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APPLICATION NUMBER:

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NON-CLINICAL REVIEW(S)

**DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH**

PHARMACOLOGY/TOXICOLOGY BLA REVIEW AND EVALUATION

Application number: 761039
Supporting document/s: 1, 36, 57
Applicant's letter date: May 3, 2018
CDER stamp date: May 3, 2018
Product: Udenyca (pegfilgrastim-cbqv)
Indication: To decrease the incidence of infection, as manifested by febrile neutropenia, in patients with non-myeloid malignancies receiving myelosuppressive anti-cancer drugs associated with a clinically significant incidence of febrile neutropenia
Applicant: Coherus BioSciences Inc
Review Division: Division of Hematology Oncology Toxicology (DHOT) for Division of Hematology Products (DHP)
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1 Executive Summary

1.1 Introduction

Coherus BioSciences Inc developed Udenyca (pegfilgrastim-cbqv, CHS-1701) as a proposed biosimilar product to the US-licensed reference product Neulasta (pegfilgrastim). BLA 761039 was submitted on August 9, 2016 for the purpose of licensure of pegfilgrastim-cbqv under section 351(k) of the Public Health Service (PHS) Act; a Complete Response (CR) letter was sent on June 9, 2017. BLA 761039 was resubmitted on May 3, 2018.

The Applicant conducted a stepwise biosimilar development program intended to demonstrate that pegfilgrastim-cbqv is highly similar to Neulasta, with no clinically meaningful differences in terms of safety, purity, or potency. The proposed formulation and dosing regimen of pegfilgrastim-cbqv are identical to that of Neulasta. The Applicant proposes pegfilgrastim-cbqv to have the same indication as Neulasta: to decrease the incidence of infection, as manifested by febrile neutropenia, in patients receiving myelosuppressive anti-cancer drugs associated with a clinically significant incidence of febrile neutropenia.

Granulocyte-colony stimulating factor (G-CSF) is an endogenous glycoprotein that regulates the survival, proliferation, differentiation, and function of cells in the neutrophil lineage. Recombinant G-CSF (Neupogen, filgrastim) is used therapeutically to stimulate the production of granulocytes in patients who are neutropenic subsequent to receiving myelosuppressive chemotherapy; Neupogen was first approved in the United States in 1991. Neulasta is a conjugate of a 20 kDa polyethylene glycol (PEG) molecule covalently bound to the N-terminal methionyl residue of filgrastim that was first approved in the United States in 2002. Neulasta has a considerably longer half-life than Neupogen (15-80 hours compared to 3-4 hours) and is advantageous in that it requires less frequent administration.

The Applicant's biosimilar development program consisted of a definitive analytical similarity assessment and abbreviated nonclinical and clinical programs. The abbreviated nonclinical program included comparative in vitro pharmacology studies and a comparative toxicity study. The in vitro pharmacology studies evaluated the comparative binding affinity and potency of pegfilgrastim-cbqv and Neulasta in a biochemical and cell-based assay, respectively. The toxicity, toxicokinetics (TK), and pharmacodynamics (PD) of pegfilgrastim-cbqv and Neulasta were evaluated in a 4-week comparative repeat-dose toxicity study in cynomolgus monkeys. The comparative toxicity study identified significant TK and PD anomalies between pegfilgrastim-cbqv and Neulasta; the cause of these anomalies was not definitively determined. From the perspective of nonclinical pharmacology and toxicology, there is residual uncertainty regarding the similarity of pegfilgrastim-cbqv to Neulasta.

1.2 Brief Discussion of Nonclinical Findings

Two comparative in vitro pharmacology studies were conducted as part of the analytical similarity assessment and were intended to complement the physicochemical analyses. As assessed by surface plasmon resonance (SPR) analysis, pegfilgrastim-cbqv and Neulasta demonstrated comparable binding affinity to the G-CSF receptor (mean $K_D=111-117$ pM), albeit with significant variability between individual lots (range $K_D= 62-157$ pM). The Applicant attributed the variability between lots to the PEGylated nature of both molecules. The potency of pegfilgrastim-cbqv and Neulasta was evaluated in a comparative cell-based proliferation assay with NFS-60 myeloid leukemia cells. Pegfilgrastim-cbqv and Neulasta demonstrated similar mean potency (96.8-98.2%) relative to a pegfilgrastim-cbqv reference standard.

The toxicity, TK, and PD of pegfilgrastim-cbqv and Neulasta were evaluated in a 4-week comparative repeat-dose toxicity study in cynomolgus monkeys. Pegfilgrastim-cbqv and Neulasta were both well tolerated when administered by subcutaneous (SC) injection once weekly, with no treatment-related clinical signs. Histopathology findings were similar between pegfilgrastim-cbqv- and Neulasta-treated monkeys; target organs included the bone marrow, spleen, lymph nodes, and thymus. Pegfilgrastim-cbqv and Neulasta were both associated with significant increases in absolute neutrophil count (ANC) and white blood cell (WBC) count. After a 4-week recovery period findings were less severe indicating partial to full reversibility. The TK of pegfilgrastim-cbqv and Neulasta were similar after the first dose, however significant variability in systemic exposure (C_{max} and AUC_{0-t}) was observed after the fourth dose; the cause of these anomalies was not definitively determined. Anti-drug antibodies (ADAs) were detected in monkeys administered either product, however the relative immunogenicity of pegfilgrastim-cbqv and Neulasta could not be formally assessed given the limited number of monkeys studied. A rapid, robust, and sustained increase in ANC was observed following administration of pegfilgrastim-cbqv or Neulasta, consistent with the expected pharmacology of pegfilgrastim. In a post-hoc similarity analysis for ANC, pegfilgrastim-cbqv and Neulasta did not fall within the recognized similarity range of 80-125%, however this study was not adequately powered to formally assess bioequivalence.

With prior agreement with the FDA, the Applicant did not conduct safety pharmacology, reproductive and developmental toxicity, carcinogenicity, or genotoxicity studies with pegfilgrastim-cbqv. In general, nonclinical safety pharmacology, reproductive and developmental toxicity, and carcinogenicity studies are not necessary to support marketing of biosimilar products¹. Genotoxicity studies are generally not necessary to support marketing of biotechnology-derived pharmaceuticals such as pegfilgrastim-cbqv².

¹ FDA Final Guidance, Scientific Considerations in Demonstrating Biosimilarity to a Reference Product, 2015.

² S6(R1) Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals, 2011.

1.3 Recommendations

1.3.1 Approvability

From the perspective of nonclinical pharmacology and toxicology, there is residual uncertainty regarding the similarity of pegfilgrastim-cbqv to Neulasta. However, the nonclinical team acknowledges biosimilarity should be evaluated using a totality-of-the-evidence approach, and agrees pegfilgrastim-cbqv may be approved for the proposed indication.

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

Section 8 of the Udenyca label is in Pregnancy and Lactation Labeling Rule format. Otherwise, the nonclinical sections of the Udenyca label will be comparable to the label of Neulasta.

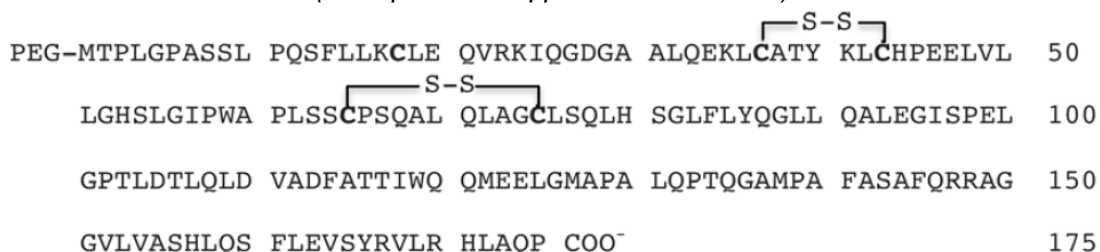
2 Drug Information

2.1 Drug

CAS Registry Number	208265-92-3
Proprietary Name	Udenyca
Generic Name	Pegfilgrastim-cbqv
Code Name	CHS-1701
Chemical Name	Recombinant N-(3-hydroxypropyl) methionylcolony-stimulating factor (human), 1-ether with α -methyl- ω -hydroxypoly(oxyethylene)
Molecular Formula	C ₈₄₅ H ₁₃₃₉ N ₂₂₃ O ₂₄₃ S ₉ (r-met-Hu-G-CSF (b) (4))
Molecular Weight	39 kDa (theoretical molecular weight of pegfilgrastim-cbqv, the sum of the (b) (4) r-met-Hu-G-CSF and a PEG group, assuming a 20 kDa nominal mass of PEG)
Structure or Biochemical Description	Pegfilgrastim-cbqv is a single chain 175 amino-acid polypeptide conjugated by the amino terminus to an approximately 20 kDa PEG group (see Figure 1).
Pharmacologic Class	Leukocyte growth factor

Figure 1: Primary structure of pegfilgrastim-cbqv

(Excerpted from Applicant's submission)



2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 115573 (pegfilgrastim-cbqv); BLA 125031 (Neulasta)

2.3 Drug Formulation

Pegfilgrastim-cbqv will be supplied in sterile, single-use, pre-filled glass syringes for SC injection. Each 1 mL syringe is ready-to-use and will contain 0.6 mL of a sterile, clear, colorless, preservative-free isotonic solution containing 6 mg pegfilgrastim-cbqv, (b) (4), (b) (4), sorbitol and (b) (4) polysorbate 20, at pH 4.0. The formulation of pegfilgrastim-cbqv is identical to that of Neulasta³. The composition of the pegfilgrastim-cbqv drug product, including the function and reference standard of each component, is listed in Table 1.

Table 1: Composition of the pegfilgrastim-cbqv drug product

Ingredients	Composition per dose		Function	Compendial reference
	Quantity per syringe	Concentration		
Pegfilgrastim-cbqv	6 mg	10 mg/mL	Active substance (b) (4)	In house specification
(b) (4) a	(b) (4)	(b) (4)		Ph. Eur., JP, BP, USP
(b) (4)	(b) (4)	(b) (4)		Ph. Eur., JP, BP, USP
Sorbitol	30 mg	(b) (4)		Ph. Eur., JP, BP, NF
Polysorbate 20	0.02 (b) (4) mg	(b) (4)		Ph. Eur., JP, BP, NF
WFI	q.s.	q.s.		USP, Ph. Eur.

^a Total acetate content per syringe is equivalent to 0.35 mg; ^b Total sodium content per syringe is equivalent to 0.02 mg; q.s. = quantum satis; WFI = Water for Injection; Ph.Eur. = European Pharmacopoeia; JP = Japanese Pharmacopoeia; USP = United States Pharmacopoeia; NF = National Formulary; BP = British Pharmacopoeia.

2.4 Comments on Novel Excipients

None

2.5 Comments on Impurities/Degradants of Concern

None

2.6 Proposed Clinical Population and Dosing Regimen

The proposed clinical population is adult and pediatric patients with cancer who are receiving myelosuppressive chemotherapy. The proposed dose of pegfilgrastim-cbqv in adult patients is a single 6 mg SC injection administered once per chemotherapy cycle. The proposed dose of pegfilgrastim-cbqv in pediatric patients is weight-based (see Table 2), and is also administered as a single SC injection once per chemotherapy cycle. The aforementioned proposed clinical population and dosing regimen is identical to that of Neulasta.

³ Piedmonte, D and M Treuheit, 2008, Formulation of Neulasta® (pegfilgrastim), Adv Drug Deliv Rev, 60(1):50-58.

Neulasta is also indicated to increase survival in patients acutely exposed to myelosuppressive doses of radiation, for which it has protected orphan status; pegfilgrastim-cbqv is not proposed for this indication.

