover PK/PD study to evaluate the PK, PD, immunogenicity, and safety of LA-EP2006 and Neulasta EU in healthy male and female subjects, aged between 18 to 45 years (inclusive). Objectives & evaluations: to determine bioequivalence of LA-EP2006 and Neulasta EU following a single 6 mg s.c. injection in terms of PK parameters AUC_{0-inf}, AUC_{0-last} and C_{max} as well as equivalence in terms of the PD parameters ANC AUEC_{0-last} and ANC E_{max} Primary: - PK: AUC_{0-inf}, AUC_{0-last} and C_{max} - PD: AUEC_{0-last} and E_{max} of ANC Secondary: - PK: t_{max} and t_{1/2} - PD: ANC t_{max,E} Safety, immunogenicity, and local tolerance	total: 184* (150w, 12b, 6a, 16o) age: 18-45 (26.7 (7.35)) 2 groups (117m, 67f): LA-EP2006/Neulasta EU: 92 (58m, 34f) Neulasta EU/LA-EP2006: 92 (59m, 33f) * Number of subjects dosed; 185 subjects were randomized, but 1 subject did not receive IMP.	2 study drug administrations: LA-EP2006: 6 mg (6 mg/0.6 mL, PFS), single s.c. injection, batch [7007843] Neulasta EU: 6 mg (6 mg/0.6 mL, PFS), single s.c. injection, batch [1061466C] Duration of therapy: Approximately 17 weeks (including a screening period of up to 5 weeks and 2 study periods, each including single dosing, PK, PD, and safety assessments and follow-up, and 8-week washout between the 2 study drug administrations)	Complete; Final report
[Module 5.3.4.1 LA-EP06-104] Study initiation date: 14-Apr-2017 Study completion date: 01-Aug-2018 Report date: 15-Feb-2019	Design, goal & population: A randomized, double-blind, single dose, 3-period, 6-sequence crossover multicenter PK/PD study to compare the PK, PD, safety and immunogenicity of the proposed biosimilar product LA-EP2006, Neulasta US and Neulasta EU in healthy subjects. Objectives & evaluations: Primary: To demonstrate PK similarity in terms of pegfilgrastim AUC_{0-inf}, AUC_{0-last} and C_{max}, as well as PD similarity based on ANC AUEC_{0-last}	total: 577 (388w, 142b, 14a, 033) age: 18-55 (33.9 (10.51)) 6 groups (378m, 199f): ABC: 96 (59m, 37f) ACB: 97 (60m, 37f) BAC: 96 (62m, 34f) BCA: 95 (68m, 27f) CAB: 96 (68m, 28f) CBA: 97 (61m, 36f)	3 study drug administrations: LA-EP2006: 6 mg (6 mg/0.6 mL, PFS), single s.c. injection, batches [7007843, 7008257] Neulasta US: 6 mg (6 mg/0.6 mL, PFS), single s.c. injection, batches [1071089, 1072044] Neulasta EU: 6 mg (6 mg/0.6 mL, PFS), single s.c. injection, batches [1073269A, 1079877A]	Complete; Final Report

Protocol number & Study dates	Design, goal & population Objectives & evaluations	Total no. & race (w, b, a, o) Age range (mean (SD)) Group no. & sex (m, f)	Test product(s); Dosage regimen; Route of administration Duration of therapy	Study status Type of report
	and ANC E_{max} between: - LA-EP2006 and Neulasta US - LA-EP2006 and Neulasta EU - Neulasta US and Neulasta EU following a single 6 mg s.c. injection in healthy male and female subjects. The PK and PD primary endpoints were tested as co-primary endpoints. Secondary: To evaluate/compare LA-EP2006 with Neulasta US, LA-EP2006 with Neulasta EU, and Neulasta US with Neulasta EU following a single 6 mg s.c. injection in healthy male and female subjects in terms of: - Descriptive statistics for PK (t_{max} and t_{1/2}), and PD ANC t_{max,E} parameters. - Safety, immunogenicity, and local tolerance.	A=LA-EP2006 B=Neulasta US C=Neulasta EU	Duration of therapy: Approximately 25 weeks (including a screening period of up to 5 weeks, 2 treatment periods of at least 8 weeks each, and 1 treatment period of at least 4 weeks, each including single dosing, PK, PD, and safety assessments and follow-up, and 8-week washout between the 3 study drug administrations)	

Table 6. Efficacy and Safety Studies in Patients Pertinent to Claimed Indication

Protocol number & Study dates	Design, goal & population Objectives & evaluations	Total no. & race (w, b, a, o) Age range (mean (SD)) Group no. & sex (m, f)	Test product(s); Dosage regimen; Route of administration Duration of therapy	Study status Type of report
[Module 5.3.5.1 LA-EP06-301] Study initiation date: 28-Jun-2012 (first patient enrolled) Completion date: 07-Sep-2013 (treatment period); 11-Feb-2014 (6-month safety follow-up period; last patient last visit) Report date: 11-Jun-2015 Addendum 1: 22-Jul-2015	Design, goal & population: randomized, double-blind, parallel-group, multi-center Phase 3 comparative study investigating efficacy and safety of LA-EP2006 and Neulasta EU. The study population consisted of patients with histologically proven breast cancer, who had an indication for neo-adjuvant or adjuvant treatment with TAC chemotherapy. Objectives & evaluations: The primary objective of the study was to assess the efficacy of LA-EP2006 compared to Neulasta EU with respect to the mean DSN, defined as the number of consecutive days with Grade 4 neutropenia (ANC < 0.5 × 10⁹/L), during Cycle 1 of the neo-adjuvant or adjuvant TAC regimen in breast cancer patients. The secondary objectives were to further compare LA-EP2006 and Neulasta with respect to the efficacy, safety, and immunogenicity of both products. Primary endpoint: Mean DSN during Cycle 1 of chemotherapy, defined as the number of consecutive days in which a patient had an ANC < 0.5 × 10⁹/L Secondary endpoints: Incidence of FN, defined as oral temperature ≥ 38.3°C while having an ANC < 0.5 × 10⁹/L (both measured on the same day) by cycle and across all cycles Number of days of fever, defined as oral	total: 316 (256w, 54a, 6o) age: 29-76 (50.2 (10.2)) 2 groups (316f): LA-EP2006: 159 (159f) Neulasta EU: 157 (157f)	LA-EP2006 (PFS, 6 mg/0.6 mL), batches [30324244, 30324245]; up to 6 s.c. injections of 6 mg Neulasta EU (PFS, 6 mg/0.6 mL), batches [10271811A, 1028053D, 1033639A]; up to 6 s.c. injections of 6 mg Duration of therapy: approximately 44 weeks, including a screening period of up to 3-weeks, an 18-week period of active treatment, i.e. 6 TAC chemotherapy cycles, and a 6-month safety follow-up period starting with the last IMP administration at the beginning of the sixth chemotherapy cycle	Complete; Final report

Protocol number & Study dates	Design, goal & population Objectives & evaluations	Total no. & race (w, b, a, o) Age range (mean (SD)) Group no. & sex (m, f)	Test product(s); Dosage regimen; Route of administration Duration of therapy	Study status Type of report
	temperature $\geq 38.3^{\circ}\text{C}$, for each cycle Depth of ANC nadir, defined as the patient's lowest ANC in Cycle 1 Time to ANC recovery, defined as the time in days from the chemotherapy administration until the patient's ANC increased to $\geq 2 \times 10^9/\text{L}$ after the nadir, in Cycle 1 Frequency of infection by cycle and across all cycles Mortality due to infection Safety: Incidence, occurrence, and severity of AEs Assessment of local tolerability at the injection site Systemic tolerance (physical examination and safety laboratory assessments) Safety follow-up 4 weeks and 6 months after the last administration of the IMP Immunology: Development of binding and neutralizing anti-drug antibodies upon IMP injection			
[Module 5.3.5.1 LA-EP06-302] Study initiation date: 05-Mar-2012 (first patient first visit) Completion date: 04-Dec-2013 (last patient last visit) Report date: 08-Jun-2015	Design, goal & population: randomized, double-blind, parallel-group, multi-center Phase 3 comparative study investigating efficacy and safety of LA-EP2006 and Neulasta EU. The study population consisted of patients with histologically proven breast cancer, who had an indication for neo-adjuvant or adjuvant treatment with TAC chemotherapy. Objectives & evaluations: The primary objective of the study was to assess the efficacy of LA-EP2006 compared to Neulasta EU with respect to the mean DSN, defined as the number of consecutive days with Grade 4 neutropenia ($\text{ANC} < 0.5 \times 10^9/\text{L}$), during Cycle 1	total: 308 (183w, 3b, 120a, 2o) age: 25-75 (48.9 (10.27)) 2 groups (308f): LA-EP2006: 155 (155f) Neulasta EU: 153 (153f)	LA-EP2006 (PFS, 6 mg/0.6 mL), batches [30324244, 30324245]; up to 6 s.c. injections of 6 mg Neulasta EU (PFS, 6 mg/0.6 mL), batches [1022627/1022626, 1028053D, 1033639A]; up to 6 s.c. injections of 6 mg Duration of therapy: approximately 22 weeks, including a screening period of up to 3-weeks, an 18-week period of active treatment, i.e. 6 TAC chemotherapy cycles, and a 4-week safety follow-up period starting with the	Complete; Final report

Protocol number & Study dates	Design, goal & population Objectives & evaluations	Total no. & race (w, b, a, o) Age range (mean (SD)) Group no. & sex (m, f)	Test product(s); Dosage regimen; Route of administration Duration of therapy	Study status Type of report
	of the neo-adjuvant or adjuvant TAC regimen in breast cancer patients. The secondary objectives were to further compare LA-EP2006 and Neulasta with respect to the efficacy, safety, and immunogenicity of both products. Primary endpoint: Mean DSN during Cycle 1 of chemotherapy, defined as the number of consecutive days in which a patient had an $\text{ANC} < 0.5 \times 10^9/\text{L}$ Secondary endpoints: Incidence of FN, defined as oral temperature $\geq 38.3^{\circ}\text{C}$ while having an $\text{ANC} < 0.5 \times 10^9/\text{L}$ (both measured on the same day) by cycle and across all cycles Number of days of fever, defined as oral temperature $\geq 38.3^{\circ}\text{C}$, for each cycle Depth of ANC nadir, defined as the patient's lowest ANC in Cycle 1 Time to ANC recovery, defined as the time in days from the chemotherapy administration until the patient's ANC increased to $\geq 2 \times 10^9/\text{L}$ after the nadir, in Cycle 1 Frequency of infection by cycle and across all cycles Mortality due to infection Safety: Incidence, occurrence, and severity of AEs Assessment of local tolerability at the injection site Systemic tolerance (physical examination and safety laboratory assessments) Immunology: Development of binding and neutralizing anti-drug antibodies upon IMP injection PK sub-study:		last IMP administration at the beginning of the sixth chemotherapy cycle	

Protocol number & Study dates	Design, goal & population Objectives & evaluations	Total no. & race (w, b, a, o) Age range (mean (SD)) Group no. & sex (m, f)	Test product(s); Dosage regimen; Route of administration Duration of therapy	Study status Type of report
	PK profile over Days 1-15 of Cycle 1, C_{max} and AUC_{0-last} of pegfilgrastim concentrations in Cycle 1, trough concentrations on Day 1 of Cycles 2-6 ECG sub-study: Heart rate, PR interval, QRS, QT, QTcF, QTcB, relationship between LA-EP2006 and Neulasta serum concentrations with $\Delta QTcF$			

The applicant conducted a randomized, double-blind, single-dose, 3-period, 6-sequence crossover multicenter PK/PD study (LA-EP06-104) to evaluate the PK, PD, safety, and immunogenicity of LA-EP2006, Neulasta US and Neulasta EU in 576 healthy male and female subjects, aged between 18 to 55 years. The study design is displayed in **Figure 1**.

A single 6 mg s.c. injection of LA-EP2006, Neulasta US or Neulasta EU was administered according to treatment allocation by a qualified unblinded administrator on Day 1 of Treatment Periods I, II and III after an at-least 10 hour fast. No food was permitted until at least 4 hours after dosing on Day 1 of Treatment Periods I, II and III. A standardized meal was provided approximately 4 and 12 hours post-dose.

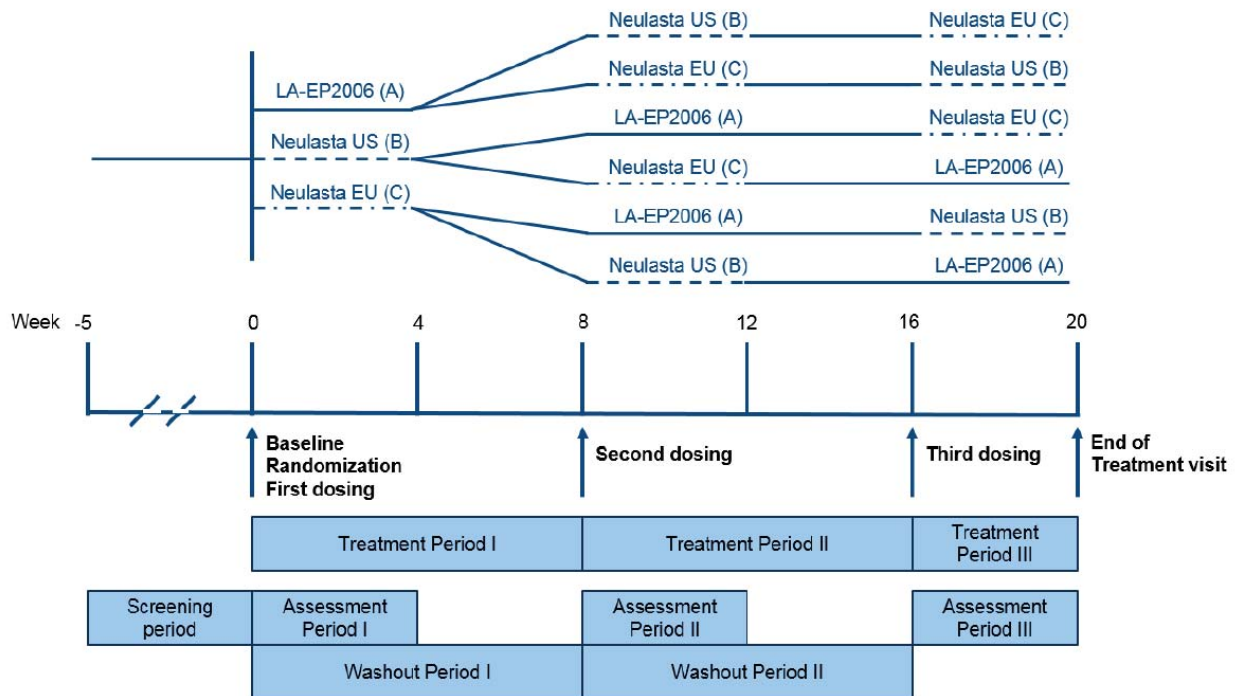


Figure 1. Study Design Schematic Diagram

The primary objective of this study was to demonstrate PK similarity in terms of pegfilgrastim AUC_{0-inf} and C_{max} , as well as PD similarity based on ANC $AUEC_{0-last}$ and ANC E_{max} between:

- LA-EP2006 and Neulasta US
- LA-EP2006 and Neulasta EU
- Neulasta US and Neulasta EU

The PK and PD primary endpoints were tested as co-primary endpoints for all pairwise comparisons among LA-EP2006, Neulasta US and Neulasta EU. The similarity for both, PK and PD among the 3 treatment groups was statistically demonstrated only if the 90% CIs for geometric mean ratio of all co-primary endpoints and for all pairwise comparisons among LA-EP2006, Neulasta US and Neulasta EU were contained within the predefined equivalence margins of 0.80 to 1.25.

The study design of Study LA-EP06-104 is considered adequate due to the following reasons:

1. 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.
2. Considering PK assay sensitivity, dose-exposure linearity, and tolerability, a single IV dose of 6 mg SC pegfilgrastim is considered appropriate for a PK similarity study.

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

In Study LA-EP06-104, the PK similarity criteria of AUC_{0-inf} and C_{max} and PD endpoints $AUEC_{0-last}$ and E_{max} were that the 90% CI of the geometric mean ratios should lie within 80-125%. This margin proposed by the applicant was acceptable.

PK serum samples were collected on Day 1 at pre-dose, 4, 8, 12, 24, 36, 48, 60, 72, 84, 96, 108, 120, 144, 168, 192, 216, 264, and 336 hours post-dose.

Blood samples for PD were collected 15 minutes prior to dosing at then 4, 8, 12, 24 hour on Day 1 and then every 12 hours on Days 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15 and Day 28 (648 hours post-injection).

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 and PD parameters?

Yes. Pegfilgrastim levels were measured in serum by a validated enzyme-linked immunosorbent assay (ELISA). The absolute neutrophil count (ANC) was measured by a validated/qualified method using commercial flow cytometers. Details of the analytical method, results including analytical within-study quality control procedures are provided in the corresponding bioanalytical reports. See **Section 4.1.1** for details.

3.2.4 Is PK/PD similarity met?

Yes. The analyses results showed that PK similarity between LA-EP2006 and US-licensed Neulasta was demonstrated, where the 90% CI of geometric mean ratios of the primary PK endpoint for each product pairwise comparison was contained within defined criteria of 80 to 125% (**Table 1**). Also, as shown in **Figure 2**, the PK profiles of LA-EP2006, US-licensed Neulasta, and EU-approved Neulasta overlay each other. Similarly, PD profiles (absolute neutrophil count vs time) for LA-EP2006, US-licensed Neulasta, and EU-approved Neulasta also overlay each other (**Figure 3**). The 90% CI of geometric mean ratios of the PD endpoints for each product pairwise comparison were contained within defined criteria of 80 to 125% (**Table 2**).

Figure 2. Arithmetic mean serum pegfilgrastim concentration vs. time profile (Study LA-EP-06-104)

Linear scale

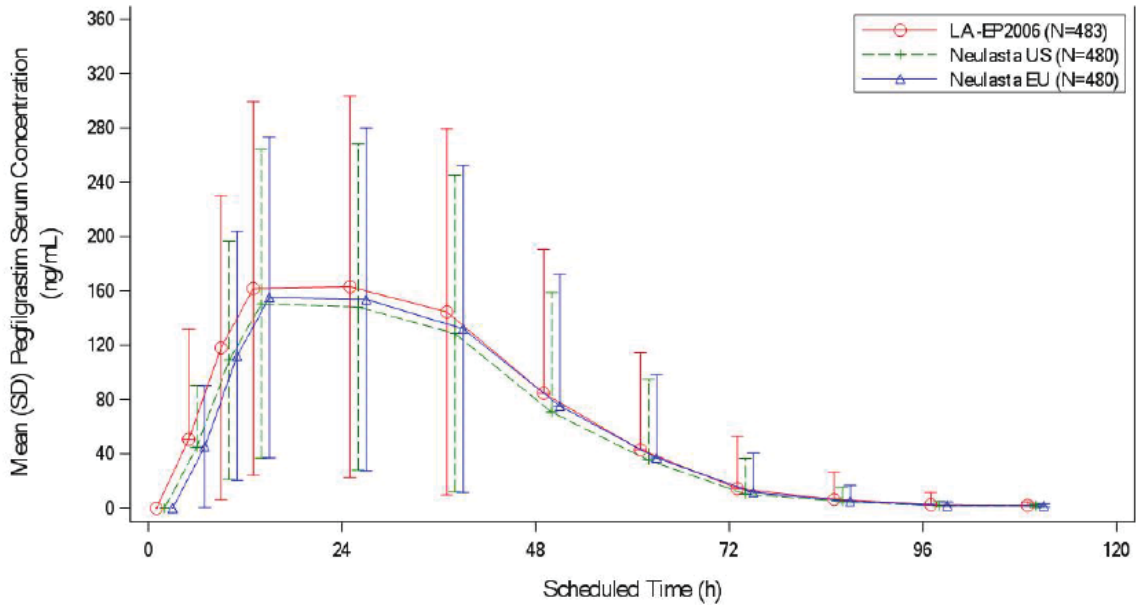
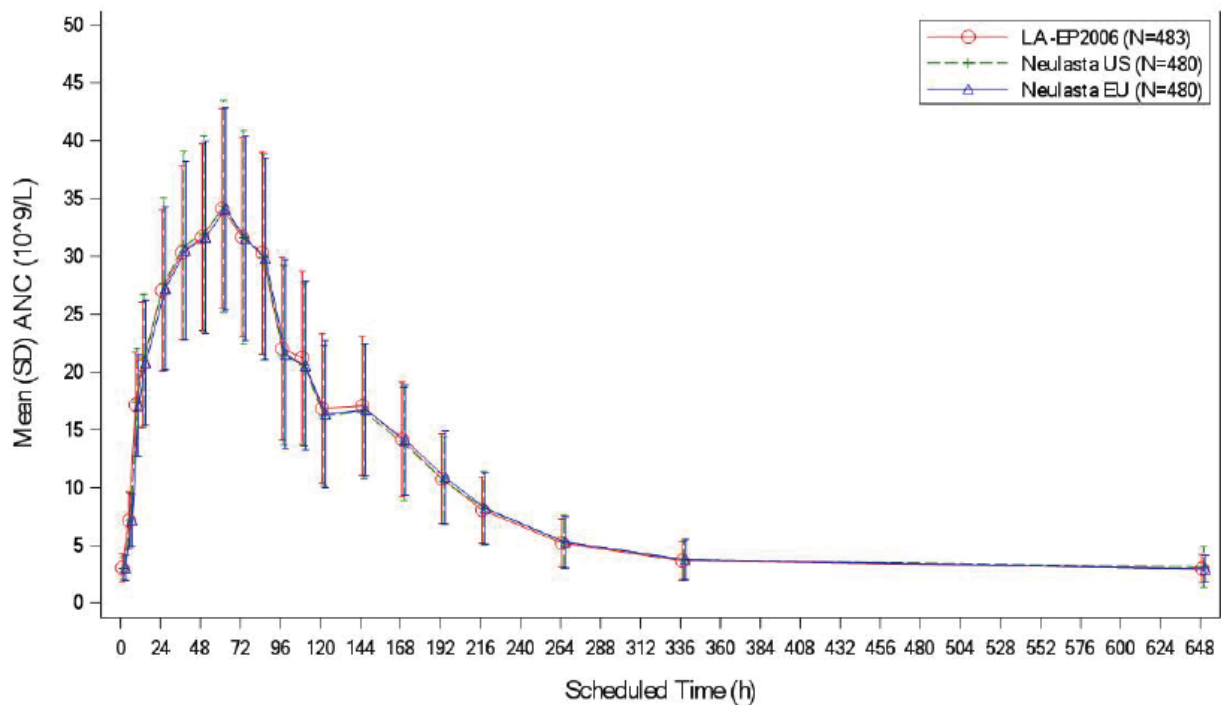


Figure 3. Arithmetic mean (SD) absolute neutrophil count profiles overall



The Office of Study Integrity and Surveillance (OSIS) stated in their inspection report that at the time of Celerion Arizona clinical site inspection (May 28 to June 5, 2019), the blinding codes for

Study LA-EP06-104 were not in Celerion's possession after study completion. Without the blinding codes and accurate dosing information, the study results from this clinical site are considered unreliable. Therefore, data from Study LA-EP06-104 was re-analyzed after excluding data from 59 patients from Celerion Tempe AZ site. The re-analyses of remaining PK data from the 429 subjects who completed all three treatment periods, demonstrated the of PK and PD similarity among LA-EP2006, US-licensed Neulasta, and EU-licensed Neulasta as shown in **Table 4** above.

Immunogenicity

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

Yes. The detection, confirmation and titration of ADAs were accomplished using an electrochemiluminescence (ECL) method. The lowest concentration at which the monoclonal anti-PEG control antibody consistently provides a positive signal was determined to be 3 µg/mL. For more details regarding the ECL assay, refer to the Office of Biotechnology Products (OBP) review.

3.2.6 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 study (for Study LA-EP06-104) was 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 on baseline Day 1, 15 and 28.

3.2.7 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.)

Immunogenicity of pegfilgrastim as determined by the formation of antibodies against the drug was evaluated using validated immunoassays. The validation procedure and serum sample analysis followed internationally accepted guidelines.

The incidences of ADAs were similar across all treatment groups (LA-EP2006: 31/512 subjects, 6.1%; Neulasta US: 36/511 subjects, 7.0%; and Neulasta EU: 39/501 subjects, 7.8%). Most of the confirmed positive ADAs were PEG specific.

Samples of subjects with confirmed specificity to pegfilgrastim alone or to filgrastim were analyzed for the neutralizing potential of ADAs. Among these subjects, 3 subjects developed nAbs: 1 in the LA-EP2006 group (1/512, 0.2%) and 2 in the Neulasta EU group (2/501, 0.4%) at the baseline (**Table 3**).

The incidence of treatment emergent adverse events (TEAEs) in ADA positive subjects was similar across all treatment groups (LA-EP2006: 41/74 subjects, 55.4%; Neulasta US: 45/78 subjects, 57.7%; Neulasta EU: 40/76 subjects, 52.6%).