Table 2: Dosing of pegfilgrastim-cbqv for pediatric patients weighing <45 kg

Body weight	Udenyca dose	Volume to administer
Less than 10 kg*	See below*	See below*
10 – 20 kg	1.5 mg	0.15 mL
21 – 30 kg	2.5 mg	0.25 mL
31 – 44 kg	4 mg	0.4 mL

*For pediatric patients weighing less < 10 kg, administer 0.1 mg/kg (0.01 mL/kg) of pegfilgrastim-cbqv

2.7 Regulatory Background

A pre-IND meeting was held on August 1, 2012 to discuss the development program for pegfilgrastim-cbqv; the Agency clarified the requirements for a BLA submitted under (b) (4) 351(k) of the PHS Act. The nonclinical team advised the Applicant the 4-week toxicity study in cynomolgus monkeys appeared adequate to support administration of single doses to healthy subjects. The nonclinical team also advised the Applicant that the proposed 2-week toxicity study in rats was unnecessary.

IND 115573 was received on October 23, 2012; a Study May Proceed letter was sent to the Applicant on November 21, 2012.

(b) (4)

A BPD-2 meeting was held on October 9, 2014 to discuss developing pegfilgrastim-cbqv under section 351(k) of the PHS Act. The nonclinical team advised the Applicant that if adequate analytical and in vitro PD similarity data between the new process material and Neulasta were provided, and there are no safety concerns raised because of uncertainties regarding similarity, then no additional nonclinical pharmacokinetic (PK) and toxicity studies would be needed to support a BLA under the 351(k) pathway.

A BPD-2 meeting was held on September 29, 2015 to discuss the Applicant's definitive analytical similarity package to support a BLA under the 351(k) pathway; no nonclinical issues were discussed.

A BPD-3 meeting was held on December 1, 2015 to discuss the ongoing clinical program for pegfilgrastim-cbqv; no nonclinical issues were discussed.

A BPD-1 meeting was held on March 21, 2016 to discuss a proposed comparative PK similarity study; no nonclinical issues were discussed.

A BPD-4 meeting was held on August 8, 2016 to discuss the format and content of a BLA submitted under the 351(k) pathway; no nonclinical issues were discussed.

BLA 761039 was submitted on August 9, 2016. The nonclinical team agreed pegfilgrastim-cbqv could be approved for the proposed indication using a totality-of-the-evidence approach; however, BLA 761039 received a CR letter on June 9, 2017.

BLA 761039 was resubmitted on May 3, 2018

3 Studies Submitted

3.1 Studies Reviewed

Pharmacology

Study number	Study title	eCTD location
Not provided	Test system: SPR Endpoint: binding affinity to CSF receptor	3.2.R.1.
Not provided	Test system: NFS-60 cells Endpoint: proliferation	3.2.R.1.

Toxicology

Study number	Study title	eCTD location
20026889	A 4-Week Toxicity Study of CHS-1701 Administered by Subcutaneous Injection in Cynomolgus Monkeys with a 4-Week Recovery Period	4.2.3.2.

3.2 Studies Not Reviewed

Pharmacokinetics

Study number	Study title	eCTD location
20026891	Validation of an Enzyme Linked Immunosorbent Assay (ELISA) Method used for the Determination of Neulasta® and CHS-1701 in Cynomolgus Monkey Plasma (K2 EDTA)	4.2.2.1.
20026893	Validation of a Qualitative ELISA for the Detection of anti-CHS-1701 and anti-Neulasta® Antibodies in Cynomolgus Monkey Plasma (K2EDTA)	4.2.2.1.
20029114	Validation of a Qualitative Electrochemiluminescent Method for the Detection of anti-CHS-1701 and anti-Pegfilgrastim Antibodies in Human Plasma (K ₂ EDTA)	4.2.2.1.

3.3 Previous Reviews Referenced

Nonclinical review for IND 115573 by Dr. Shawna Weis dated November 13, 2012.

4 Pharmacology

4.1 Primary Pharmacology

The PD effect of CHS-1701 and Neulasta was assessed by evaluating changes in ANC as part of the repeat-dose toxicity study in cynomolgus monkeys (refer to section 6.2 Repeat-Dose Toxicity).

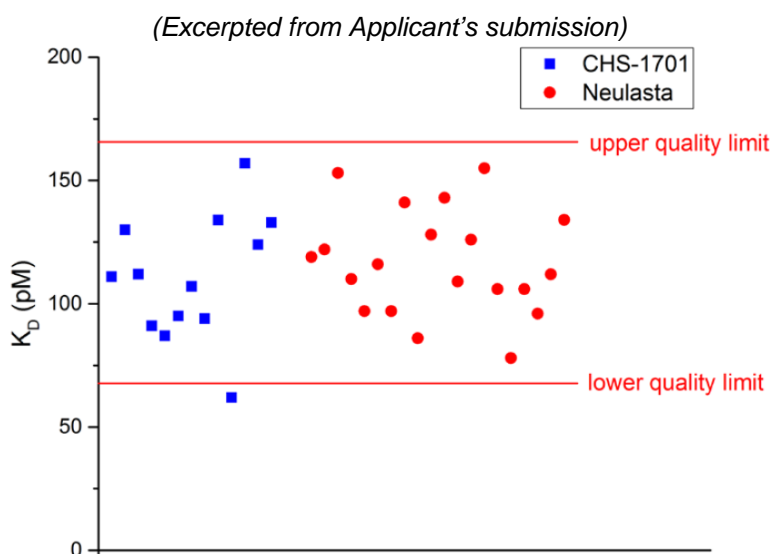
CHS-1701 and Neulasta were compared in two in vitro pharmacology assays as part of the analytical similarity assessment; these study reports were not submitted. The following information was obtained from the Analytical Similarity Assessment (eCTD 3.2.R.1).

The comparative binding affinity of CHS-1701 (13 lots) and Neulasta (20 lots) to the G-CSF receptor was evaluated by SPR analysis using a Biacore T200 label-free optical biosensor. Recombinant human G-CSF receptor was coupled to the surface of sensor chips at three different surface densities. Binding of CHS-1701 and Neulasta to the G-CSF receptor was evaluated over a range of ~1.4 to ~37 ng/mL at 25°C. The summary statistics for G-CSF receptor binding are in Table 3. All lots of CHS-1701 except one fell within the acceptance criteria (± 2.3 standard deviations) for Neulasta binding (see Figure 2). The variability between individual lots was relatively high, which the Applicant attributed to the PEGylated nature of both molecules.

Table 3: Summary statistics for G-CSF receptor binding

	CHS-1701	Neulasta
Mean K_D	111 pM	117 pM
K_D range (min-max)	62-157 pM	78-155 pM
Standard deviation	25.2 pM	21.3 pM

Figure 2: Receptor binding of CHS-1701 and Neulasta, as determined by SPR



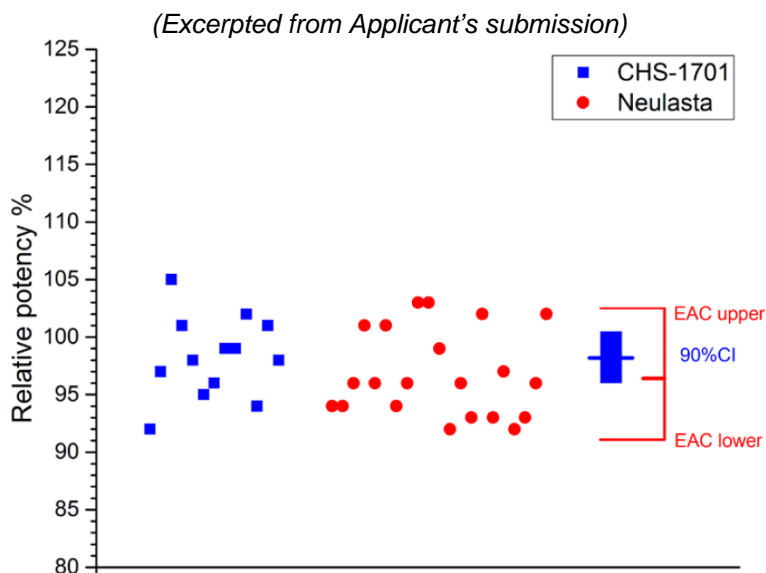
The comparative potency of CHS-1701 and Neulasta was evaluated in a cell-based proliferation assay using NFS-60 myeloid leukemia cells; NFS-60 cells are dependent on G-CSF for growth and maintenance of viability in vitro. NFS-60 cells were treated with CHS-1701 (13 lots), Neulasta (21 lots), or CHS-1701 reference standard and incubated for 3 days. MTT reagent was added for 3 hours, after which proliferation was inferred from the optical density at 550 nm. The mean relative potency of CHS-1701 was similar to that of Neulasta (see Table 4 and Figure 3).

Table 4: Summary statistics for relative biological potency

	CHS-1701	Neulasta
Mean relative potency	98.2%	96.8%
Relative potency range (min-max)	92-105%	92-103%
Standard deviation	3.5%	3.8%

Potency is relative to a CHS-1701 reference standard

Figure 3: Relative biological potency of CHS-1701 and Neulasta



4.2 Secondary Pharmacology

No studies were submitted for review.

4.3 Safety Pharmacology

Standalone safety pharmacology studies were not conducted. Electrocardiograms (ECGs) were recorded as part of the repeat-dose toxicity study in cynomolgus monkeys (refer to section 6.2 Repeat-Dose Toxicity).

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Standalone PK studies were not conducted. The PK effect of CHS-1701 and Neulasta was assessed as part of the repeat-dose toxicity study in cynomolgus monkeys (refer to section 6.2 Repeat-Dose Toxicity).

6 General Toxicology

6.1 Single-Dose Toxicity

No studies were submitted for review.

6.2 Repeat-Dose Toxicity

Study title: A 4-Week Toxicity Study of CHS-1701 Administered by Subcutaneous Injection in Cynomolgus Monkeys with a 4-Week Recovery Period

Study no.:	20026889
Study report location:	4.2.3.2.
Conducting laboratory and location:	 (b) (4)
Date of study initiation:	April 25, 2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	CHS-1701 (test article): lot # DS-12040BM-043012A, purity = 99.16% (SEC-HPLC) Neulasta (reference article): lot # 1026654, purity not provided

Key Study Findings

- CHS-1701 and Neulasta were well tolerated when administered by SC injection once weekly, with no treatment-related clinical signs; the high dose level (750 µg/kg) was determined to be the no observed adverse effect level for both CHS-1701 and Neulasta.
- Target organs identified by histopathology included the bone marrow, spleen, lymph nodes, and thymus. Findings were mostly limited to increased cellularity and immune cell infiltration, which were consistent with the marked and reversible increase in neutrophil (NEUT) count (the expected pharmacology of pegfilgrastim). Histopathology findings were similar between CHS-1701- and Neulasta-treated monkeys.
- Cataracts were observed in one Neulasta-treated monkey (750 µg/kg) and in two CHS-1701-treated monkeys (750 µg/kg); the relationship of these findings to CHS-1701 and/or Neulasta remains unknown.

- TK parameters for CHS-1701 and Neulasta were similar on Day 1; on Day 22, TK parameters for CHS-1701 and Neulasta were variable, with systemic exposure markedly lower in female CHS-1701-treated monkeys at the 750 µg/kg dose level. ADAs were detected in monkeys administered either product, however the relative immunogenicity of CHS-1701 and Neulasta could not be formally assessed given the limited number of monkeys studied.
- Dose formulations were evaluated by spectrophotometric absorption which the Reviewer disagrees is a selective or specific enough method to conclusively assess the identity, quality, or quantity of the test or reference article.