The incidences of TEAEs and of study drug-related TEAEs were lower in subjects with ADAs than in the overall study population, given some limitations of the comparison. In ADA positive subjects, there were no SAEs, no TEAE resulted in premature discontinuation, and all TEAEs were either mild or moderate.

Immunogenicity in Patients:

In studies LA-EP06-301 and LA-EP06-302, serum samples were collected prior to the first administration on day 1 cycle 1, at day 15 of cycle 6, at the EOS visit 4 weeks after the last administration, and at the early termination visit in case of early termination. The positive confirmatory tests for anti-product antibodies were listed in **Table 7**.

One patient in the LA-EP2006 treatment group had a positive nAb result at cycle 1, day 1, i.e. at pre-dose. The characterization of the ADA response in the binding assay had demonstrated that ADAs were targeted against pegfilgrastim and/or PEG, but not filgrastim.

All post-dose sampling time points of this patient were determined to be negative for binding ADA. Neutralizing antibody results of the other patients with confirmed positive results for ADA binding were negative.

Table 7. Immunogenicity in patients with breast cancer: studies LA-EP06-301 and LA-EP06-02 (SAF set)

Phase 3 studies	LA-EP06-301		LA-EP06-302	
Confirmatory assay positive n (%)	LA-EP2006 N=159	EU-approved Neulasta N=155	LA-EP2006 N=157	EU-approved Neulasta N=153
Pegfilgrastim specific ADA				
Pre-dose	15 (9.4)	19 (12.1)	8 (5.2)	10 (6.5)
EOS	1 (0.7)	0	0	0
6-mo SFU*	1 (0.8)	0	n.a.	n.a.
Filgrastim specific ADA				
Pre-dose	8 (5.0)	5 (3.2)	0	1 (0.7)
Cycle 6, Day 15	0	0	0	1 (0.7)
EOT	1 (0.7)	0	0	1 (0.7)
EOS	0	0	0	1 (0.7)
6-mo SFU	2 (1.7)	0	n.a.	n.a.
PEG specific ADA				
Pre-dose	18 (11.3)	20 (12.7)	13 (8.4)	18 (11.8)
EOS	1 (0.7)	1 (0.7)	0	0
6-mo SFU*	1 (0.8)	0	n.a.	n.a.

Notes: ADA, ADA=anti-drug antibody; LA-EP=LA-EP2006; n=number of patients with an ADA positive sample; N=number of patients in a treatment group; n.a.=not applicable; Neulasta=EU-Neulasta; PEG=polyethylene glycol; SAF set=safety analysis set Only time points with confirmed positive results in the confirmatory binding antibody enzyme-linked immunosorbent assay are shown. Patients could have events in more than one category. a Samples with pegfilgrastim specific ADA were further differentiated into: also filgrastim specific ADA; also PEG specific ADA; and only pegfilgrastim specific ADA.

Overall, the data indicate that there is no increase in immunogenicity risk for LA-EP2006 as compared to Neulasta US, and supports the demonstration that there are no clinically meaningful differences between LA-EP2006 and Neulasta US. Of note, a scientific bridge was established between LA-EP2006, Neulasta US, and Neulasta EU, supporting the relevance of comparative

data, including immunogenicity data to support a demonstration of no clinically meaningful differences between LA-EP2006 and Neulasta US.

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

No patient tested positive for nAbs following administration of LA-EP2006 or Neulasta US on Day 1 or Day 28 post injection of drug (**Table 3**). One patient tested positive at baseline for nAb in LA-EP2006 arm. Two patients tested positive for nAb in Neulasta EU arm at the baseline. Also, 1 patient tested positive for nAb for Neulasta-EU on Day 28.

3.2.9 What is the impact of anti-drug antibodies (ADA) on the PK, activity, and safety of the therapeutic protein?

No apparent impact of ADA on PK, activity, or safety endpoints was observed from Study LA-EP206-104 (data not shown).

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?

A description of the bioanalytical methods used to quantify the G-CSF in human plasma is described in **Table 8**.

Table 8. Bioanalytical Method Validation Report for PK

Bioanalytical method validation report and amendments	[BA17014-R]; [BA09013-R]; [N-A-IMM-16-001]
Method description	The Human Quantikine® G-CSF ELISA employs the quantitative sandwich enzyme immunoassay technique. Microplate wells, coated with a monoclonal (mouse) antibody specific for G-CSF were incubated with calibration standard samples, quality control samples or validation samples. LA-rhG-CSF (pegfilgrastim) in the samples bind to the immobilized antibody on the plate. After removing excess sample, wells were incubated with an anti-hG-CSF polyclonal antibody conjugated to horseradish peroxidase. During this second incubation, the antibody-enzyme conjugate bind to immobilized LA-rhG-CSF. Excess conjugate was removed by washing. A chromogen was added into the wells and was oxidized by horseradish peroxidase to form a blue colored complex. The reaction was stopped by the addition of acid, which turns the blue coloration to yellow. The intensity of the yellow color is directly proportional to the amount of conjugate bound to the antibody complex, which, in turn, is directly proportional to the amount of LA-rhG-CSF in the samples. The absorbance was measured with a microplate reader and a calibration curve was generated by plotting absorbance versus the known concentrations of the calibration standard samples.
Materials used for calibration curve & concentration	LA-EP2006-03.REF spiked in 100% healthy human serum pool. The concentration of the calibration curve below considers the minimum required dilution (MRD): • S01: 48,000 pg/mL • S02: 24,000 pg/mL • S03: 12,000 pg/mL • S04: 6,000 pg/mL • S05: 3,000 pg/mL • S06: 1,500 pg/mL • S07: 750 pg/mL (anchor point)
Validated assay range	1,500 pg/mL – 48,000 pg/mL
Material used for QCs & concentration	LA-EP2006-03.REF spiked in 100% healthy human serum pool. The concentration of the QCs below considers the minimum required dilution (MRD): • QC1: 36,000 pg/mL • QC2: 9,000 pg/mL • QC3: 4,500 pg/mL
Minimum required dilutions (MRDs)	1:10
Source & lot of reagents (LBA)	The reagents along with the lot no for the ELISA assay are listed below: • Human serum pool (b) (4) lot no: 16080557 • Human G-CSF Quantikine® ELISA kit (R&D Systems), lot no: P151567

	QCs (including the MRD) of the Neulasta® US from 2 different lots: • VS-ULOQ: 48,000 pg/mL • VS1: 36,000 pg/mL • VS2: 9,000 pg/mL • VS3: 4,500 pg/mL • VS-LLOQ: 1,500 pg/mL	90 to 98%	
	<u>Inter-batch %CV</u> QCs (including the MRD) of the LA-EP2006.03REF material: • VS-ULOQ: 48,000 pg/mL • VS1: 36,000 pg/mL • VS2: 9,000 pg/mL • VS3: 4,500 pg/mL • VS-LLOQ: 1,500 pg/mL QCs (including the MRD) of the LA-EP2006 drug product (DP) from 2 different lots: • VS-ULOQ: 48,000 pg/mL • VS1: 36,000 pg/mL • VS2: 9,000 pg/mL • VS3: 4,500 pg/mL • VS-LLOQ: 1,500 pg/mL QCs (including the MRD) of the Neulasta® EU from 2 different lots: • VS-ULOQ: 48,000 pg/mL • VS1: 36,000 pg/mL • VS2: 9,000 pg/mL • VS3: 4,500 pg/mL • VS-LLOQ: 1,500 pg/mL QCs (including the MRD) of the Neulasta® US from 2 different lots: • VS-ULOQ: 48,000 pg/mL • VS1: 36,000 pg/mL • VS2: 9,000 pg/mL • VS3: 4,500 pg/mL • VS-LLOQ: 1,500 pg/mL	≤ 8% ≤ 10% ≤ 8% ≤ 6%	BA17014-R Appendix 6
	<u>Total error</u> QCs (including the MRD) of the LA-EP2006.03REF material: • VS-ULOQ: 48,000 pg/mL • VS1: 36,000 pg/mL	≤ 12%	BA17014-R Appendix 6

	• VS2: 9,000 pg/mL • VS3: 4,500 pg/mL • VS-LLOQ: 1,500 pg/mL QCs (including the MRD) of the LA-EP2006 drug product (DP) from 2 different lots: ≤ 15% • VS-ULOQ: 48,000 pg/mL • VS1: 36,000 pg/mL • VS2: 9,000 pg/mL • VS3: 4,500 pg/mL • VS-LLOQ: 1,500 pg/mL QCs (including the MRD) of the Neulasta® EU from 2 different lots: ≤ 15% • VS-ULOQ: 48,000 pg/mL • VS1: 36,000 pg/mL • VS2: 9,000 pg/mL • VS3: 4,500 pg/mL • VS-LLOQ: 1,500 pg/mL QCs (including the MRD) of the Neulasta® US from 2 different lots: ≤ 15% • VS-ULOQ: 48,000 pg/mL • VS1: 36,000 pg/mL • VS2: 9,000 pg/mL • VS3: 4,500 pg/mL • VS-LLOQ: 1,500 pg/mL	
Selectivity & matrix effect	10 different individual serum lots were tested, including 2 lipemic and 2 hemolytic individual serums. Range of observed bias: • LA-EP2006: ○ VS1 (36,000 pg/mL): 87% - 100% ○ VS-LLOQ (1,500 pg/mL): 88% - 106% • Neulasta® EU: ○ VS1 (36,000 pg/mL): 93% - 110% ○ VS-LLOQ (1,500 pg/mL): 101% - 119% • Neulasta® US: ○ VS1 (36,000 pg/mL): 91% - 106% ○ VS-LLOQ (1,500 pg/mL): 100% - 120%	BA17014-R Section 5.3 Appendix 11
Interference & specificity	To determine the impact of pegfilgrastim specific (anti-pegfilgrastim antibodies) ADAs in human serum of healthy volunteers on the quantification of total pegfilgrastim, different amounts of anti-LA-EP2006 and anti-PEG antibodies were spiked to two different concentrations (VS1 and VS3) of the LA-EP2006 DP, Neulasta EU and Neulasta	BA17014-R Section 5.16 Appendix 13

	<u>LA-EP2006</u> <u>ADA anti-LA-EP2006</u> VS1(3,600 pg/mL) drug: up to 3,600 pg/mL tolerated VS3(450 pg/mL) drug: up to 4,500pg/mL tolerated <u>ADA anti-PEG</u> VS1(3,600 pg/mL) drug: up to at least 144,000 pg/mL tolerated (highest tested concentration) VS3(450 pg/mL) drug: up to at least 18,000 pg/mL tolerated (highest tested concentration) <u>Neulasta® EU</u> <u>ADA anti-LA-EP2006</u> VS1(3,600 pg/mL) drug: up to 3,600 pg/mL tolerated VS3(450 pg/mL) drug: up to 4,500pg/mL tolerated <u>ADA anti-PEG</u> VS1(3,600 pg/mL) drug: up to at least 144,000 pg/mL tolerated (highest tested concentration) VS3(450 pg/mL) drug: up to at least 18,000 pg/mL tolerated (highest tested concentration) <u>Neulasta® US</u> <u>ADA anti-LA-EP2006</u> VS1(3,600 pg/mL) drug: up to 3,600 pg/mL tolerated VS3(450 pg/mL) drug: up to 4,500pg/mL tolerated <u>ADA anti-PEG</u> VS1(3,600 pg/mL) drug: up to at least 144,000 pg/mL tolerated (highest tested concentration) VS3(450 pg/mL) drug: up to at least 18,000 pg/mL tolerated (highest tested concentration) <u>Note:</u> Concentrations of validation samples and ADAs are given excluding MRD (1:10)	
Hemolysis effect	2 different serum lots were tested. Range of observed bias: • LA-EP2006: ○ VS1 (36,000 pg/mL): 94% - 100% ○ VS-LLOQ (1,500 pg/mL): 91% - 104% • Neulasta® EU ○ VS1 (36,000 pg/mL): 93% - 102% ○ VS-LLOQ (1,500 pg/mL): 108% - 111% • Neulasta® US ○ VS1 (36,000 pg/mL): 94% - 96% ○ VS-LLOQ (1,500 pg/mL): 100% - 100%	BA17014-R Section 5.3 Appendix 11
Lipemic effect	2 different serum lots were tested.	BA17014-R Section 5.3 Appendix 11