Methods

Doses: 0, 75, 250, or 750 µg/kg (see Table 5)
 Frequency of dosing: Once weekly for 4 weeks (Days 1, 8, 15, and 22)
 Route of administration: SC injection
 Dose volume: 0.5 mL/kg/dose
 Formulation/Vehicle: (b) (4), (b) (4) Sorbitol, (b) (4) % Polysorbate 20, pH 4.0; the vehicle was used as control article and to dilute the test and reference articles.
 Species/Strain: Cynomolgus monkey (China)
 Number/Sex/Group: Main study: 3/sex/group
 Recovery: 2/sex/group (control, low, and high groups only)
 Age: Males: 3.1 to 4.9 years
 Females: 2.5 to 3.4 years
 Weight: Males: 2.7 to 4.1 kg
 Females: 2.2 to 3.1 kg
 Satellite groups: None
 Unique study design: No
 Deviation from study protocol: None that impacted the overall integrity of the study or the interpretation of the study results and conclusions

Table 5: 4-week monkey study, experimental design

Group	Test material	Dose level (µg/kg/dose)	Dose concentration (mg/mL)	Dose volume (mL/kg/dose)	Number of animals	
					Main study	Recovery
1	Control article	0	0	0.50	3/sex	2/sex
2	Neulasta	75	0.15	0.50	3/sex	2/sex
3	Neulasta	250	0.5	0.50	3/sex	---
4	Neulasta	750	1.5	0.50	3/sex	2/sex
5	CHS-1701	75	0.15	0.50	3/sex	2/sex
6	CHS-1701	250	0.5	0.50	3/sex	---
7	CHS-1701	750	1.5	0.50	3/sex	2/sex

Observations and times

The first day of dosing was designated as study Day 1. Sacrifice and necropsies were performed on Day 29 (main study animals) or Day 57 (recovery animals).

Mortality and morbidity:	• Twice daily
Cage side observations:	• Once daily
Detailed clinical examinations:	• Once weekly
Injection site observations:	• Once prestudy • 24 hours after each dose
Body weight:	• Twice prestudy • Weekly thereafter
Food consumption:	• Once daily
Ophthalmoscopic examinations:	• Once prestudy • Near the end of Week 4
Electrocardiology:	• Once prestudy • Days 1 and 22, 1-2 hours after dosing
Clinical pathology: (hematology, coagulation, clinical chemistry, urinalysis)	• See Table 6
TK sample collection:	• Same as hematology
Sacrifice:	• Day 29 or 57

Table 6: 4-week monkey study, clinical pathology collection time points

Parameter	Time point
Hematology / TK	Day -8 (hematology only)
	Day 1 (predose and 2, 6, 12 hrs postdose)
	Day 2 (24, 36 hrs postdose)
	Day 3 (48, 60 hrs postdose)
	Days 4-7
	Days 8, 15 (predose)
	Day 22 (predose and 2, 6, 12 hrs postdose)
	Day 23 (24, 36 hrs postdose)
	Day 24 (48, 60 hrs postdose)
	Days 25-28
	Days 30, 34, 40, 48, 57
Coagulation	Days -8, 28, 57
Clinical chemistry	Days -8, 28, 57

Parameter	Time point
Urinalysis	Days 29, 57

Results

Mortality

All monkeys survived until scheduled necropsy.

Clinical signs

There were no CHS-1701- or Neulasta-related clinical observations. The incidences of abrasions and bruising were similar between groups. The incidences of injection site reactions (swelling or redness) were low and similar between groups.

Body weights

Unremarkable

Food consumption

Unremarkable

Ophthalmoscopy

Examinations were performed by slit lamp biomicroscopy and indirect ophthalmoscopy; findings were subject to peer review.

At week 4, a cataract was observed in one Neulasta-treated monkey (750 µg/kg) and in two CHS-1701-treated monkeys (750 µg/kg), including one monkey in which cataracts formed in both eyes. The relationship of these findings to CHS-1701 or Neulasta could not be definitively determined, however the following suggests these findings were incidental: 1) the incidence of cataracts was not dose-dependent; 2) the spontaneous development of cataracts has been observed previously at the testing facility; 3) there were no histologic correlates in the eyes of the affected or unaffected study animals.

All other ophthalmic findings were observed prestudy or were present in control monkeys and therefore determined to be unrelated to the test or reference articles.

ECG

Unremarkable

Hematology

The following changes in hematology parameters were considered treatment-related (see Table 7):

- At doses of CHS-1701 or Neulasta ≥ 75 µg/kg, a large increase in NEUT was observed in male and female monkeys (the expected PD effect); this finding correlated with a large increase in WBC count, and smaller increases in monocyte (MONO), basophil (BASO), and large unstained cell (LUC) counts. These observations were made 24 hours after the first and fourth doses of CHS-1701 or Neulasta.

- At doses of CHS-1701 or Neulasta ≥ 250 $\mu\text{g}/\text{kg}$, decreases in red blood cell (RBC) count, hemoglobin (HGB), and hematocrit (HCT) were observed in male and female monkeys and were generally not dose-related. These findings correlated with increases in reticulocyte (RETI) counts and red cell distribution width (RDW). These observations were made 24 hours after the fourth dose of CHS-1701 or Neulasta, but not after the first dose.
- Findings were generally similar between CHS-1701- and Neulasta-treated monkeys, with the exception of the relatively small increase in NEUT, WBC, and MONO in female monkeys treated with 750 $\mu\text{g}/\text{kg}$ CHS-1701. The relatively small increases in this group correlated with diminished systemic exposure of pegfilgrastim.
- After the 4-week recovery period there were no treatment-related changes in hematology parameters indicating all changes were fully reversible.

Table 7: 4-week monkey study, statistically significant percent change in hematology parameters versus concurrent control

(Adapted from IND review)

Group	Males - $\Delta\%$ from control		Females - $\Delta\%$ from control	
	Day 1 (24 hours postdose)	Day 22 (24 hours postdose)	Day 1 (24 hours postdose)	Day 22 (24 hours postdose)
RBC count				
1	N/A	N/A	N/A	N/A
2	-1.97	-6.69	-1.03	-7.43
3	-8.26	-30.07*	8.82	-5.86
4	-3.53	-25.92*	-1.70	-26.63*
5	-2.73	-13.11*	2.88	-7.56
6	-2.45	-17.86*	-2.17	-11.37
7	1.21	-21.11*	-3.59	-19.43*
HGB				
1	N/A	N/A	N/A	N/A
2	1.18	-4.10	-1.71	-11.11*
3	-0.51	-22.95*	6.91	-8.00
4	-1.52	-23.28*	-0.85	-25.93*
5	0.51	-10.00	1.54	-10.44*
6	-0.25	-15.33*	-2.99	-14.14*
7	3.37	-18.03*	-1.54	-20.03*
HCT				
1	N/A	N/A	N/A	N/A
2	1.80	-0.74	-2.17	-6.85
3	-2.44	-21.64*	6.64	-4.78
4	-1.13	-19.27*	0.16	-21.15*
5	1.90	-7.49	3.20	-7.20
6	0.64	-12.10*	-1.11	-9.73
7	3.19	-14.79*	-1.14	-14.97*
RDW				
1	N/A	N/A	N/A	N/A
2	4.72	15.73	-1.74	6.49
3	-3.35	41.23*	-4.57	15.66*
4	2.89	36.46*	-1.74	35.12*

Group	Males - Δ% from control		Females - Δ% from control	
	Day 1 (24 hours postdose)	Day 22 (24 hours postdose)	Day 1 (24 hours postdose)	Day 22 (24 hours postdose)
5	-0.30	10.29	-5.51	2.40
6	4.79	32.15*	-0.51	17.07*
7	1.07	42.21*	-1.88	26.80*
RETI count				
1	N/A	N/A	N/A	N/A
2	67.10	34.02	-21.39	101.96
3	47.07	241.60*	14.43	83.82
4	41.37	148.36*	4.73	183.82*
5	4.23	13.52	-5.97	70.59
6	36.32	108.40*	-5.10	119.85
7	6.84	95.90*	12.19	164.22*
WBC count				
1	N/A	N/A	N/A	N/A
2	248.77*	769.09*	306.05*	855.93*
3	244.74*	1053.23*	264.15*	1259.84*
4	312.81*	1373.74*	296.09*	1610.96*
5	194.74*	721.62	305.52*	838.03*
6	249.12*	930.61*	245.46*	1166.55*
7	196.67*	1322.63*	243.77*	378.08*
NEUT count				
1	N/A	N/A	N/A	N/A
2	460.60*	1462.31*	856.84*	2132.80*
3	470.94*	1989.28*	757.70*	3110.24*
4	608.52*	2633.03*	829.12*	3919.82*
5	408.81*	1388.87	848.43*	2078.61*
6	482.92*	1751.39*	703.79*	2862.93*
7	405.63*	2507.16*	702.45*	945.43
MONO count				
1	N/A	N/A	N/A	N/A
2	85.24	134.45*	388.69*	339.62*
3	138.63*	190.22*	221.57*	494.40*
4	135.68*	300.72*	233.50*	1322.31*
5	101.54*	185.53*	295.43*	303.73*
6	155.04*	180.52*	229.47*	738.93*
7	109.03*	413.69*	171.82*	79.05
BASO count				
1	N/A	N/A	N/A	N/A
2	541.76*	2556.59	519.23*	2786.13
3	519.71*	6891.09	511.75*	3607.23
4	652.35*	7518.60	458.97*	2168.21
5	260.00	1996.12	492.74*	2761.27
6	589.12*	5697.29	324.57	2050.29
7	383.53*	3398.45	348.29	908.09
LUC count				
1	N/A	N/A	N/A	N/A
2	163.88*	215.96	163.96	356.79
3	167.66*	380.92	98.28	352.38
4	179.70*	358.13	151.22	164.64
5	83.28	190.06	125.47	327.50

Group	Males - $\Delta\%$ from control		Females - $\Delta\%$ from control	
	Day 1 (24 hours postdose)	Day 22 (24 hours postdose)	Day 1 (24 hours postdose)	Day 22 (24 hours postdose)
6	171.15*	387.45	218.88	498.21
7	137.91*	293.98	44.17	222.14

* $p \leq 0.05$ (ANOVA with Dunnett's/Dunn's test)

Coagulation

Unremarkable

Clinical chemistry

Unremarkable

Urinalysis

Unremarkable

Gross pathology

There were no gross observations at terminal necropsy.

At recovery necropsy, red discolored left lung lobes were observed in one male monkey treated with 750 $\mu\text{g}/\text{kg}$ Neulasta; this finding correlated with histologically observable multifocal arterial thrombosis. Lungs from all other animals were examined histologically with no similar findings, therefore the relationship of this finding to Neulasta is unlikely.

Organ weights

The following organ weight alterations at terminal necropsy were considered treatment-related (see Table 8):

- The absolute spleen, spleen to body, and spleen to brain weights and ratios were increased in most groups, however this finding only achieved statistical significance in female monkeys dosed with 750 $\mu\text{g}/\text{kg}$ CHS-1701 or Neulasta.
- The absolute thymus, thymus to body, and thymus to brain weights and ratios were decreased in most female monkeys, however this finding did not achieve statistical significance. Increased absolute thymus, thymus to body, and thymus to brain weights and ratios were observed in male monkeys dosed with 250 $\mu\text{g}/\text{kg}$ CHS-1701, however this observation was not considered treatment-related due to the lack of dose-responsiveness and the isolated nature of the finding.
- The changes in spleen and thymus weights correlated with histologic observations of increased splenic red pulp mixed cell infiltrates and lymphoid depletion of the thymus, respectively.
- After the 4-week recovery period there were no treatment-related changes in organ weights indicating all changes were fully reversible.

Table 8: 4-week monkey study, statistically significant percent change in organ weights versus concurrent control

Group	Males - Δ% from control			Females - Δ% from control		
	Absolute weight	Relative to body weight	Relative to brain weight	Absolute weight	Relative to body weight	Relative to brain weight
Spleen						
1	N/A	N/A	N/A	N/A	N/A	N/A
2	15.71	26.84	8.16	12.07	6.26	19.69
3	41.48	49.27	42.24	25.32	11.45	25.38
4	53.92	70.10	50.41	89.61*	74.25*	91.03*
5	13.75	17.40	12.24	22.91	13.42	22.54
6	18.20	23.22	7.55	17.23	6.05	11.60
7	45.88	42.43	38.78	60.88	44.62	64.11*
Thymus						
1	N/A	N/A	N/A	N/A	N/A	N/A
2	40.77	54.54	34.43	14.35	7.86	22.08
3	-2.97	3.32	-4.76	-46.40	-53.32	-46.37
4	32.93	47.18	32.97	-6.44	-16.41	-8.52
5	-14.49	-11.23	-14.65	-15.28	-24.18	-13.88
6	95.20*	99.44*	76.92*	-23.92	-32.88	-27.44
7	-42.01	-43.52	-45.05	-15.75	-29.37	-12.62

* p≤0.05 (ANOVA with Dunnett's/Dunn's test)

Histopathology

Adequate Battery: Yes

Peer Review: No

Histological Findings

The following treatment-related histological findings were observed at terminal necropsy on Day 29 (see Table 9).