	Range of observed bias: • LA-EP2006: ○ VS1 (36,000 pg/mL): 97% - 98% ○ VS-LLOQ (1,500 pg/mL): 104% - 106% • Neulasta[®] EU: ○ VS1 (36,000 pg/mL): 102% - 110% ○ VS-LLOQ (1,500 pg/mL): 113% - 119% • Neulasta[®] US: ○ VS1 (36,000 pg/mL): 104% - 106% ○ VS-LLOQ (1,500 pg/mL): 116% - 120%	
Dilution linearity & hook effect	Samples with a starting pegfilgrastim concentration of 900 ng/mL in 100% healthy human serum pool were prepared and diluted to fit the linear calibration range of the assay. Each dilution series was prepared three times and measured in one run together with a calibration curve prepared in healthy human serum pool. The following concentrations of pegfilgrastim (incl. MRD 1:10) diluted in human serum pool were analyzed: 450,000 pg/mL, 225,000 pg/mL, 45,000 pg/mL, 22,500 pg/mL and 4,500 pg/mL • <u>LA-EP2006</u> ○ mean precision (conc. includes MRD): 450,000pg/mL: n/a ; 225,000 pg/mL: n/a; 45,000 pg/mL: 2%; 22,500 pg/mL: 4%;4,500pg/mL: 2% ○ mean accuracy (conc. includes MRD): 450,000pg/mL: n/a ; 225,000 pg/mL: n/a; 45,000 pg/mL: 89%; 22,500 pg/mL: 88%;4,500pg/mL: 94% Slope: 0.8872 Prozone effect (pg/mL: 450,000 / 225,000): >ULOQ • <u>Neulasta[®] EU</u> ○ mean precision (conc. includes MRD) 450,000pg/mL: n/a ; 225,000 pg/mL: n/a; 45,000 pg/mL: 4%; 22,500 pg/mL: 6%; 4,500pg/mL: 8% ○ mean accuracy (conc. includes MRD): 450,000pg/mL: n/a ; 225,000 pg/mL: n/a; 45,000 pg/mL: 87%; 22,500 pg/mL: 85%;4,500pg/mL: 90% Slope: 0.8624 Prozone effect (pg/mL: 450,000 / 225,000): >ULOQ • <u>Neulasta[®] US</u> ○ mean precision (conc. includes MRD) 450,000pg/mL: n/a ; 225,000 pg/mL: n/a; 45,000 pg/mL: 2%; 22,500 pg/mL: 2%; 4,500pg/mL: 7%	BA17014-R Section 5.14

	○ mean accuracy (conc. includes MRD): 450,000pg/mL: n/a ; 225,000 pg/mL: n/a; 45,000 pg/mL: 95%; 22,500 pg/mL: 93%;4,500pg/mL: 96% Slope: 0.945 Prozone effect (pg/mL: 450,000 / 225,000): >ULOQ Hook effect criteria: Samples with a concentration > ULOQ had to be measured > ULOQ. Hook effect was excluded for all test items tested	
Bench-top/process stability	To test the short term stability of the test items at room temperature, two concentrations (VS1: 36,000 pg/mL and VS3: 4,500 pg/mL) of LA-EP2006 Drug product, Neulasta® EU and Neulasta® US spiked in 100% serum pool were analyzed three times in one run after sample storage for 0 h, 24 h and 48 h at room temperature. % Precision <u>LA-EP2006: (0 h / 24 h / 48 h)</u> VS1: 0% / 2% / 1% VS3: 2% / 2% / 0% <u>Neulasta® EU: (0 h / 24 h / 48 h)</u> VS1: 1% / 1% / 2% VS3: 1% / 1% / 1% <u>Neulasta® US: (0 h / 24 h / 48 h)</u> VS1: 2% / 1% / 1% VS3: 2% / 1% / 0% % Accuracy <u>LA-EP2006 DP: (0 h / 24 h / 48 h)</u> VS1: 102% / 101% / 97% VS3: 99% / 97% / 95% <u>Neulasta® EU: (0 h / 24 h / 48 h)</u> VS1: 94% / 92% / 91% VS3: 91% / 89% / 90% <u>Neulasta® US: (0 h / 24 h / 48 h)</u> VS1: 90% / 91% / 90% VS3: 90% / 87% / 85%	BA17014-R Section 5.17
Freeze-Thaw stability	To test the freeze / thaw stability of test item LA-EP2006, 3 aliquots of two concentrations (VS-LR: 4,500 pg/mL and VS-HR: 36,000 pg/mL including MRD) spiked in 100% human serum pool were tested freshly prepared (this value served as a reference value (t0), after one, three and five freeze / thaw cycles at -75°C±15°C and -20°C±5°C (thawing at room temperature for at least 30 minutes). All samples had to be frozen for at least 12 h before thawing and freezing again (first thawing was performed at least 24 h after first freezing). For analysis, the samples were measured undiluted each in three duplicates against a calibration curve in one run.	N-A-IMM-16-001. (b) (4) validation report for the 103 study Section 5.12

	The analyte is considered to be stable when the mean of the measured concentration is between 80.0% and 120% of the nominal value (% recovery). Recovery rate at 0h VSLR-ST: 105% VSHR-ST: 104% Recovery rate 1xF/T at -20°C±5°C VSLR-ST: 104% VSHR-ST: 100% Recovery rate 3xF/T at -20°C±5°C VSLR-ST: 104% VSHR-ST: 102% Recovery rate 5xF/T at -20°C±5°C VSLR-ST: 103% VSHR-ST: 102% Recovery rate 1xF/T at -75°C±15°C VSLR-ST: 105% VSHR-ST: 104% Recovery rate 3xF/T at -75°C±15°C VSLR-ST: 103% VSHR-ST: 105% Recovery rate 5xF/T at -75°C±15°C VSLR-ST: 105% VSHR-ST: 105% Pegfilgrastim remains stable after 5 freeze / thaw cycles at -20°C ±5°C and at -75°C ±15°C. Note: Data for freeze.thaw stability was included in validation report N-A-IMM-16-001 and therefore lots for reference materil and drug product differ from the ones used during the BA17014 validation.	
Long-term storage	To test the long term at -70°C stability for LA-EP2006-02.REF, LA-EP2006 and Neulasta®, two concentrations (i.e. VS1 and VS3) of each test item were analyzed three times in one run after storage at -70 °C for different time periods: 0h (start value), 1 month, 3 months and 6 months Additionally, the stability of LA-EP2006-02.REF after 12, 15, 19 and 24 months was determined. % Accuracy <u>LA-EP2006-02.REF: (0 h / 1 / 3 / 6 / 12 / 15 / 19 / 24 months)</u> VS1: 102% / 106% / 105% /100% / 104%/106% /105% / 109% VS3: 104% / 109% / 98% / 93 / 106% /110% / 107% / 119% <u>LA-EP2006-DP: (0 h / 1 / 3 / 6 months)</u> VS1: 108% / 114% / 104% / 111% VS3: 113% /117% / 101% / 104%	BA09013-R Section 5.1.12 Amendment 2

	<u>Neulasta®: (0 h / 1 / 3 / 6 months)</u> VS1: 96% / 100% / 95% / 91% VS3: 94% / 99% / 93% / 89% To test the long term at -20°C of LA-EP2006 , two concentrations (i.e. VS1 and VS3) were analyzed three times in one run after storage at -20 °C for different time periods: h (start value), 1 month, 3 months, 6 months and 12 months % Accuracy <u>LA-EP2006-DP: (0 h / 1 / 3 / 6 / 12 months)</u> VS1: 93% / 97% / 95% / 96% / 89% VS3: 99% / 98% / 96% / 99% / 90% Note: Data from long term storage was included in validation report BA09013 and therefore lots for reference material and drug product differ from the ones used during the BA17014 validation.	
Parallelism	Six study samples were diluted 6 times in a serial manner, all samples were quantified within the allowed precision limits, therefore excluding any possible matrix effect	BA17015-R Section 4.3 Appendix 11
Carry over	NA	
Method performance in study (LA-EP06-104) (Provide bioanalytical report link) BA17015-R		
Assay passing rate	All samples (including ISR) were measured in 1094 runs (version 2 will contain an additional run, corresponding to 1095 runs). From these, 4 runs were rejected due to failure to meet acceptance criteria either for QC (quality controls) or calibration curve.	BA17015-R Appendix 03
Standard curve performance	- Cumulative bias range: 0 to 1% - Cumulative precision: ≤ 2% CV Note: Data corresponds to maximum and minimum accuracy and maximum CV for the calibration curve of all accepted plates only	BA17015-R Appendix 4
QC performance	- Cumulative bias range: -4 to -2% - Cumulative precision: ≤ 6% CV - TE: ≤ 9% (LBA only) Note: : Data corresponds to maximum and minimum accuracy and maximum CV for the quality controls of all accepted plates only	BA17015-R Appendix 5
Method reproducibility	Incurred sample reanalysis was performed in 6% of study samples and 99.3 % of samples met the pre-specified criteria	BA17015-R Section 4.4 Appendix 10
Study sample analysis/ stability	Study sample stability Samples are always kept under the conditions described in the lab manual. During shipment, the PK samples will be surrounded with sufficient dry ice to maintain a temperature of ≤-80°C_or below during transport and receipt. It is not allowed to send different aliquots of one sample within one shipment. A temperature logger (provided by (b)(4)) will be included in each shipment. The data logger evaluation will be sent to the receiving site automatically by PRA/Celerion. Long-term stability of 24 months covers sample collection until analysis.	

	Stability of working solutions To test the long-term stability of the standard working solution, three aliquots of the frozen WS480STD (pre-dilution for calibration curve) were tested at different time points against a standard curve prepared from aliquots of freshly frozen reference item working solution (WS480STD_F) and freshly frozen QC working solution (WS360QC_F, pre-dilution for quality controls). WS480STD_F and WS360QC_F will be prepared as described for WS480STD and WS360QC respectively, but stored at -70°C for up to 1 week only. The standards prepared from the WS480STD_F served as calibration curve in the assay. The frozen WS480STD (three aliquots) for the respective time point was used to prepare serial dilutions with the concentrations of the standard curve samples S01-S06. In order to monitor the quality of the assay two sets of QCs were prepared from WS360QC_F and analyzed in the same experiment. The working solution for preparation of calibration curve samples can be stored in a -70°C freezer for at least 9 months (prolongation up to 18 months of the working solution is addressed in the version 3 of BA17014-R) without impact on pegfilgrastim concentration.

For PD determination the applicant provided the details of the bioanalytical method used to determine pharmacodynamics, to list acceptance of runs and to display results, i.e. ANC over time in the blood of the subjects included in the LA-EP06-104 study over the three periods.

Absolute Neutrophil Counts are enumerated using an ADVIA 2120i Hematology System in (b) (4) or a Sysmex XE2100 hematology analyzer in (b) (4). The method used is flow cytometry that differentiates the white blood cell population into five categories, one of which is neutrophils. Method validation summary is presented in **Table 9**.

Table 9. Method Validation for PD to support Study LA-EP06-104

Review summary	Method validation was adequate to support the Study LA-EP06-104
Method performance summary	
Functional sensitivity	0.02 x 10 ⁹ cells/L (for WBC)
Linear range	0.02-400 x 10 ⁹ cells/L for WBC
Percent bias	< 20% for neutrophils
Intra-batch %CV	< 2% for neutrophils
Inter-batch %CV	< 2% for neutrophils
Performance in study LA-EP06-104	
Inter-batch %CV	< 2% for neutrophils
Sample analysis	Whole blood samples were collected and stabilized at room temperature for approximately one hour prior to analysis and then were analyzed immediately.

The ANC was measured by a validated method using a commercial flow cytometer (ADVIA 2120i Hematology System) at the Clinical Chemistry Laboratory of (b) (4) of the method for the determination of hematology parameters on the ADVIA 2120i Hematology System were performed by the (b) (4) and results of these validations are reported in the submission. ADVIA technology utilizes peroxidase staining, the gold-standard in differential testing. This primary methodology of the ADVIA Hematology Systems also provides a secondary total white cell count that acts as an internal QC check to

monitor sample integrity. The ADVIA 2120i performs a 6-part differential count by lysing erythrocytes and analyzing the light scatter/fluorescence or size/myeloperoxidase content of the leukocytes, respectively. The ADVIA 2120i is capable of precisely counting very low numbers of neutrophils. An example of ANC validation summary is shown in **Table 10**.

Table 10. ANC validation summary

CRO/site	Hematology Analyzer
(b) (4)	Commercial flow cytometer (Sysmex XN-11 Hematology Analyzer)

Hematology Analyzer	Validation Parameter	Specification	Validation result
Sysmex XN-11 Hematology System (A1129)	Reproducibility (within run %CV)	WBC: 3.0%	WBC: 1.06
		RBC: 3.0%	RBC: 0.56
		HgB: 3.0%	HgB: 0.43
		MCV: 3.0%	MCV: 0.28
		PLT: 3.0%	PLT: 1.05
		Retic%: 5.0%	Retic%: 2.46
Accuracy and Precision (within run %CV)		HB: <5%	<u>70371101 control sample:</u> HB: 0.47
		HCT: <5%	HCT: 0.64
		MCV: <5%	MCV: 0.37
		MPV: <5%	MPV: 0.48
		PLT: <7.5%	PLT: 1.06
		PLYM: <7.5%	PLYM: 2.05
		PMON: <12%	PMON: 4.74
		PNEU: <7.5%	PNEU: 1.82
		RBC: <5%	RBC: 0.80
		RDW: <5%	RDW: 0.49
		RETA: <5%	RETA: 2.38
		WBC: <5%	WBC: 1.02
			<u>70371102 control sample:</u> HB: 0.56
			HCT: 1.14
	MCV: 0.38		
	MPV: 0.97		
	PLT: 1.93		
	PLYM: 4.83		
	PMON: 6.21		
	PNEU: 1.74		
	RBC: 0.99		
	RDW: 0.59		
	RETA: 2.99		
	WBC: 1.41		

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CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW	
BLA	761045 (SDN1)
Submission Date	8/27/2015: \\CDSESUB1\evsprod\BLA761045\0000
Type/Category	BLA 351 (k)
Brand (generic) Name	ZIEXTENZO (LA-EP2006)
Dosage Form /Strength	6 mg/0.6 mL (10 mg/mL) in a single-use pre-filled syringe
Dosing Regimen	6 mg per chemotherapy cycle
Proposed Indication	Decrease the incidence of infection, as manifested by febrile neutropenia, in patients with non-myeloid malignancies receiving myelosuppressive anti-cancer drugs associated with a clinically significant incidence of febrile neutropenia
Applicant	Sandoz, Inc.
Clinical Pharmacology Reviewer	Xianhua (Walt) Cao, Ph.D.
Clinical Pharmacology Team Leader	Bahru Habtemariam, Pharm.D.
OCP Division	DCPV
OND Division	Division of Hematology Products
BsUFA Goal Date	June 26, 2016
Action Goal Date	June 26, 2016

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1 EXECUTIVE SUMMARY

To support the 351(k) Biologics License Application (BLA) for ZIEXTENZO (LA-EP2006) as a biosimilar to US-licensed Neulasta (licensed under BLA 125031 by Amgen Inc.), the applicant submitted data from two clinical studies that included pharmacokinetic (PK) and pharmacodynamic (PD) similarity assessments in healthy subjects and in patients with breast cancer. Study LA-EP06-101 was a single dose PK and PD study in healthy subjects (N=279, 93/arm) that compared the PK and PD of LA-EP2006, US-licensed Neulasta, and EU-approved Neulasta in a 3-way parallel study design following the administration of 6 mg subcutaneous dose. The pre-specified PK and PD similarity criteria were that the 90% confidence interval (CI) of the geometric mean ratio (GMR) of the test and the comparator products in pairwise comparisons be within the 80 to 125% range. A second study, LA-EP06-302 compared the efficacy, safety, and PK of LA-EP2006 and EU-approved Neulasta in 308 breast cancer patients. In this study, PK data were collected in a sub-set of 58 patients and PK assessment was considered supportive of the healthy subject PK and PD study (LA-EP06-101).

All three pairwise PK comparisons for both AUC and Cmax in the single dose PK/PD study LA-EP06-101 failed to meet the pre-specified assessment criteria. The upper bounds of the 90% CI for both Cmax and AUC were over the 125% acceptance range. The 90% CI of the three pairwise PD comparisons (i.e., absolute neutrophil counts (ANC)), were within the 80 to 125% range, meeting the pre-specified similarity criteria.

Although PK assessment in study LA-EP06-302 was intended to be supportive, the PK data showed unusually high GMR between LA-EP2006 and EU-approved Neulasta. The data in **Table 1** show high AUC GMRs and a trend toward higher exposure of LA-EP2006 when compared to US-licensed Neulasta and EU-approved Neulasta both in animal and human studies.

Table 1. Cross study comparison of LA-EP2006 PK similarity assessments

Study	Subjects	Comparison	Geometric Mean Ratio (90% CI)	
			AUC _{0-inf} (ng*h/mL)	Cmax (ng/mL)
Pivotal PK/PD Study LA-EP06-101	Healthy subjects	LA-EP2006 vs EU Neulasta	111 (90 – 136)	102 (81 - 127)
		LA-EP2006 vs US Neulasta	115 (92 – 142)	107 (85 -136)
		EU Neulasta vs US Neulasta	104 (84 – 129)	106 (85 - 132)
Supportive PK/PD Study LA-EP06-302	Breast Cancer Patients	LA-EP2006 vs EU Neulasta	121 (96 -180)	123 (85 -179)
Supportive * (Rat PK/PD)	Neutropenic Rats	LA-EP2006 vs EU Neulasta	1.20 (NA)	1.27 (NA)

*rat study: 4 µg/kg dose

After examination of the submitted human and animal PK and PD data, the Clinical Pharmacology review team concluded that the applicant failed to support a demonstration of PK similarity between LA-EP2006, US-licensed Neulasta, and EU-approved Neulasta. The applicant has not adequately explained the trend of higher AUC and Cmax of LA-EP2006 when compared to US-licensed Neulasta and EU approved Neulasta. In addition, the healthy subject PK and PD

study (LA-EP06-101) was not adequately powered to assess the PK similarity between LA-EP2006 and the US-licensed Neulasta.

1.1 RECOMMENDATIONS

The pharmacokinetic similarity assessment is a critical element in the demonstration of biosimilarity for proposed biosimilar products to US-licensed Neulasta. The applicant has not demonstrated PK similarity between their proposed biosimilar product and US-licensed Neulasta. The Office of Clinical Pharmacology, Division of Clinical Pharmacology V recommends non-approval of the application.

Recommendation to the Applicant:

1. We recommend that you conduct further root cause analyses to identify factors that may have led to the inability to demonstrate PK similarity.
2. Once the root causes are identified, you should conduct a feasibility assessment to determine whether developing your product as a proposed biosimilar product under section 351(k) of the PHS Act is still a viable option for licensure of LA-EP2006.
3. If the results of the feasibility assessment are supportive of continued development of LA-EP2006 as a proposed biosimilar to US-licensed Neulasta, you should consider conducting an adequately designed study to demonstrate PK and PD similarity of LA-EPO2006 and US-licensed Neulasta in healthy subjects. You should consider a cross-over study with a sample size estimation based on the expected mean AUC and Cmax differences and publicly available PK variability of pegylated G-CSF.

1.2 SUMMARY OF IMPORTANT CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS FINDINGS

This Biologics License Application (BLA) for LA-EP2006 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 LA-EP2006 as a biosimilar to US-licensed Neulasta (Amgen Inc. BLA 125031), and is seeking licensure for all the indications for which US-licensed Neulasta has been previously licensed. LA-EP2006 drug product was developed as an aqueous solution for subcutaneous (s.c.) administration, filled in a single-use prefilled syringe in the strength of 6 mg/0.6 mL.

The applicant submitted the pharmacokinetic (PK) assessment results from two clinical studies. The first study (LA-EP06-101) was a 3 arm-parallel PK - pharmacodynamic (PD) study in healthy subjects in which a single s.c. dose of 6 mg was given to evaluate the PK and PD similarity for LA-EP2006, EU-approved Neulasta and US-licensed Neulasta with n=93 for each treatment arm. The pre-specified PK and PD similarity criteria were that the 90% confidence interval (CI) of the geometric mean ratio (GMR) of the test and the comparator products should be within the 80 to 125% range. The second clinical study (LA-EP06-302) was an efficacy and safety comparison study for LA-EP2006 and EU-approved Neulasta in patients with breast cancer receiving TAC (docetaxel, doxorubicin, and cyclophosphamide) anticancer chemotherapy in adjuvant setting. In this trial, PK data were collected in a sub-set of 58 patients and the PK assessment was considered supportive of the healthy subject PK and PD study (LA-EP06-101).

PK similarity assessment: A variability of ~70-90 % for AUC and C_{max} were observed for all treatment arms in the healthy subjects. The geometric mean ratios (90% CI) of AUC and C_{max} indicated higher exposures for LA-EP2006 when compared with US-licensed Neulasta or EU-approved Neulasta. None of the 90% CIs of the GMR ratio for C_{max} or AUC fell within the pre-specified margins of 80 -125% for PK similarity (**Table 4**, page 11).

PD similarity assessment: The 90% CI for the geometric mean ratios of the ANC E_{max} and area under the effect curve ($AUEC_{0-last}$) for LA-EP2006, EU-approved Neulasta and US-licensed Neulasta were within the pre-specified boundaries of 80 -125% for PD similarity (**Table 5**, page 12). PD response is dependent on the exposure, target engagement and potency of a molecule. In this instance, PD is less sensitive than PK for pegfilgrastim biosimilar product assessment.

The applicant attributed the AUC differences between LA-EP2006 and comparator products (i.e., US- or EU- Neulasta) to the difference in six product-related variants. Although each product variant individually showed small differences (<3%), together there were 4.4 to 5.8 % higher product variants in the US or EU Neulasta comparator products compared to LA-EP2006 (**Table 8**). The applicant further indicated that these product-related variants are leading to reduced exposure of the comparator products when compared to LA-EP2006. However, there were no data submitted by the applicant to support that these variants alter (reduce) the PK of the comparator products.

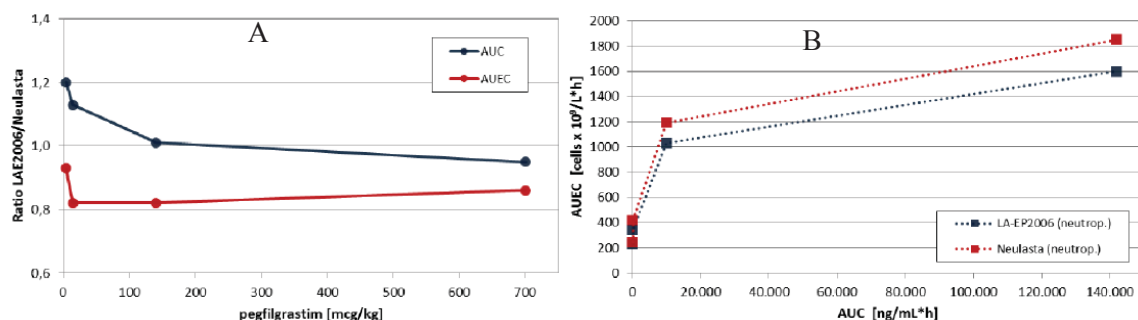
A single dose PK/PD study in neutropenic rats showed that LA-EP2006 has higher AUC than EU-Neulasta at low doses. Furthermore, at any given dose, the AUEC of LA-EP2006 was lower than AUEC of EU-Neulasta (**Figure 1A**) in this animal study. Results of the applicant's

exposure-response analyses using rat PK and ANC data also indicate that LA-EP2006 has reduced exposure-response compared to EU-Neulasta (**Figure 1B**).

The animal PK and PD data raise uncertainties regarding the following:

- The applicant did not explain the observed differences in exposure-response between LA-EP2006 and EU-Neulasta observed in Study LA-EP06-008.
- It may be possible that, in healthy subjects, the higher exposure (AUC and Cmax) observed for LA-EP2006 could be compensating for a lower in vivo activity and thus achieving similar PD response. Therefore, the demonstration of PD similarity could be a result of the higher LA-EP2006 exposure.

Figure 1. AUC and AUEC ratios (LA-EP2006/EU-approved Neulasta) for the dose-range (A) and exposure-response relationship for LA-EP2006 & EU-approved Neulasta (B) (Data from study LA-EP06-008)



Source: Applicant's responses to IR (2/3/2016) for clinical pharmacology

Overall, submitted data do not support a demonstration of PK similarity between LA-EP2006 and US-licensed Neulasta.

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2 QUESTION BASED REVIEW

2.1 GENERAL ATTRIBUTES

2.1.1 What are the highlights of the chemistry and physical-chemical properties of the drug substance and the formulation of the drug product as they relate to clinical pharmacology and biopharmaceutics review?

LA-EP2006 is a covalent conjugate of recombinant methionyl human granulocyte colony-stimulating factor (r-met-HuG-CSF, filgrastim) covalently linked to a 20 kDa polyethylene glycol (PEG). The molecular weight of LA-EP2006 is approximately 39 kDa. LA-EP2006 was developed to be biosimilar to the US-licensed Neulasta[®] (pegfilgrastim; BLA 125031), a registered trademark of Amgen Inc.

LA-EP2006 drug product is supplied as an aqueous solution in a single-use prefilled syringe with strength of 6 mg/0.6 mL (10 mg/mL) for subcutaneous (s.c.) administration.