- Minimal to mild increased cellularity in the bone marrow of the sternum and femur was observed; the incidence of this observation increased with dose. This observation was considered the result of increased hematopoiesis and was expected given the established pharmacology of pegfilgrastim.
- Minimal to mild increased mixed cell infiltrates were observed within the red pulp of the spleen; this observation was characterized by increased numbers of NEUT and MONO, and correlated with hematology findings.
- Minimal to mild mixed cell infiltrates were observed in the liver; the infiltrates were characterized by mixed WBCs and extramedullary hematopoiesis. Kupffer cells containing a golden-brown granular pigment were observed in some monkeys, however this finding was not dose-related.
- Minimal to moderate neutrophil infiltration and/or extramedullary hematopoiesis were observed in the axillary, mandibular, and mesenteric lymph nodes. The incidences of these findings were generally similar between dose groups.

- Minimal to mild mixed cell and/or mononuclear cell infiltrates were observed in the SC injection sites. The frequency of observations was similar at the two injection sites.
- Minimal lymphoid depletion of the thymus was observed, characterized by reduced cortex, and to a lesser extent, reduced medulla.
- Overall, the histological findings at terminal necropsy were comparable between CHS-1701- and Neulasta-treated monkeys.

Table 9: 4-week monkey study, histology findings at terminal necropsy

Observations	Severity	Dose group							
		Ctrl	Neulasta (µg/kg/dose)				CHS-1701 (µg/kg/dose)		
		0	75	250	750	75	250	750	
		1	2	3	4	5	6	7	
Bone Marrow, Sternum Increased Hematopoietic Cellularity	Minimal	0/6	2/6	0/6	1/6	4/6	0/6	0/6	
	Mild	0/6	3/6	6/6	5/6	1/6	6/6	6/6	
Bone Marrow, Femur Increased Hematopoietic Cellularity	Minimal	0/6	3/6	3/6	3/6	2/6	3/6	2/6	
	Mild	0/6	2/6	2/6	3/6	0/6	3/6	4/6	
Red Pulp, Spleen Mixed Cell Infiltrates and/or Extramedullary Hematopoiesis	Minimal	0/6	1/6	4/6	2/6	5/6	1/6	5/6	
	Mild	0/6	1/6	0/6	3/6	0/6	1/6	0/6	
Liver Mixed Cell Infiltrates	Minimal	0/6	2/6	2/6	2/6	1/6	2/6	4/6	
	Mild	0/6	0/6	3/6	2/6	0/6	2/6	1/6	
Liver Kupffer Cell Cytoplasmic Pigment	Minimal	0/6	2/6	1/6	2/6	3/6	1/6	0/6	
Axillary Lymph Node Neutrophil Infiltrate and/or Extramedullary Hematopoiesis	Minimal	0/6	3/6	3/6	2/6	1/6	2/6	2/6	
	Moderate	0/6	0/6	0/6	1/6	0/6	1/6	1/6	
Mandibular Lymph Node Neutrophil Infiltrate and/or Extramedullary Hematopoiesis	Minimal	1/6	1/6	4/6	1/6	1/6	3/6	3/6	
	Mild	0/6	0/6	0/6	1/6	0/6	2/6	2/6	
	Moderate	0/6	0/6	0/6	2/6	1/6	0/6	0/6	
Mesenteric Lymph Node Neutrophil Infiltrate and/or Extramedullary Hematopoiesis	Minimal	0/6	0/6	0/6	0/6	0/6	0/6	2/6	
Subcutaneous Injection Site No. 1 Mixed Cell and/or Mononuclear Cell Infiltrates	Minimal	0/6	0/6	2/6	1/6	0/6	1/6	2/6	
	Mild	0/6	0/6	0/6	0/6	1/6	0/6	0/6	
Subcutaneous Injection Site No. 2 Mixed Cell and/or Mononuclear Cell Infiltrates	Minimal	0/6	0/6	1/6	2/6	0/6	2/6	4/6	
	Mild	0/6	0/6	0/6	1/6	0/6	0/6	2/6	
Thymus	Minimal	0/6	0/6	2/6	0/6	2/6	1/6	3/6	

Observations	Severity	Dose group						
		Ctrl	Neulasta (µg/kg/dose)			CHS-1701 (µg/kg/dose)		
		0	75	250	750	75	250	750
		1	2	3	4	5	6	7
Lymphoid Depletion								

Male and female data were pooled because there were no distribution alterations between sexes.

The following treatment-related histological findings were observed at recovery necropsy on Day 57 (see Table 10).

- Increased hematopoietic cellularity in the bone marrow of the femur was observed; this finding was of minimal severity in contrast to the minimal to mild severity observed at the terminal necropsy.
- Minimal to mild increased mixed cell and/or neutrophil infiltrates were observed in the spleen, liver, and axillary and mandibular lymph nodes; in contrast, similar observations of minimal to moderate severity were observed at the terminal necropsy.
- Minimal to mild macrophage infiltrates were observed in the SC injection sites; this observation is indicative of ongoing clearance of injection site inflammation.
- Ungraded multifocal arterial thrombosis was observed in the left lung of a single monkey treated with 750 µg/kg Neulasta; the relationship of this finding to Neulasta is unlikely.
- Overall, the histological findings at recovery necropsy were less severe than at terminal necropsy, and were comparable between CHS-1701- and Neulasta-treated monkeys.

Table 10: 4-week monkey study, histology findings at recovery necropsy

Observations	Severity	Dose group						
		Ctrl	Neulasta (µg/kg/dose)			CHS-1701 (µg/kg/dose)		
		0	75	250	750	75	250	750
		1	2	3	4	5	6	7
Bone Marrow, Femur Increased Hematopoietic Cellularity	Minimal	0/4	1/4	N/A	3/4	1/4	N/A	2/4
Red Pulp, Spleen Neutrophil Infiltrates	Minimal	0/4	2/4	N/A	1/4	0/4	N/A	0/4
Liver Mixed Cell Infiltrates	Minimal	0/4	1/4	N/A	1/4	1/4	N/A	0/4
	Mild	0/4	0/4	N/A	1/4	0/4	N/A	0/4
Axillary Lymph Node Neutrophil Infiltrate and/or Extramedullary Hematopoiesis	Minimal	0/4	0/4	N/A	1/4	0/4	N/A	1/4

Observations	Severity	Dose group						
		Ctrl	Neulasta ($\mu\text{g}/\text{kg}/\text{dose}$)			CHS-1701 ($\mu\text{g}/\text{kg}/\text{dose}$)		
		0	75	250	750	75	250	750
		1	2	3	4	5	6	7
Lung Thrombus; Arteriole	Ungraded	0/4	0/4	N/A	1/4	0/4	N/A	0/4
Mandibular Lymph Node Neutrophil Infiltrate and/or Extramedullary Hematopoiesis	Minimal	1/4	0/4	N/A	0/4	0/4	N/A	1/4
Subcutaneous Injection Site No. 1 Macrophage Infiltrates	Mild	0/4	0/4	N/A	0/4	1/4	N/A	0/4
Subcutaneous Injection Site No. 2 Macrophage Infiltrates	Minimal	0/4	0/4	N/A	0/4	0/4	N/A	1/4

Male and female data were pooled because there were no distribution alterations between sexes.

Toxicokinetics

Plasma TK parameters were calculated after the first (Day 1) and fourth (Day 22) doses of CHS-1701 and Neulasta (see Table 11 and Figure 4).

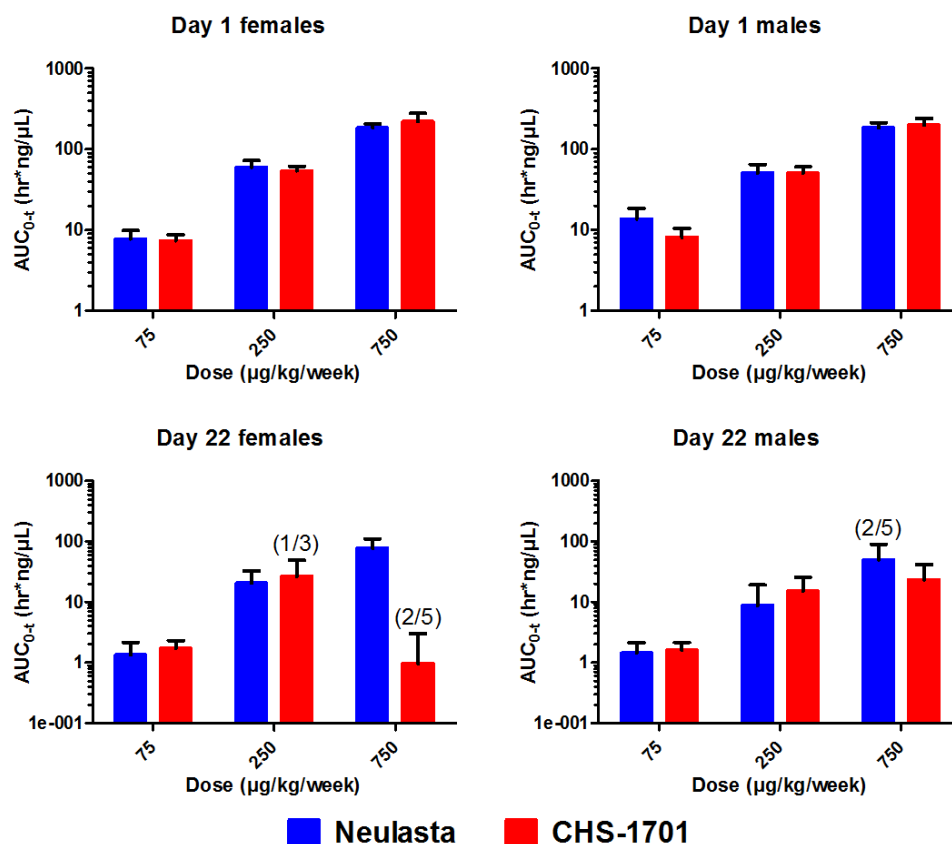
- On Day 1, the mean $T_{1/2}$ of CHS-1701 and Neulasta ranged from 11.6-23.9 hrs and 13.5-24.6 hrs, respectively, and trended lower with increasing dose. The mean T_{max} of CHS-1701 and Neulasta ranged from 7.2-13.2 hrs and 7.2-15.6 hrs, respectively, and generally increased with increasing dose. In terms of exposure, the mean C_{max} for CHS-1701 and Neulasta increased in a dose-proportional manner, however greater than dose-proportional increase in mean AUC_{0-t} was observed. There were no apparent gender differences in the TK of CHS-1701 or Neulasta.
- On Day 22, the mean TK parameters for both CHS-1701 and Neulasta were more variable than on Day 1. The mean $T_{1/2}$ trended lower with increasing dose (similar to that observed on Day 1), however the variability in mean $T_{1/2}$ widened for both CHS-1701 and Neulasta to 5.3-26.1 hrs and 9.1-20.7 hrs, respectively. The mean T_{max} of CHS-1701 and Neulasta ranged from 6.0-14.4 hrs and 5.2-19.2 hrs, respectively, and generally increased with increasing dose, which was similar to that observed on Day 1. The largest variability and differences from Day 1 were observed for the exposure parameters C_{max} and AUC_{0-t} . In females at the 750 $\mu\text{g}/\text{kg}$ dose level, markedly lower C_{max} and AUC_{0-t} were observed in all CHS-1701-treated monkeys relative to Neulasta-treated monkeys. For this group, mean C_{max} and AUC_{0-t} were >40-fold and >80-fold lower (respectively) than the group mean for Neulasta-treated monkeys at the same dose level. In this group, ADAs were confirmed in 2/5 monkeys determined to have no or low systemic exposure.
- Although a formal statistical analysis was not conducted, the TK parameters for CHS-1701 and Neulasta were similar on Day 1, but the variability for both products on Day 22 prevented comparison.