2.1.2 What are the proposed mechanism(s) of action and therapeutic indication(s)?

Pegfilgrastim is a colony-stimulating factor that acts on hematopoietic cells by binding to specific cell surface receptors, thereby stimulating proliferation, differentiation, commitment, and end cell functional activation.

The currently proposed indication is the same to US-licensed Neulasta[®]: to decrease the incidence of infection, as manifested by febrile neutropenia, in patients with non-myeloid malignancies receiving myelosuppressive anti-cancer drugs that are associated with a clinically significant incidence of febrile neutropenia.

2.1.3 What are the proposed dosage(s) and route(s) of administration?

The applicant proposes the same dosing regimen, 6 mg s.c. during every chemotherapy cycle, as that approved for US-licensed Neulasta.

2.2 GENERAL CLINICAL PHARMACOLOGY

2.2.1 What are the design features of the clinical pharmacology and clinical studies used to support dosing or claims?

To demonstrate pharmacokinetics (PK) and pharmacodynamics (PD) similarity, a single-dose parallel PK-PD study (LA-EP06-101) in healthy subjects (n=93 per arm) was conducted to evaluate the PK and resultant absolute neutrophil counts (ANC) of LA-EP2006, EU-approved Neulasta and US-licensed Neulasta (**Table 1**). For PK similarity following single s.c. dose of 6 mg in healthy subjects, the primary PK endpoint was AUC_{0-last} and the secondary PK parameters included AUC_{0-inf} and C_{max} . For the PD endpoint, the ANC was considered the PD measure of interest given the relationship between ANC and duration of severe neutropenia. The primary PD exposure parameters are area under the effect curve (AUEC) and maximum ANC count (ANC_{max}).

To demonstrate equivalent efficacy and safety, LA-EP2006 was compared to EU-approved Neulasta in patients with breast cancer who were eligible for neo-adjuvant or adjuvant treatment

with TAC ((Taxotere[®] [docetaxel 75 mg/m²] in combination with Adriamycin[®] [doxorubicin 50 mg/m²] and Cytosan[®] [cyclophosphamide 500 mg/m²])) anticancer chemotherapy in studies LA-EP06-301 and LA-EP06-302 (**Table 2**). Patients received 6 mg s.c. doses LA-EP2006 or EU-approved Neulasta on the day following TAC chemotherapy for up to six cycles. A comparison of the duration of severe neutropenia (DSN) was then conducted with a pre-specified margin of -1 to 1 days with 90% CI. The DSN was calculated as the number of consecutive days from the first day when a patient's ANC was $< 0.5 \times 10^9/L$ until the patient reached an ANC $\geq 0.5 \times 10^9/L$ in Cycle 1. Refer to the Clinical and Statistical reviews for more detail.

Table 2. LA-EP2006 clinical studies in the submission					
Study No.	Design /Population	Comparators	Assessment	Dosage and Regimen	N
LA-EP06-101	Randomized, parallel, PK and PD similarity study in healthy subjects	LA-EP2006 EU-approved Neulasta US-licensed Neulasta	PK, PD (ANC), safety & immunogenicity	6 mg s.c. single dose	279 (93/arm)
LA-EP06-301	Randomized, parallel, comparative safety and efficacy in patients with breast cancer	LA-EP2006 EU-approved Neulasta	Efficacy (DSN), Safety & Immunogenicity	6 mg s.c. per chemotherapy cycle for up to 6 cycles	316; No PK subjects
LA-EP06-302	Randomized, parallel, comparative safety and efficacy in patients with breast cancer	LA-EP2006 EU-approved Neulasta	Efficacy (DSN), safety & Immunogenicity, PK (PK subset)	6 mg s.c. per chemotherapy cycle for up to 6 cycles	308; PK substudy n=58

Abbreviations: PK: pharmacokinetics; PD: pharmacodynamics; ANC: absolute neutrophil counts; DSN: duration of severe neutropenia, during cycle 1 of the chemotherapy.

2.2.2 What are the PK and PD predefined similarity margins?

In the PK-PD study, the predefined similarity criteria for both PK (AUC and C_{max}) and PD were that the 90% CI of the geometric mean ratio should lie within 80-125%. A 95% CI of the geometric mean ratio for PK comparison was also conducted by the applicant in the BLA submission. For ANC, the predefined similarity criteria for both the area under the effect curve (AUEC) and maximum ANC count (ANC_{max}) were that 90% CI of for the ratio of the geometric means should lie within 80-125%. A 95% CI of the ratio of the geometric means for PD comparison was also conducted.

2.2.3 Are the active moieties in the plasma (or other biological fluid) appropriately identified and measured to assess pharmacokinetic parameters and exposure response relationships?

Pegfilgrastim serum concentrations were measured to characterize the PK of LA-EP2006, EU-approved Neulasta and US-licensed Neulasta using a validated enzyme-linked immunosorbent assay (ELISA) method with a lower limit of quantification (LLOQ) of 150 pg/mL.

2.2.4 Exposure-response

2.2.4.1 Are the dose(s) selected for PK and PD studies consistent with the known relationship between dose-concentration-response, and is there any unresolved dosing or administration issue?

A single dose of 6 mg for LA-EP2006 and Neulasta® had been selected in the PK-PD study as it represented the recommended dose of Neulasta® used to treat patients with non-myeloid malignancies receiving myelosuppressive anti-cancer drugs associated with a clinically significant incidence of febrile neutropenia. Pegfilgrastim is administered once per chemotherapy cycle, therefore it was appropriate to investigate a single dose in this clinical study. In addition, the 6 mg dose, which corresponds to 80 to 100 µg/kg, has been demonstrated previously to fall within the steep part of the dose-response curve for pegfilgrastim PK and PD.

2.2.5 What are the PK characteristics of the drug and its major metabolite?

2.2.5.1 What are the single dose PK and PD (ANC) parameters in healthy volunteers?

PK parameters and similarity assessment:

The summary of PK parameters derived from the non-compartmental analysis after single s.c. injection of 6 mg LA-EP2006, US-licensed Neulasta, or EU-approved Neulasta in 279 healthy volunteers in Study LA-EP06-101 is presented in **Table 3**, and the serum concentration-time profiles (mean (+SD)) are shown in **Figure 2**. The exposure levels as indicated by the parameter values of AUC_{0-last}, AUC_{0-inf} and C_{max}, were higher for LA-EP2006 than those for either US-licensed Neulasta or EU-approved Neulasta. A variability of ~70-90 % for AUC and C_{max} were observed for all treatment arms.

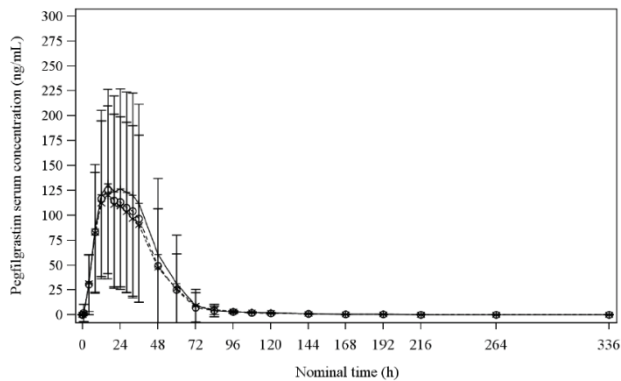
Table 3. Summary of PK parameters in healthy subjects – Study LA-EP06-101 (PP population).

Parameters	Treatment Arms (Mean (CV%))		
	LA-EP2006 N=93	US-licensed Neulasta N=91	EU-approved Neulasta N=93
AUC _{0-last} (ng*h/mL)	8216 (89%)	7213 (92%)	7277 (77%)
AUC _{0-inf} (ng*h/mL)	8289 (88%)	7251 (92%)	7295 (77%)
C _{max} (ng/mL)	229 (82%)	207 (82%)	215 (73%)
t _{1/2} (h)	43 (38%)	41 (41%)	45 (44%)

Notes: AUC_{0-inf}, area under the curve measured from the time of dosing and extrapolated to infinity; AUC_{0-last}, area under curve measured from the time of dosing to the last measured concentration; C_{max}, measured maximum serum concentration after administration; CV%, coefficient of variation as percentage; N, number of subjects analyzed; PK, pharmacokinetics; PP population, per protocol population; t_{1/2}, apparent terminal half-life of elimination phase;

Source: Data adapted from applicant's Summary of Clinical Pharmacology, Table 2-4, Page 18

Figure 2. Time course of pegfilgrastim serum concentrations (mean ($\pm$ SD)) in healthy subjects – Study LA-EP06-101 (PP population).



Source: Applicant's Summary of Clinical Pharmacology, Figure 2-1, Page 19

The geometric means (90% CI and 95% CI) of the ratios of the C_{max} , AUC_{0-last} and AUC_{0-inf} for LA-EP2006, EU-approved Neulasta and US-licensed Neulasta, are shown in **Table 4**. As shown in **Table 4**, none of the CIs of the ratio for C_{max} or AUC falls within the pre-specified margins of 80 -125%.

The geometric mean ratios of AUC and C_{max} indicated higher exposures for LA-EP2006 when compared with US-licensed Neulasta (15% and 7%, respectively) and EU-approved Neulasta (11% and 2%, respectively) (**Table 4**). When comparing AUC and C_{max} of the two comparator products, EU-approved Neulasta were approximately 4% and 6% higher exposures than US-licensed Neulasta.

Table 4. PK similarity assessment in healthy subjects – Study LA-EP06-101 (PP population).

Comparison	Parameters	GeoMean Ratio (%)	[95% CI]	[90% CI]
LA-EP2006 / US-licensed Neulasta	AUC_{0-last}	115	[89; 149]	[93; 143]
	AUC_{0-inf}	115	[90; 150]	[92; 142]
	C_{max}	107	[83; 142]	[85; 135]
LA-EP2006 / EU-approved Neulasta	AUC_{0-last}	110	[85; 142]	[88; 136]
	AUC_{0-inf}	111	[86; 142]	[89; 136]
	C_{max}	102	[78; 133]	[81; 127]
EU-approved Neulasta / US-licensed Neulasta	AUC_{0-last}	105	[81; 135]	[85; 130]
	AUC_{0-inf}	104	[82; 140]	[84; 129]
	C_{max}	106	[82; 140]	[85; 132]

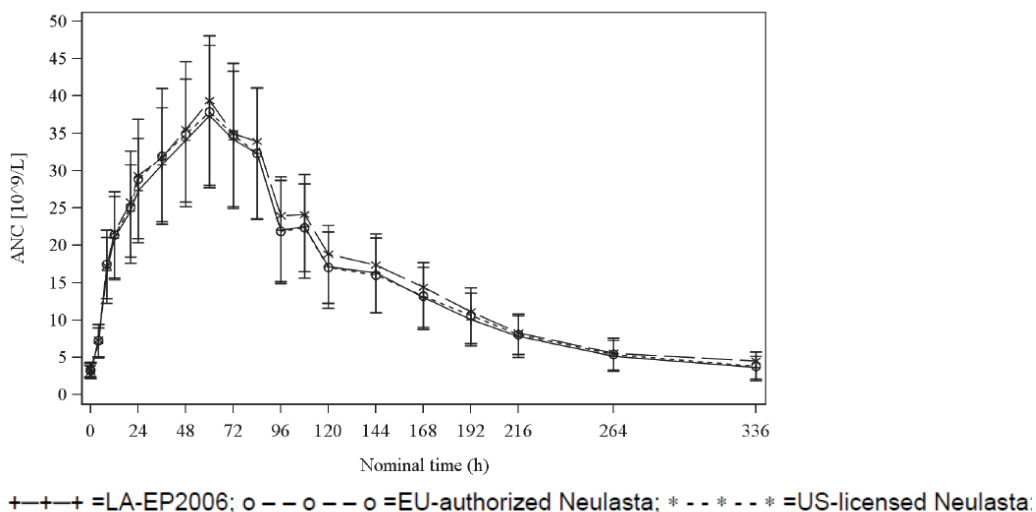
Notes: AUC_{0-inf} , area under the curve measured from the time of dosing and extrapolated to infinity; AUC_{0-last} , area under curve measured from the time of dosing to the last measured concentration; C_{max} , measured maximum serum concentration after administration; GeoMean, geometric mean; PK, pharmacokinetics; PP population, per protocol population;

Source: FDA's analysis combined with data from applicant's Summary of Clinical Pharmacology, Table 2-5, Page 20

PD parameters and similarity assessment:

As shown in **Figure 3**, the PD profiles (ANC response time curves) of LA-EP2006, EU-approved Neulasta and US-licensed Neulasta after single s.c. injection of 6 mg in healthy subjects overlay with each other. The geometric means (90% and 95% CI) of the ratios of the ANC E_{max} and area under the effect curve (AUEC_{0-last}) for LA-EP2006, EU-approved Neulasta and US-licensed Neulasta, are shown in **Table 5**. The 90 and 95% CI of the ratio for E_{max} and AUEC_{0-last} fall within the pre-specified boundaries of 80 -125%.

Figure 3. Time course of ANC (mean (± SD)) in healthy subjects– Study LA-EP06-101 (PP population).



Source: Applicant’s Summary of Clinical Pharmacology, Figure 2-4, Page 29

Table 5. PD similarity assessment in healthy subjects– Study LA-EP06-101 (PP population).

Comparison	Parameters	GeoMean Ratio (%)	[95% CI]	[90% CI]
LA-EP2006 / US-licensed Neulasta	AUEC _{0-last}	99	[93; 105]	[94; 104]
	E _{max}	101	[94; 108]	[95; 106]
LA-EP2006 / EU-approved Neulasta	AUEC _{0-last}	101	[95; 107]	[96; 106]
	E _{max}	102	[96; 109]	[97; 108]
EU-approved Neulasta / US-licensed Neulasta	AUEC _{0-last}	99	[92; 104]	[93; 103]
	E _{max}	98	[92; 105]	[93; 104]

Source: Data adapted from applicant’s Summary of Clinical Pharmacology, Table 2-6, Page 22

2.2.5.2 How does the PK and PD (ANC) of the drug and its major active metabolites in healthy volunteers compare to that in patients?

PK parameters in patients:

PK profiles were assessed for LA-EP2006 and EU-approved Neulasta in breast cancer patients in the PK substudy (n=58) of Study LA-EP06-302 (**Table 6** and **Figure 4**). Blood samples for PK

measurements were collected in cycle 1 prior to chemotherapy (Day 1), on Day 2 prior to pegfilgrastim administration (Day 2) and on each day until Day 15. Further samples were taken prior to chemotherapy of cycles 2 to 6. As indicated in **Table 6**, LA-EP2006 had greater than 20% higher exposure levels than EU-approved Neulasta in breast cancer patients. The variability of C_{max} and AUC for pegfilgrastim in patients is ~90-110%. The upper bounds of 90% CIs of the ratios of the C_{max} and AUC_{0-last} for LA-EP2006 and EU-approved Neulasta are up to 180%.

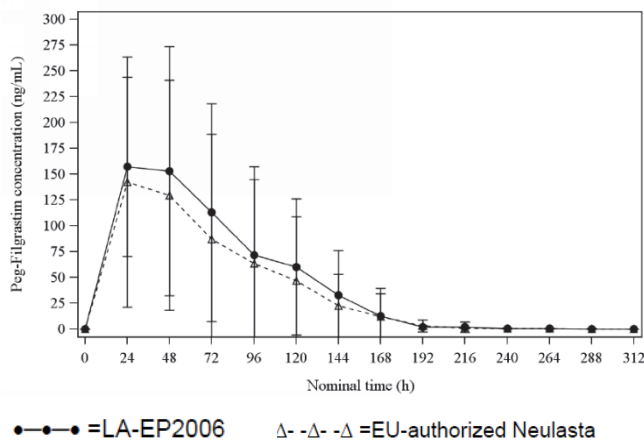
Table 6. Summary of PK comparison in patients – study LA-EP06-302 (PK Set).

Parameters	LA-EP2006 N=27	EU-approved Neulasta N=31	LA-EP2006 / EU-approved Neulasta	
			GeoMean Ratio (%)	[90% CI]
AUC_{0-last} (ng*h/mL)				
GeoMean	9612	7930	121	[82; 180]
CV%	113%	110%		
C_{max} (ng/mL)				
GeoMean	144	117	123	[85; 179]
CV%	93%	113%		

Notes: AUC_{0-last}, area under curve measured from the time of dosing to the last measurable concentration; C_{max}, measured maximum serum concentration after administration; CV%, coefficient of variation as percentage; GeoMean, geometric mean; N, number of subjects analyzed; PK, pharmacokinetics;

Source: Data adapted from applicant's Summary of Clinical Pharmacology, Table 2-6 and 2-7, Page 22-23

Figure 4. Time course of pegfilgrastim serum concentrations (mean (± SD)) in patients – cycle 1 in Study LA-EP06-302 PK set

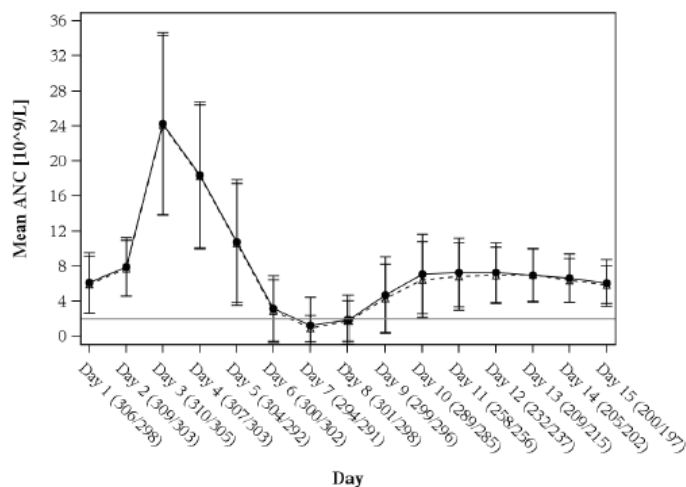


Source: Applicant's Summary of Clinical Pharmacology, Figure 2-2, Page 22

PD parameters in patients:

Mean areas under the ANC efficacy curves ($AUEC_{0-last}$), calculated from time of pegfilgrastim administration to last scheduled blood sample during Cycle 1, were calculated. As indicated in **Figure 5** and **Table 7**, the results showed lower and upper limits of the 90% CIs of the ratio of $AUEC_{0-last}$ were contained within a range of 80-125% for the acceptance of PD equivalence.

Figure 5. Time course of arithmetic mean (+/- SD) of ANC count – Pool 1 (FAS set)



—●— = LA-EP2006; --Δ-- = EU-approved Neulasta; Day X (N=LA-EP2006/N=Neulasta). The grey line indicates the threshold of $2 \times 10^9/L$ defined for ANC recovery.
 Source: Applicant's Clinical Overview, Figure 4-1, Page 31

Table 7. Comparison of PD parameters of LA-EP2006 and EU-approved Neulasta – Area under the ANC efficacy curve (AUEC_{0-last}) in patients (FAS set)

Study number	Adjusted GeoMean		Point Estimate# (%)	[90% CI]	CV%
	LA-EP2006	EU- Neulasta			
LA-EP06-301	94.4	91.5	103.3	[94.5; 112.8]	47.1
LA-EP06-301	92.8	90.5	102.6	[96.1; 109.5]	34.5

ANC=absolute neutrophil count; AUEC_{0-last}=area under the efficacy curve calculated from time of IMP administration to last scheduled blood sample during Cycle 1; CI=confidence interval; CV%=coefficient of variation as percentage; GeoMean=geometric mean; FAS=full analysis set
 #:The comparison is based on the geometric means of AUEC_{0-last} and reflects the ratio of LA-EP2006 to EU-Neulasta multiplied by 100
 Source: Adapted from applicant's Clinical Overview, Table 4-2, Page 31

Investigations related to the PK difference of LA-EP2006 to the comparator products

An information request (IR) was sent to the applicant on March 8, 2016 to address the causes and identify the factors that lead to higher exposure of LA-EP2006 compared to the comparator products, as LA-EP2006 shows a consistently higher mean AUC than US-licensed Neulasta or EU-approved Neulasta in both preclinical and clinical studies (**Table 1**). The PK variability of pegylated filgrastim alone is not a sufficient explanation for the observed consistently higher systemic exposure of LA-EP2006 as compared to US-licensed Neulasta and EU-approved Neulasta. It is important to note that the comparison of the US-licensed and EU-approved Neulasta showed AUC GMR of 4% although the upper bound of 90% CI was 129%. The data show a consistent trend of higher AUC of LA-EP2006 when compared to US-licensed and EU-approved products, indicating potential product differences.

In response to the IR, the applicant concurred with FDA's assessment that the products may be different and the applicant attributed AUC differences between LA-EP2006 and US-licensed or

EU-approved Neulasta to the difference in the six product variants (**Table 8** below). Although each product variant showed a small difference (<3%), together there were 4.4 to 5.8 % higher product variants in the US-licensed and EU-approved Neulasta products compared to LA-EP2006. The applicant further indicated that these product variants are leading to reduced exposure (AUC and Cmax) of the comparator products when compared to LA-EP2006. However, there were no data to support that these variants alter the PK of the comparator products.

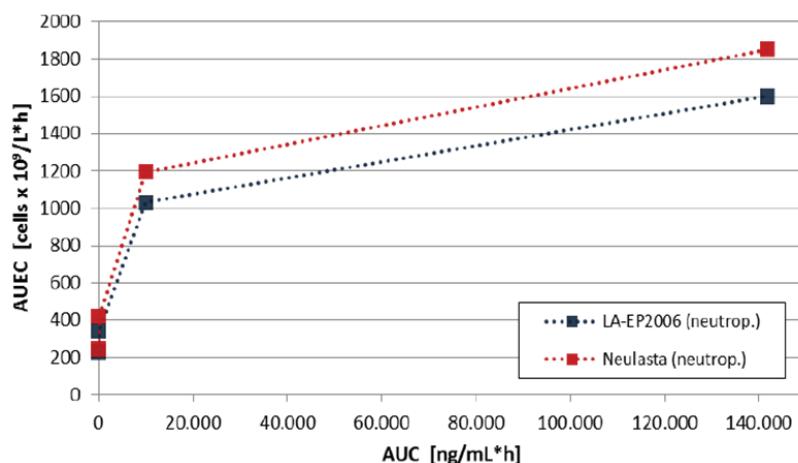
Table 8. Summary of the potential impact of individual product-related variants on the PK

Variant	Abundance in batches used in clinical study LA-EP06-101	Detectability by PK serum ELISA	Potential impact on AUC
Di-pegylated	0.3% higher in Neulasta US and 0.4% higher in Neulasta EU (RP-HPLC)	Lowered to approximately 50%	Between 0.2% and 0.8% lower exposure in Neulasta
Oxidized	0.5% higher in Neulasta US and 0.7% higher in Neulasta EU(RP-HPLC)	Lowered to approximately 80%	Only slight impact on PK of up to 0.2% lower exposure in Neulasta
Wrongly pegylated	0.4-0.6% higher in Neulasta US and 0.6% higher in Neulasta EU(CEX)	Unknown	No impact on PK expected
Aggregates/ oligomers	1.0% higher in Neulasta US and 1.2% higher in Neulasta EU (SEC)	Lowered to approximately 75%	Up to 2.3% lower exposure in Neulasta
De-amidated	Up to 2.4% higher in Neulasta US and 2.6% higher in Neulasta EU	Fully detectable	Up to 4.9% lower exposure in Neulasta
Free filgrastim	0.1-0.2% higher in Neulasta US and 0.3% higher in Neulasta EU (SEC)	Fully detectable	Approximately 3% lower exposure in Neulasta
Sum of all variants	Between 4.4% and 5.0% higher in Neulasta US and 5.8% higher in Neulasta EU	n.a.	Up to 11.2% lower exposure in Neulasta

Source: Applicant's responses to IR (3/8/2016) for clinical pharmacology

Animal PK and PD data indicate LA-EP2006 has different clearance and potency properties compared to the EU approved Neulasta. As depicted in **Figure 1**, LA-EP2006 has high GMR at low doses. At all of the studied doses in the single dose rat study, the AUEC of LA-EP2006 is lower than AUEC of EU-approved Neulasta, indicating that LA-EP2006 has lower potency as compared to EU-approved Neulasta. **Figure 6** below also shows that the exposure-response curve for LA-EP2006 is shifted to the right, also showing reduced potency of LA-EP2006 as compared to EU-approved Neulasta. Therefore, PK and PD data from the single dose rat study indicate LA-EP2006 has reduced clearance and potency when compared to EU-approved Neulasta. The reduced clearance of LA-EP2006 in rats is consistent with the clinical PK data, in which the AUC of LA-EP2006 is greater when compared to either US-licensed or EU-approved Neulasta.

Figure 6. Exposure response relationship in study LA-EP06-008 (25622)



Source: Applicant's responses to IR (2/3/2016) for clinical pharmacology

2.3 INTRINSIC FACTORS

2.3.1 Immunogenicity

2.3.1.1 What is the incidence (rate) of the formation of the anti-product antibodies (APA), including the rate of pre-existing antibodies, the rate of APA formation during and after the treatment, time profiles and adequacy of the sampling schedule?