- The Applicant attributed the high variability in TK parameters for CHS-1701 and Neulasta on Day 22 to ADA formation and/or target-mediated clearance.

Table 11: 4-week monkey study, TK parameters for CHS-1701 and Neulasta

Dose level	Test article	Sex	T _{1/2} (hr)	T _{max} (hr)	C _{max} (ng/mL)	AUC _{0-t} (hr*ng/mL)
Day 1						
75 µg/kg	Neulasta	F	21.47	7.20	445.53	7804.31
		M	23.71	10.80	635.55	13703.82
	CHS-1701	F	19.76	7.20	372.19	7359.43
		M	19.50	7.20	366.33	8096.16
250 µg/kg	Neulasta	F	14.91	12.00	1806.91	59871.45
		M	24.59	8.00	1575.09	51220.29
	CHS-1701	F	23.86	12.00	1712.74	54315.91
		M	18.63	10.00	1228.91	50635.49
750 µg/kg	Neulasta	F	15.25	15.60	4286.75	184773.78
		M	13.53	14.40	4146.78	184351.00
	CHS-1701	F	11.58	12.00	5118.10	221676.59
		M	16.89	13.20	4532.55	199193.39
Day 22						
75 µg/kg	Neulasta	F	19.65	5.20	132.02	1346.27
		M	20.72	6.00	129.37	1440.63
	CHS-1701	F	26.08	6.00	183.16	1726.27
		M	18.69	6.00	180.19	1613.92
250 µg/kg	Neulasta	F	14.49	8.00	981.74	20520.43
		M	12.70	12.00	491.36	8863.84
	CHS-1701	F	12.00	8.00	1799.21	26681.81
		M	10.74	6.00	1138.35	15180.35
750 µg/kg	Neulasta	F	9.13	19.20	2644.98	78181.64
		M	10.55	15.00	1721.44	50240.47
	CHS-1701	F	5.32	14.40	57.21	957.94
		M	5.89	12.00	1080.27	23537.75

Shaded cells = notable anomalies in exposure

Figure 4: 4-week monkey study, mean pegfilgrastim exposure (AUC_{0-t})

(X/X): (Number confirmed ADA-positive / total number screened) on Day 28

A validated analytical procedure was used to detect the presence of ADAs. No ADAs were detected in monkeys treated with CHS-1701 or Neulasta on Days 1 or 15 (see Table 12 and Table 13). On Day 28, ADAs were detected in 2/26 (7.7%) monkeys treated with Neulasta and in 3/26 (11.5%) monkeys treated with CHS-1701. On Day 57, ADAs were detected in 1/8 (12.5%) monkeys treated with Neulasta and in 3/8 (37.5%) monkeys treated with CHS-1701. The relative immunogenicity of the two products cannot be accurately assessed given the limited number of monkeys studied. The presence of ADAs did not always correlate with lower circulating concentrations of CHS-1701 and Neulasta, suggesting multiple mechanisms affect the clearance of CHS-1701 and Neulasta.

Table 12: 4-week monkey study, incidence of ADAs in Neulasta treatment groups

Day	Males				Females			
	0 μg/kg	75 μg/kg	250 μg/kg	750 μg/kg	0 μg/kg	75 μg/kg	250 μg/kg	750 μg/kg
1	(0/5)	(0/5)	(0/3)	(0/5)	(0/5)	(0/5)	(0/3)	(0/5)
15	(0/5)	(0/5)	(0/3)	(0/5)	(0/5)	(0/5)	(0/3)	(0/5)
28	(0/5)	(0/5)	(0/3)	(2/5)	(0/5)	(0/5)	(0/3)	(0/5)
57	(0/2)	(0/2)	(0/0)	(1/2)	(0/2)	(0/2)	(0/0)	(0/2)

(Number confirmed/total number screened)

Table 13: 4-week monkey study, incidence of ADAs in CHS-1701 treatment groups

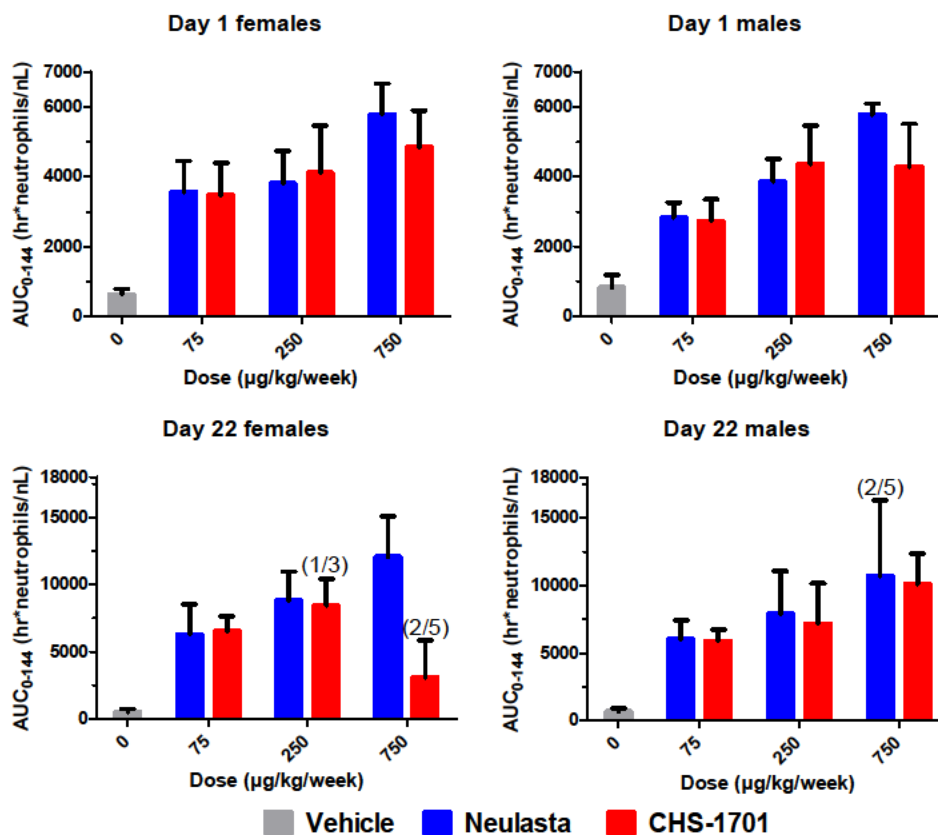
Day	Males				Females			
	0 µg/kg	75 µg/kg	250 µg/kg	750 µg/kg	0 µg/kg	75 µg/kg	250 µg/kg	750 µg/kg
1	(0/5)	(0/5)	(0/3)	(0/5)	(0/5)	(0/5)	(0/3)	(0/5)
15	(0/5)	(0/5)	(0/3)	(0/5)	(0/5)	(0/5)	(0/3)	(0/5)
28	(0/5)	(0/5)	(0/3)	(0/5)	(0/5)	(0/5)	(1/3)	(2/5)
57	(0/2)	(0/2)	(0/0)	(1/2)	(0/2)	(0/2)	(0/0)	(2/2)

(Number confirmed/total number screened)

Special Evaluation

PD response was evaluated by monitoring ANC after the first (Day 1) and fourth (Day 22) doses of CHS-1701 or Neulasta (see Figure 5).

- Following the first dose of CHS-1701 or Neulasta, an increase in mean ANC was observed at all dose levels, however the increase was not dose-responsive in females and less than dose-proportional in males.
- Following the fourth dose of Neulasta a less than dose-proportional increase in mean ANC was observed, while no trend in mean ANC was observed for CHS-1701.
- Notably lower group mean ANC was observed in female monkeys treated with CHS-1701 at the 750 µg/kg dose level, mirroring the diminished pegfilgrastim exposure observed in this group.
- In a post-hoc similarity analysis of the geometric mean ratios for ANC AUC₀₋₁₄₄, the 90% confidence intervals for CHS-1701 and Neulasta did not fall within the recognized similarity range of 80-125% (see Table 14); however, it should be noted that this study was not adequately powered to formally assess bioequivalence.

Figure 5: 4-week monkey study, mean ANC (AUC₀₋₁₄₄)

(X/X): (Number confirmed ADA-positive / total number screened) on Day 28

Table 14: 4-week monkey study, post-hoc similarity analysis of ANC for CHS-1701 and Neulasta

Dose (µg/kg)	Day	Parameter	Geometric mean ratio (%)	90% confidence intervals
75	1	AUC ₀₋₁₄₄	96.40	78.44, 118.47
	22	AUC ₀₋₁₄₄	104.76	83.80, 130.95
250	1	AUC ₀₋₁₄₄	108.60	84.07, 140.28
	22	AUC ₀₋₁₄₄	93.40	64.93, 134.35
750	1	AUC ₀₋₁₄₄	77.09	65.65, 90.52
	22	AUC ₀₋₁₄₄	51.71	26.00, 102.83

Dosing Solution Analysis

Dose formulations were evaluated by spectrophotometric absorption at 280 nm (A₂₈₀). This method determines the total protein concentration and cannot distinguish between CHS-1701, Neulasta, or contaminating proteins. The Applicant indicates mean CHS-

1701 and Neulasta dose formulations were 104% to 108% of the target theoretical concentration (and thus within the $\pm 10\%$ acceptance range), and control dose formulations had no significant amount of protein. The Reviewer disagrees this method is selective or specific enough to assess the identity, quality, or quantity of test or reference article.

7 Genetic Toxicology

7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

Not applicable.

7.2 *In Vitro* Assays in Mammalian Cells

Not applicable.

7.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

Not applicable.

7.4 Other Genetic Toxicity Studies

Not applicable.

8 Carcinogenicity

Not applicable.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Not applicable.

9.2 Embryonic Fetal Development

Not applicable.

9.3 Prenatal and Postnatal Development

Not applicable.

10 Special Toxicology Studies

No studies were submitted for review.

11 Integrated Summary and Safety Evaluation

The Applicant conducted a stepwise approach to support the demonstration of similarity between pegfilgrastim-cbqv and Neulasta; this approach consisted of a definitive analytical similarity assessment and abbreviated nonclinical and clinical programs. As part of the definitive analytical similarity assessment two comparative in vitro pharmacology studies were conducted; these studies demonstrated pegfilgrastim-cbqv and Neulasta bound to the G-CSF receptor with similar affinity and had similar in vitro

potency. It should be noted in vitro studies such as these may not be able to detect product differences with potential to impact in vivo TK and PD profiles.

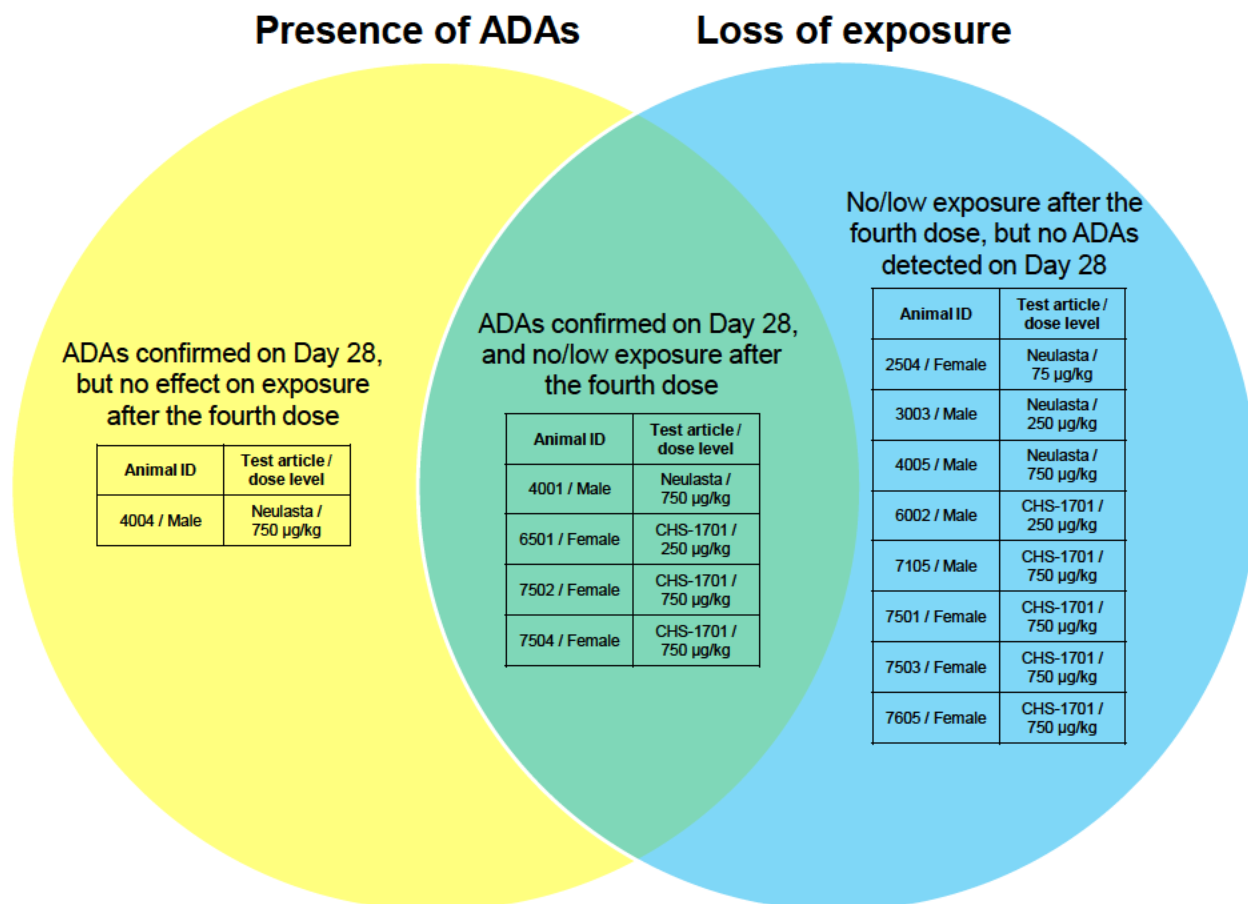
To further support the demonstration of similarity a 4-week comparative repeat-dose toxicity study was conducted in cynomolgus monkeys. The cynomolgus monkey was chosen for comparative toxicological assessment as pegfilgrastim is pharmacologically active in this species with TK and PD profiles comparable to that observed in humans. The low and high dose levels evaluated in the toxicity study were intended to approximate the human clinical dose and the high dose used in the toxicity studies to support the approval of Neulasta, respectively. Administration by the SC route is appropriate as this is the same route pegfilgrastim is administered to humans.

Target organs, histological findings, and most changes in hematology parameters were similar between pegfilgrastim-cbqv- and Neulasta-treated monkeys, and were consistent with the well-characterized PD activity of pegfilgrastim. While the TK of pegfilgrastim-cbqv and Neulasta were similar after the first dose, the TK of both products were variable after the fourth dose. Systemic exposures of pegfilgrastim were markedly lower in isolated animals at the 250 and 750 µg/kg dose levels, as well as a single animal at the 75 µg/kg dose level. In addition, no or very low systemic exposure was observed in the entire group of female monkeys treated with pegfilgrastim-cbqv at the 750 µg/kg dose level. The exposure of pegfilgrastim is expected to decrease after repeated administration due to pharmacodynamics-mediated drug disposition⁴, however the differences between pegfilgrastim-cbqv- and Neulasta-treated monkeys were unexpected. On Day 28, ADAs were detected in 2/26 (7.7%) monkeys treated with Neulasta and in 3/26 (11.5%) monkeys treated with pegfilgrastim-cbqv. Diminished exposure of pegfilgrastim-cbqv and Neulasta did not always correlate with the presence of ADAs (see Figure 6), indicating target-mediated clearance and/or other uncharacterized mechanisms of clearance were functioning. Indeed, the elimination of pegfilgrastim in healthy volunteers is primarily attributed to target-mediated clearance⁵. The relative immunogenicity of the two products cannot be accurately assessed given the limited number of monkeys studied. Lower ANC correlated with low systemic pegfilgrastim exposure, however there were only modest differences between the ANC of affected and unaffected animals. In a similarity analysis for ANC response (AUC_{0-144}) the 90% confidence intervals for pegfilgrastim-cbqv and Neulasta did not fall within the recognized similarity range of 80-125%, however this study was not adequately powered to formally assess bioequivalence.

⁴ Yang, BB and A Kido, 2011, Pharmacokinetics and Pharmacodynamics of Pegfilgrastim, Clin Pharmacokinet, 50(5):295-306.

⁵ Roskos, LK, P Lum, P Lockbaum, G Schwab, and B Yang, 2006, Pharmacokinetic/Pharmacodynamic Modeling of Pegfilgrastim in Healthy Subjects, J Clin Pharmacol, 46:747-757.

Figure 6: 4-week monkey study, correlation between presence of ADAs and diminished pegfilgrastim exposure



Conclusion

Despite sharing similar toxicity profiles pegfilgrastim-cbqv and Neulasta demonstrated dissimilar TK profiles, and to a lesser degree, PD profiles. The root of residual uncertainty between pegfilgrastim-cbqv and Neulasta primarily lays in the loss of exposure in females administered pegfilgrastim-cbqv at the 750 µg/kg dose level. The Applicant suggested the diminished exposure was the result of target-mediated clearance and/or formation of ADAs, however it is unclear why Neulasta-treated monkeys were not equally affected by these mechanisms. Given the limited number of monkeys per group the nonclinical team acknowledges the loss of exposure in this group may have been a chance occurrence.

The nonclinical team recognizes the TK and PD profiles of pegfilgrastim-cbqv and Neulasta are similar at the 75 µg/kg dose level, and that the 75 µg/kg dose level represents a clinically meaningful dose. However, the lack of exposure to pegfilgrastim-cbqv in females at the 750 µg/kg dose level indicates exposure was not maintained throughout the study, drawing into question the other conclusions of the study.

From the perspective of nonclinical pharmacology and toxicology, there is residual uncertainty regarding the similarity of pegfilgrastim-cbqv to Neulasta. However, the nonclinical team acknowledges biosimilarity should be evaluated using a totality-of-the-evidence approach, and agrees pegfilgrastim-cbqv may be approved for the proposed indication.

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

/s/

MICHAEL L MANNING
10/16/2018

CHRISTOPHER M SHETH
10/16/2018
I concur

**DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH**

PHARMACOLOGY/TOXICOLOGY BLA REVIEW AND EVALUATION

Application number: 761039
Supporting document/s: 1, 36
Applicant's letter date: August 9, 2016
CDER stamp date: August 9, 2016
Product: Udenyca (pegfilgrastim- (b) (4))
Indication: To decrease the incidence of infection, as manifested by febrile neutropenia, in patients with non-myeloid malignancies receiving myelosuppressive anti-cancer drugs associated with a clinically significant incidence of febrile neutropenia
Applicant: Coherus BioSciences Inc
Review Division: Division of Hematology Oncology Toxicology (DHOT) for Division of Hematology Products (DHP)
Reviewer: Michael L Manning, PhD
Supervisor/Team Leader: Christopher M Sheth, PhD
Division Director: John Leighton, PhD, DABT (DHOT)
Ann Farrell, MD (DHP)
Project Manager: Natasha Kormanik, MSN, RN, OCN

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1 Executive Summary

1.1 Introduction

Coherus BioSciences Inc has developed Udenyca (pegfilgrastim- (b) (4) CHS-1701) as a proposed biosimilar product to the US-licensed reference product Neulasta (pegfilgrastim). BLA 761039 was submitted for the purpose of licensure of pegfilgrastim- (b) (4) under section 351(k) of the Public Health Service Act. The Applicant has conducted a stepwise biosimilar development program intended to demonstrate that pegfilgrastim- (b) (4) is highly similar to Neulasta, with no clinically meaningful differences in terms of safety, purity, or potency. The proposed formulation and dosing regimen of pegfilgrastim- (b) (4) are identical to that of Neulasta. The Applicant proposes pegfilgrastim- (b) (4) to have the same indication as Neulasta: to decrease the incidence of infection, as manifested by febrile neutropenia, in patients receiving myelosuppressive anti-cancer drugs associated with a clinically significant incidence of febrile neutropenia. Pegfilgrastim- (b) (4) has not been marketed or withdrawn in any other country.

Granulocyte-colony stimulating factor (G-CSF) is an endogenous glycoprotein that regulates the survival, proliferation, differentiation, and function of cells in the neutrophil lineage. Recombinant G-CSF (Neupogen, filgrastim) is used therapeutically to stimulate the production of granulocytes in patients who are neutropenic subsequent to receiving myelosuppressive chemotherapy; Neupogen was first approved in the United States in 1991. Neulasta is a conjugate of a 20 kDa polyethylene glycol (PEG) molecule covalently bound to the N-terminal methionyl residue of filgrastim that was first approved in the United States in 2002. Neulasta has a considerably longer half-life than Neupogen (15-80 hours compared to 3-4 hours) and is advantageous in that it requires less frequent administration.

The Applicant's biosimilar development program consisted of a definitive analytical similarity assessment and abbreviated nonclinical and clinical programs. The abbreviated nonclinical program included comparative in vitro pharmacology studies and a comparative toxicity study. The in vitro pharmacology studies evaluated the comparative binding affinity and potency of pegfilgrastim- (b) (4) and Neulasta in a biochemical and cell-based assay, respectively. The toxicity, toxicokinetics (TK), and pharmacodynamics (PD) of pegfilgrastim- (b) (4) and Neulasta were evaluated in a 4-week comparative repeat-dose toxicity study in cynomolgus monkeys. The comparative toxicity study identified significant TK and PD anomalies between pegfilgrastim- (b) (4) and Neulasta; the cause of these anomalies was not definitively determined. From the perspective of nonclinical pharmacology and toxicology, there is residual uncertainty regarding the similarity of pegfilgrastim- (b) (4) to Neulasta.

1.2 Brief Discussion of Nonclinical Findings

Two comparative in vitro pharmacology studies were conducted as part of the analytical similarity assessment and were intended to complement the physicochemical analyses. As assessed by surface plasmon resonance (SPR) analysis, pegfilgrastim- (b) (4) and Neulasta demonstrated comparable binding affinity to the G-CSF receptor (mean

$K_D=111-117$ pM), albeit with significant variability between individual lots (range $K_D=62-157$ pM). The Applicant attributed the variability between lots to the PEGylated nature of both molecules. The potency of pegfilgrastim-^{(b) (4)} and Neulasta was evaluated in a comparative cell-based proliferation assay with NFS-60 myeloid leukemia cells. Pegfilgrastim-^{(b) (4)} and Neulasta demonstrated similar mean potency (96.8-98.2%) relative to a pegfilgrastim-^{(b) (4)} reference standard.