Immunogenicity in health volunteers:

In study LA-EP06-101, blood samples for the assessment of immunogenicity were collected at 15 minutes pre-dose on Day 1, and on Days 15 and 28 post s.c. administration. The positive confirmatory tests for anti-pegfilgrastim antibodies are listed in **Table 9** below. No neutralizing antibodies binding filgrastim were detected for the samples with positive confirmatory test results. There was no unusual behavior noted in individual PK profiles indicative of potential effects of antibodies on pegfilgrastim systemic clearance.

Table 9. Immunogenicity in healthy volunteers – study LA-EP06-101 (Safety population)

Pegfilgrastim specific ADA+ n (%)	Confirmatory Test		
	LA-EP2006 (n=93)	Neulasta-US (n=93)	Neulasta-EU (n=93)
Pre-dose	0 (0)	3(3.2)	3 (3.2)
Day 15	5 (5.4)	6 (6.5)	2 (2.2)
Day 28	2 (2.2)	3 (3.2)	1 (1.1)

Notes: ADA, anti-drug antibody; n, number of subjects with an ADA positive sample; n=number of subjects in a treatment group; Neulasta-EU=EU-approved Neulasta; Neulasta-US=US-licensed Neulasta.

Source: Data adapted from applicant's Summary of Clinical Safety, Table 4-1, Page 63

Immunogenicity in patients:

In studies LA-EP06-301 and LA-EP06-302, serum samples were collected prior to the first administration on day 1 cycle 1, at day 15 of cycle 6, at the EOS visit 4 weeks after the last

administration, and at the early termination visit in case of early termination. The positive confirmatory tests for anti-product antibodies were listed in **Table 10** below.

One patient in the LA-EP2006 treatment group had a positive neutralizing antibody result at cycle 1, day 1, i.e. at pre-dose. The characterization of the APA response in the binding assay had demonstrated that APAs were targeted against pegfilgrastim and/or PEG, but not filgrastim. All post-dose sampling time points of this patient were determined to be negative for binding APA.

Neutralizing antibody results of the other patients with confirmed positive results for APA binding were negative.

Table 10. Immunogenicity in patients with breast cancer: studies LA-EP06-301 and LA-EP06-302 (SAF set)

Phase 3 studies	LA-EP06-301		LA-EP06-302	
	LA-EP2006 N=159	EU-approved Neulasta N=155	LA-EP2006 N=157	EU-approved Neulasta N=153
Pegfilgrastim specific ADA				
Pre-dose	15 (9.4)	19 (12.1)	8 (5.2)	10 (6.5)
EOS	1 (0.7)	0	0	0
6-mo SFU*	1 (0.8)	0	n.a.	n.a.
Filgrastim specific ADA				
Pre-dose	8 (5.0)	5 (3.2)	0	1 (0.7)
Cycle 6, Day 15	0	0	0	1 (0.7)
EOT	1 (0.7)	0	0	1 (0.7)
EOS	0	0	0	1 (0.7)
6-mo SFU	2 (1.7)	0	n.a.	n.a.
PEG specific ADA				
Pre-dose	18 (11.3)	20 (12.7)	13 (8.4)	18 (11.8)
EOS	1 (0.7)	1 (0.7)	0	0
6-mo SFU*	1 (0.8)	0	n.a.	n.a.

Notes: ADA, ADA=anti-drug antibody; LA-EP=LA-EP2006; n=number of patients with an ADA positive sample; N=number of patients in a treatment group; n.a.=not applicable; Neulasta=EU-Neulasta; PEG=polyethylene glycol; SAF set=safety analysis set Only time points with confirmed positive results in the confirmatory binding antibody enzyme-linked immunosorbent assay are shown. Patients could have events in more than one category. a Samples with pegfilgrastim specific ADA were further differentiated into: also filgrastim specific ADA; also PEG specific ADA; and only pegfilgrastim specific ADA.

Source: Data adapted from applicant's Summary of Clinical Safety, Table 4-2, Page 64

2.3.1.2 Does the immunogenicity affect the PK and/or PD of the therapeutic protein?
APAs did not influence the serum concentrations of pegfilgrastim in the PK-PD study. See

response to Question 2.3.1.1

2.3.1.3 Do the anti-product antibodies have neutralizing activity?

In the PK-PD study in healthy subjects, no neutralizing antibodies were detected for the confirmed positive APA+ samples. One patient was detected as a positive neutralizing antibody at predose only, with negative results for binding APA for all the post-dose sampling time points of this patient.

2.3.1.4 What is the impact of anti-product antibodies on clinical efficacy?

No indication of any impact on clinical efficacy.

2.3.1.5 What is the impact of anti-product antibodies on clinical safety (e.g., infusion-related reactions, hypersensitivity reactions, cross-reactivity to endogenous counterparts, etc.)?

No indication of any impact on the clinical safety.

2.4 EXTRINSIC FACTORS

Not applicable for this submission.

2.5 GENERAL BIOPHARMACEUTICS

2.5.1 What are the manufacturing differences between the to-be-marketed formulation and the formulation used in the pivotal clinical trial?

2.5.2 What is the in vivo relationship of the proposed to-be-marketed formulation to the pivotal clinical trial formulation in terms of comparative exposure?

Not applicable for this submission.

2.6 ANALYTICAL SECTION

2.6.1 What bioanalytical methods are used to assess concentrations and absolute neutrophil counts?

PK Assay:

The concentrations of pegfilgrastim in Study LA-EP06-101 were assayed using a validated ELISA assay. A summary of the bioanalytical methods for pegfilgrastim is shown in **Table 11**.

The MSD® 96-Well Human G-CSF Ultrasensitive Assay Kit (Meso Scale Discovery, USA), an electrochemiluminescence (ECL) – based immunoassay was adapted and validated for the determination of pegfilgrastim in breast cancer patient serum for Study LA-EP06-302. The detailed summary of all validation results of the assay is described in **Table 12** below

Table 11. Summary of the pegfilgrastim ELISA validation results (human serum)

Validation parameter	Specification	Validation result	Evaluation
Selectivity	Precision: CV $\leq$ 20% Accuracy: 80% - 120%	Serum sample matrix interferences were expected if the serum is used undiluted; after diluting the serum samples 1:5 (using human serum pool) interferences were eliminated.	Passed with restrictions
Intra-assay precision	Precision: CV $\leq$ 15%	All test items fulfilled the acceptance criteria.	Passed
Inter-assay precision	Precision: CV $\leq$ 15%	All test items fulfilled the acceptance criteria.	Passed
Intra- and inter-assay accuracy	85% - 115%, at LOQ 80% - 120%	All test items fulfilled the acceptance criteria.	Passed
Linearity	Precision: CV $\leq$ 15%, at LOQ $\leq$ 20%	All standard curves fulfilled the acceptance criteria.	Passed
Lower limit of quantification (LLOQ)	Precision: CV $\leq$ 20% Accuracy: 80% - 120%	All test items fulfilled the acceptance criteria at 150 pg/mL.	Passed
Upper limit of quantification (ULOQ)	Precision: CV $\leq$ 20% Accuracy: 80% - 120%	All test items fulfilled the acceptance criteria at 4,800 pg/mL.	Passed
Short-term stability at RT	Accuracy: 80% - 120%	All test items fulfilled the acceptance criteria for 4 h storage at RT.	Passed
Short-term stability at 2-8°C	Accuracy: 80% - 120%	All test items fulfilled the acceptance criteria for 3 days storage at 2-8°C.	Passed
Freeze/thaw stability	Accuracy: 80% - 120%	All test items fulfilled the acceptance criteria for 3 freeze/thaw cycles.	Passed
Long-term stability at -70°C	Accuracy: 80% - 120%	The acceptance criteria for 24 months storage at -70°C were fulfilled.	Passed
Long-term stability at -20°C	Accuracy: 80% - 120%	The acceptance criteria for 12 months storage at -20°C were fulfilled.	Passed
Robustness	n/a	Different microplate readers, wash devices and different analysts had no impact on the results; filtered vs. non-filtered human serum pool.	Passed
Comparability (LA-EP2006, EU-authorized and US-licensed Neulasta)	Mean ratio 0.80 to 1.20	0.97 to 1.14	Passed

Table 12. Validation results for the quantification of pegfilgrastim in patient serum

Validation parameter	Specification	Validation result	Evaluation
Selectivity	Precision: CV ≤ 25% Bias ±25% 80% acceptable	80% of test sera fulfilled the acceptance criteria.	Passed
Intra-assay precision	Precision: CV ≤ 25%	All test items fulfilled the acceptance criteria	Passed
Inter-assay precision	Precision: CV ≤ 25%	All test items fulfilled the acceptance criteria	Passed
Intra- and inter-assay accuracy	Bias ± 25%	All test items fulfilled the acceptance criteria	Passed
Out of range dilution (dilution linearity)	Bias ± 25%	All test items fulfilled the acceptance criteria; Recovery: 93.4% to 99.2%	Passed
Lower limit of quantification (LLOQ)	150 pg/mL	All test items fulfilled the acceptance criteria	Passed
Upper limit of quantification (ULOQ)	4,800 pg/mL	All test items fulfilled the acceptance criteria	Passed
Comparability	Ratio 0.80 to 1.20	Ratio 0.824 to 1.15	Passed

Note: CV, coefficient of variation; ELISA, enzyme-linked immunosorbent assay; LOQ, limit of quantification
Source: Applicant's Summary of Biopharmaceutics Studies, Table 1-4, Page 8

PD Assay

The absolute neutrophil count was determined using the Abbott Cell Dyn Sapphire system in study LAEP06- 101. Validation is described in **Table 13** below

Table 13. Summary of the validation results of ANC measurement

Validation parameter	Specification	Validation result	Evaluation
Intra-assay precision	CV ≤ 15%	Level 1: 2.0%	Passed
		Level 2: 1.6%	Passed
		Level 3 : 1.4%	Passed
		Internal material: 1.7%	Passed
Inter-assay precision	CV ≤ 15%	Level 1: 1.5%	Passed
		Level 2: 0.9%	Passed
		Level 3 : 0.9%	Passed
Bias from an assigned mean	Difference from target value: 85% - 115%,	Level 1: 0.7%	Passed
		Level 2: 0.3%	Passed
		Level 3 : 0.0%	Passed

Notes: ANC, absolute neutrophil count; CV, coefficient of variation
Source: Applicant's Summary of Biopharmaceutics Studies, Table 1-18, Page 39-40

2.6.2 What bioanalytical methods are used to assess the formation of the anti-product antibodies?

APA against pegylated filgrastim in human serum was evaluated by a three-step procedure comprising a validated binding anti-pegfilgrastim antibody ELISA (screening and confirmatory assay) and a validated cell based neutralizing antibody (NAB) assay:

i. Screening anti-pegfilgrastim binding ELISA:

All study samples were first analyzed in a screening assay against the calibration curve of the rabbit anti-pegfilgrastim polyclonal antibody (reference item). In case of a result equal to or above the validated screening cut-point, a confirmatory/specificity assay was additionally performed. Serum samples with a result above the ULOQ were analyzed (in the confirmatory assay) in a higher dilution prepared in human serum pool.

ii. Confirmatory/specificity anti-pegfilgrastim binding ELISA

To prove the specificity of the anti-pegfilgrastim antibodies detected in the screening assay, all samples with a result equal to or above the screening cut-point were reanalyzed in the confirmatory assay. Specificity of the binding was confirmed if the rate of the assay signal depletion was above the validated confirmatory cut-point after spiking excess of free drug (pegfilgrastim) into the medium. Additionally, positive samples were tested with the confirmatory assay regarding their specificity against the drug (filgrastim) and/or against polyethylene glycol (PEG). In case of confirmed positive anti-pegfilgrastim antibody samples the titer of binding anti-pegfilgrastim antibodies was determined for the respective study samples.

iii. NAB assay

If positive results (confirmed anti-PEG, anti-pegfilgrastim or anti-filgrastim antibodies) were obtained in the binding antibody ELISA, a validated anti-pegfilgrastim cell-based NAB assay for determination of potential neutralizing antibodies was applied.

Refer to the CMC immunogenicity review for further details on the assays.

2.6.2.1 What is the performance of the binding assay(s)?

Refer to the CMC immunogenicity review for information and details regarding the performance of the binding assays.

2.6.2.2 What is the performance of the neutralizing assay(s)?

Refer to the CMC immunogenicity review for information and details regarding the performance of the neutralizing assays.

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/s/

XIANHUA W CAO
06/02/2016

BAHRU A HABTEMARIAM
06/02/2016

NAM ATIQRUR RAHMAN
06/02/2016
I agree with the Review Team's recommendation.