The toxicity, TK, and PD of pegfilgrastim-^{(b) (4)} and Neulasta were evaluated in a 4-week comparative repeat-dose toxicity study in cynomolgus monkeys. Pegfilgrastim-^{(b) (4)} and Neulasta were both well tolerated when administered by subcutaneous (SC) injection once weekly, with no treatment-related clinical signs. Histopathology findings were similar between pegfilgrastim-^{(b) (4)} and Neulasta-treated monkeys; target organs included the bone marrow, spleen, lymph nodes, and thymus. Pegfilgrastim-^{(b) (4)} and Neulasta were both associated with significant increases in absolute neutrophil count (ANC) and white blood cell (WBC) count. After a 4-week recovery period findings were less severe indicating partial to full reversibility. The TK of pegfilgrastim-^{(b) (4)} and Neulasta were similar after the first dose, however significant variability in systemic exposure (C_{max} and AUC_{0-t}) was observed after the fourth dose; the cause of these anomalies was not definitively determined. Anti-drug antibodies (ADAs) were detected in monkeys administered either product, however the relative immunogenicity of pegfilgrastim-^{(b) (4)} and Neulasta could not be formally assessed given the limited number of monkeys studied. A rapid, robust, and sustained increase in ANC was observed following administration of pegfilgrastim-^{(b) (4)} or Neulasta, consistent with the expected pharmacology of pegfilgrastim. In a post-hoc similarity analysis for ANC, pegfilgrastim-^{(b) (4)} and Neulasta did not fall within the recognized similarity range of 80-125%, however this study was not adequately powered to formally assess bioequivalence.

With prior agreement with the FDA, the Applicant did not conduct safety pharmacology, reproductive and developmental toxicity, carcinogenicity, or genotoxicity studies with pegfilgrastim-^{(b) (4)}. In general, nonclinical safety pharmacology, reproductive and developmental toxicity, and carcinogenicity studies are not necessary to support marketing of biosimilar products¹. Genotoxicity studies are generally not necessary to support marketing of biotechnology-derived pharmaceuticals such as pegfilgrastim^{(b) (4)}².

1.3 Recommendations

1.3.1 Approvability

From the perspective of nonclinical pharmacology and toxicology, there is residual uncertainty regarding the similarity of pegfilgrastim-^{(b) (4)} to Neulasta. However, the nonclinical team acknowledges biosimilarity should be evaluated using a totality-of-the-

¹ FDA Final Guidance, Scientific Considerations in Demonstrating Biosimilarity to a Reference Product, 2015.

² S6(R1) Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals, 2011.

evidence approach, and agrees pegfilgrastim- (b) (4) may be approved for the proposed indication.

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

Section 8 of the Udenyca label is in Pregnancy and Lactation Labeling Rule format. Otherwise, the nonclinical sections of the Udenyca label will be comparable to the label of Neulasta.

2 Drug Information

2.1 Drug

CAS Registry Number	208265-92-3
Proprietary Name	Udenyca
Generic Name	Pegfilgrastim- (b) (4)
Code Name	CHS-1701
Chemical Name	Recombinant N-(3-hydroxypropyl) methionylcolony-stimulating factor (human), 1-ether with α -methyl- ω -hydroxypoly(oxyethylene)
Molecular Formula	C ₈₄₅ H ₁₃₃₉ N ₂₂₃ O ₂₄₃ S ₉ (r-met-Hu-G-CSF (b) (4))
Molecular Weight	39 kDa (theoretical molecular weight of pegfilgrastim- (b) (4) the sum of the (b) (4) r-met-Hu-G-CSF and a PEG group, assuming a 20 kDa nominal mass of PEG)
Structure or Biochemical Description	Pegfilgrastim- (b) (4) is a single chain 175 amino-acid polypeptide conjugated by the amino terminus to an approximately 20 kDa PEG group (see Figure 1).
Pharmacologic Class	Leukocyte growth factor

Figure 1: Primary structure of pegfilgrastim- (b) (4)

(Excerpted from Applicant's submission)

```

PEG-MTPLGPASSL PQSFLKCLE QVRKIQGDGA ALQEKLCATY KLCHPEELVL 50
                                     S-S
LGHSLGIPWA PLSSCPSQAL QLAGCLSOLH SGLFLYQGLL QALEGISPEL 100
                                     S-S
GPTLDTLQLD VADFATTIWQ QMEELGMAPA LQPTQGAMPA FASAFQRRAG 150
GVLVASHLQS FLEVSRYVLR HLAQP COO- 175

```

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 115573 (pegfilgrastim- (b) (4) BLA 125031 (Neulasta)

2.3 Drug Formulation

Pegfilgrastim- (b) (4) will be supplied in sterile, single-use, pre-filled glass syringes for SC injection. Each 1 mL syringe is ready-to-use and will contain 0.6 mL of a sterile, clear, colorless, preservative-free isotonic solution containing 6 mg pegfilgrastim- (b) (4) (b) (4), (b) (4), (b) (4) sorbitol and (b) (4) polysorbate 20, at pH 4.0. The formulation of pegfilgrastim- (b) (4) is identical to that of Neulasta³. The composition of the pegfilgrastim- (b) (4) drug product, including the function and reference standard of each component, is listed in Table 1.

Table 1: Composition of the pegfilgrastim- (b) (4) drug product

Ingredients	Composition per dose		Function	Compendial reference
	Quantity per syringe	Concentration		
Pegfilgrastim- (b) (4)	6 mg	10 mg/mL	Active substance (b) (4)	In house specification
(b) (4) a	(b) (4)	(b) (4)		Ph. Eur., JP, BP, USP
(b) (4)	(b) (4)	(b) (4)		Ph. Eur., JP, BP, USP
Sorbitol	30 mg			Ph. Eur., JP, BP, NF
Polysorbate 20	0.02 (b) (4) mg			Ph. Eur., JP, BP, NF
WFI	q.s.	q.s.		USP, Ph. Eur.

^a Total acetate content per syringe is equivalent to 0.35 mg; ^b Total sodium content per syringe is equivalent to 0.02 mg; q.s. = quantum satis; WFI = Water for Injection; Ph.Eur. = European Pharmacopoeia; JP = Japanese Pharmacopoeia; USP = United States Pharmacopoeia; NF = National Formulary; BP = British Pharmacopoeia.

2.4 Comments on Novel Excipients

None

2.5 Comments on Impurities/Degradants of Concern

None

2.6 Proposed Clinical Population and Dosing Regimen

The proposed clinical population is adult and pediatric patients with cancer who are receiving myelosuppressive chemotherapy. The proposed dose of pegfilgrastim- (b) (4) in adult patients is a single 6 mg SC injection administered once per chemotherapy cycle. The proposed dose of pegfilgrastim- (b) (4) in pediatric patients is weight-based (see Table 2), and is also administered as a single SC injection once per chemotherapy cycle. The aforementioned proposed clinical population and dosing regimen is identical to that of Neulasta.

³ Piedmonte, D and M Treuheit, 2008, Formulation of Neulasta® (pegfilgrastim), Adv Drug Deliv Rev, 60(1):50-58.

Neulasta is also indicated to increase survival in patients acutely exposed to myelosuppressive doses of radiation, for which it has protected orphan status; pegfilgrastim-^{(b) (4)} is not proposed for this indication.

Table 2: Dosing of pegfilgrastim-^{(b) (4)} for pediatric patients weighing <45 kg

Body weight	Udenyca dose	Volume to administer
Less than 10 kg*	See below*	See below*
10 – 20 kg	1.5 mg	0.15 mL
21 – 30 kg	2.5 mg	0.25 mL
31 – 44 kg	4 mg	0.4 mL

*For pediatric patients weighing less < 10 kg, administer 0.1 mg/kg (0.01 mL/kg) of pegfilgrastim-^{(b) (4)}

2.7 Regulatory Background

A pre-IND meeting was held on August 1, 2012 to discuss the development program for pegfilgrastim-^{(b) (4)} the Agency clarified the requirements for a BLA submitted under ^{(b) (4)} 351(k) of the Public Health Service Act. The nonclinical team advised the Applicant the 4-week toxicity study in cynomolgus monkeys appeared adequate to support administration of single doses to healthy subjects. The nonclinical team also advised the Applicant that the proposed 2-week toxicity study in rats was unnecessary.

IND 115573 was received on October 23, 2012; a Study May Proceed letter was sent to the Applicant on November 21, 2012.

A BPD-2 meeting was held on October 9, 2014 to discuss developing pegfilgrastim-^{(b) (4)} under section 351(k) of the Public Health Service Act. The nonclinical team advised the Applicant that if adequate analytical and in vitro PD similarity data between the new process material and Neulasta were provided, and there are no safety concerns raised because of uncertainties regarding similarity, then no additional nonclinical pharmacokinetic (PK) and toxicity studies would be needed to support a BLA under the 351(k) pathway.

A BPD-2 meeting was held on September 29, 2015 to discuss the Applicant's definitive analytical similarity package to support a BLA under the 351(k) pathway; no nonclinical issues were discussed.

A BPD-3 meeting was held on December 1, 2015 to discuss the ongoing clinical program for pegfilgrastim-^{(b) (4)} no nonclinical issues were discussed.

A BPD-1 meeting was held on March 21, 2016 to discuss a proposed comparative PK similarity study; no nonclinical issues were discussed.

A BPD-4 meeting was held on August 8, 2016 to discuss the format and content of a BLA submitted under the 351(k) pathway; no nonclinical issues were discussed.

3 Studies Submitted

3.1 Studies Reviewed

Pharmacology

Study number	Study title	eCTD location
Not provided	Test system: SPR Endpoint: binding affinity to CSF receptor	3.2.R.1.
Not provided	Test system: NFS-60 cells Endpoint: proliferation	3.2.R.1.

Toxicology

Study number	Study title	eCTD location
20026889	A 4-Week Toxicity Study of CHS-1701 Administered by Subcutaneous Injection in Cynomolgus Monkeys with a 4-Week Recovery Period	4.2.3.2.

3.2 Studies Not Reviewed

Pharmacokinetics

Study number	Study title	eCTD location
20026891	Validation of an Enzyme Linked Immunosorbent Assay (ELISA) Method used for the Determination of Neulasta® and CHS-1701 in Cynomolgus Monkey Plasma (K2 EDTA)	4.2.2.1.
20026893	Validation of a Qualitative ELISA for the Detection of anti-CHS-1701 and anti-Neulasta® Antibodies in Cynomolgus Monkey Plasma (K2EDTA)	4.2.2.1.
20029114	Validation of a Qualitative Electrochemiluminescent Method for the Detection of anti-CHS-1701 and anti-Pegfilgrastim Antibodies in Human Plasma (K ₂ EDTA)	4.2.2.1.

3.3 Previous Reviews Referenced

Nonclinical review for IND 115573 by Dr. Shawna Weis dated November 13, 2012.

4 Pharmacology

4.1 Primary Pharmacology

The PD effect of CHS-1701 and Neulasta was assessed by evaluating changes in ANC as part of the repeat-dose toxicity study in cynomolgus monkeys (refer to section 6.2 Repeat-Dose Toxicity).

CHS-1701 and Neulasta were compared in two in vitro pharmacology assays as part of the analytical similarity assessment; these study reports were not submitted. The

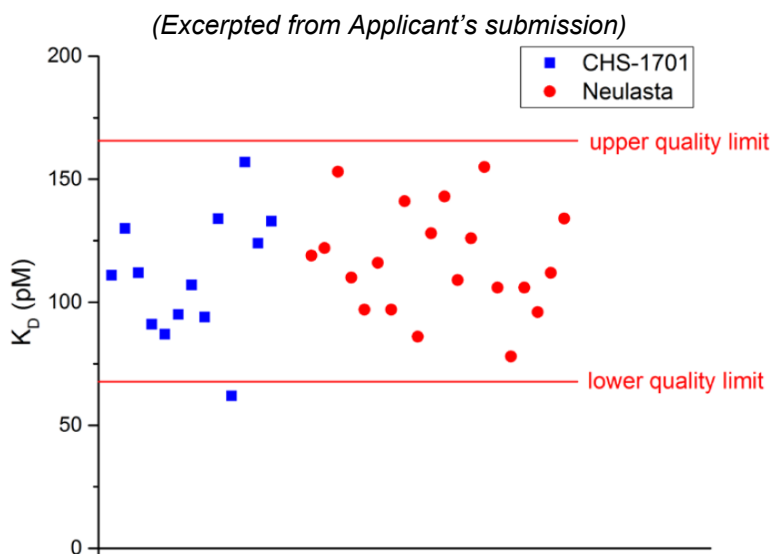
following information was obtained from the Analytical Similarity Assessment (eCTD 3.2.R.1).

The comparative binding affinity of CHS-1701 (13 lots) and Neulasta (20 lots) to the G-CSF receptor was evaluated by SPR analysis using a Biacore T200 label-free optical biosensor. Recombinant human G-CSF receptor was coupled to the surface of sensor chips at three different surface densities. Binding of CHS-1701 and Neulasta to the G-CSF receptor was evaluated over a range of ~1.4 to ~37 ng/mL at 25°C. The summary statistics for G-CSF receptor binding are in Table 3. All lots of CHS-1701 except one fell within the acceptance criteria (± 2.3 standard deviations) for Neulasta binding (see Figure 2). The variability between individual lots was relatively high, which the Applicant attributed to the PEGylated nature of both molecules.

Table 3: Summary statistics for G-CSF receptor binding

	CHS-1701	Neulasta
Mean K_D	111 pM	117 pM
K_D range (min-max)	62-157 pM	78-155 pM
Standard deviation	25.2 pM	21.3 pM

Figure 2: Receptor binding of CHS-1701 and Neulasta, as determined by SPR

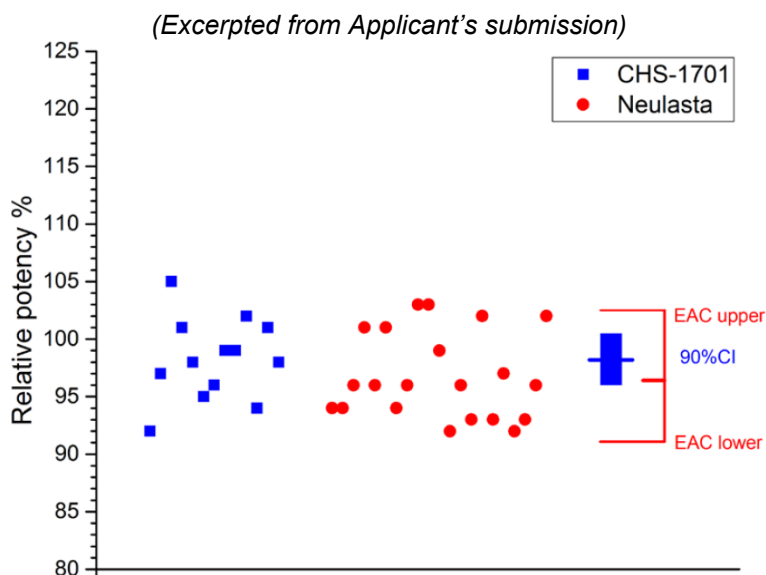


The comparative potency of CHS-1701 and Neulasta was evaluated in a cell-based proliferation assay using NFS-60 myeloid leukemia cells; NFS-60 cells are dependent on G-CSF for growth and maintenance of viability in vitro. NFS-60 cells were treated with CHS-1701 (13 lots), Neulasta (21 lots), or CHS-1701 reference standard and incubated for 3 days. MTT reagent was added for 3 hours, after which proliferation was inferred from the optical density at 550 nm. The mean relative potency of CHS-1701 was similar to that of Neulasta (see Table 4 and Figure 3).

Table 4: Summary statistics for relative biological potency

	CHS-1701	Neulasta
Mean relative potency	98.2%	96.8%
Relative potency range (min-max)	92-105%	92-103%
Standard deviation	3.5%	3.8%

Potency is relative to a CHS-1701 reference standard

Figure 3: Relative biological potency of CHS-1701 and Neulasta

4.2 Secondary Pharmacology

No studies were submitted for review.

4.3 Safety Pharmacology

Standalone safety pharmacology studies were not conducted. Electrocardiograms (ECGs) were recorded as part of the repeat-dose toxicity study in cynomolgus monkeys (refer to section 6.2 Repeat-Dose Toxicity).

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Standalone PK studies were not conducted. The PK effect of CHS-1701 and Neulasta was assessed as part of the repeat-dose toxicity study in cynomolgus monkeys (refer to section 6.2 Repeat-Dose Toxicity).

6 General Toxicology

6.1 Single-Dose Toxicity

No studies were submitted for review.

6.2 Repeat-Dose Toxicity

Study title: A 4-Week Toxicity Study of CHS-1701 Administered by Subcutaneous Injection in Cynomolgus Monkeys with a 4-Week Recovery Period

Study no.: 20026889
Study report location: 4.2.3.2.
Conducting laboratory and location:  (b) (4)
Date of study initiation: April 25, 2012
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: CHS-1701 (test article): lot # DS-12040BM-043012A, purity = 99.16% (SEC-HPLC)
Neulasta (reference article): lot # 1026654, purity not provided

Key Study Findings

- CHS-1701 and Neulasta were well tolerated when administered by SC injection once weekly, with no treatment-related clinical signs; the high dose level (750 µg/kg) was determined to be the no observed adverse effect level for both CHS-1701 and Neulasta.
- Target organs identified by histopathology included the bone marrow, spleen, lymph nodes, and thymus. Findings were mostly limited to increased cellularity and immune cell infiltration, which were consistent with the marked and reversible increase in neutrophil (NEUT) count (the expected pharmacology of pegfilgrastim). Histopathology findings were similar between CHS-1701- and Neulasta-treated monkeys.
- Cataracts were observed in one Neulasta-treated monkey (750 µg/kg) and in two CHS-1701-treated monkeys (750 µg/kg); the relationship of these findings to CHS-1701 and/or Neulasta remains unknown.
- TK parameters for CHS-1701 and Neulasta were similar on Day 1; on Day 22, TK parameters for CHS-1701 and Neulasta were variable, with systemic exposure markedly lower in female CHS-1701-treated monkeys at the 750 µg/kg dose level. ADAs were detected in monkeys administered either product, however the relative immunogenicity of CHS-1701 and Neulasta could not be formally assessed given the limited number of monkeys studied.
- Dose formulations were evaluated by spectrophotometric absorption which the Reviewer disagrees is a selective or specific enough method to conclusively assess the identity, quality, or quantity of the test or reference article.

Methods

Doses: 0, 75, 250, or 750 µg/kg (see Table 5)
 Frequency of dosing: Once weekly for 4 weeks (Days 1, 8, 15, and 22)
 Route of administration: SC injection
 Dose volume: 0.5 mL/kg/dose
 Formulation/Vehicle: (b) (4) (b) (4) Sorbitol, (b) (4)
 Polysorbate 20, pH 4.0; the vehicle was used as control article and to dilute the test and reference articles.
 Species/Strain: Cynomolgus monkey (China)
 Number/Sex/Group: Main study: 3/sex/group
 Recovery: 2/sex/group (control, low, and high groups only)
 Age: Males: 3.1 to 4.9 years
 Females: 2.5 to 3.4 years
 Weight: Males: 2.7 to 4.1 kg
 Females: 2.2 to 3.1 kg
 Satellite groups: None
 Unique study design: No
 Deviation from study protocol: None that impacted the overall integrity of the study or the interpretation of the study results and conclusions

Table 5: 4-week monkey study, experimental design

Group	Test material	Dose level (µg/kg/dose)	Dose concentration (mg/mL)	Dose volume (mL/kg/dose)	Number of animals	
					Main study	Recovery
1	Control article	0	0	0.50	3/sex	2/sex
2	Neulasta	75	0.15	0.50	3/sex	2/sex
3	Neulasta	250	0.5	0.50	3/sex	---
4	Neulasta	750	1.5	0.50	3/sex	2/sex
5	CHS-1701	75	0.15	0.50	3/sex	2/sex
6	CHS-1701	250	0.5	0.50	3/sex	---
7	CHS-1701	750	1.5	0.50	3/sex	2/sex

Observations and times

The first day of dosing was designated as study Day 1. Sacrifice and necropsies were performed on Day 29 (main study animals) or Day 57 (recovery animals).

Mortality and morbidity:	Twice daily
Cage side observations:	Once daily
Detailed clinical examinations:	Once weekly

Injection site observations:	- Once prestudy 24 hours after each dose
Body weight:	- Twice prestudy - Weekly thereafter
Food consumption:	Once daily
Ophthalmoscopic examinations:	- Once prestudy - Near the end of Week 4
Electrocardiology:	- Once prestudy - Days 1 and 22, 1-2 hours after dosing
Clinical pathology: (hematology, coagulation, clinical chemistry, urinalysis)	See Table 6
TK sample collection:	Same as hematology
Sacrifice:	Day 29 or 57

Table 6: 4-week monkey study, clinical pathology collection time points

Parameter	Time point
Hematology / TK	Day -8 (hematology only)
	Day 1 (predose and 2, 6, 12 hrs postdose)
	Day 2 (24, 36 hrs postdose)
	Day 3 (48, 60 hrs postdose)
	Days 4-7
	Days 8, 15 (predose)
	Day 22 (predose and 2, 6, 12 hrs postdose)
	Day 23 (24, 36 hrs postdose)
	Day 24 (48, 60 hrs postdose)
	Days 25-28
	Days 30, 34, 40, 48, 57
Coagulation	Days -8, 28, 57
Clinical chemistry	Days -8, 28, 57
Urinalysis	Days 29, 57

Results

Mortality

All monkeys survived until scheduled necropsy.

Clinical signs

There were no CHS-1701- or Neulasta-related clinical observations. The incidences of abrasions and bruising were similar between groups. The incidences of injection site reactions (swelling or redness) were low and similar between groups.

Body weights

Unremarkable

Food consumption

Unremarkable

Ophthalmoscopy

Examinations were performed by slit lamp biomicroscopy and indirect ophthalmoscopy; findings were subject to peer review.

At week 4, a cataract was observed in one Neulasta-treated monkey (750 µg/kg) and in two CHS-1701-treated monkeys (750 µg/kg), including one monkey in which cataracts formed in both eyes. The relationship of these findings to CHS-1701 or Neulasta could not be definitively determined, however the following suggests these findings were incidental: 1) the incidence of cataracts was not dose-dependent; 2) the spontaneous development of cataracts has been observed previously at the testing facility; 3) there were no histologic correlates in the eyes of the affected or unaffected study animals.

All other ophthalmic findings were observed prestudy or were present in control monkeys and therefore determined to be unrelated to the test or reference articles.

ECG

Unremarkable

Hematology

The following changes in hematology parameters were considered treatment-related (see Table 7):

- At doses of CHS-1701 or Neulasta ≥ 75 µg/kg, a large increase in NEUT was observed in male and female monkeys (the expected PD effect); this finding correlated with a large increase in WBC count, and smaller increases in monocyte (MONO), basophil (BASO), and large unstained cell (LUC) counts. These observations were made 24 hours after the first and fourth doses of CHS-1701 or Neulasta.
- At doses of CHS-1701 or Neulasta ≥ 250 µg/kg, decreases in red blood cell (RBC) count, hemoglobin (HGB), and hematocrit (HCT) were observed in male and female monkeys and were generally not dose-related. These findings correlated with increases in reticulocyte (RETI) counts and red cell distribution width (RDW). These observations were made 24 hours after the fourth dose of CHS-1701 or Neulasta, but not after the first dose.
- Findings were generally similar between CHS-1701- and Neulasta-treated monkeys, with the exception of the relatively small increase in NEUT, WBC,

and MONO in female monkeys treated with 750 µg/kg CHS-1701. The relatively small increases in this group correlated with diminished systemic exposure of pegfilgrastim.

- After the 4-week recovery period there were no treatment-related changes in hematology parameters indicating all changes were fully reversible.

Table 7: 4-week monkey study, statistically significant percent change in hematology parameters versus concurrent control

(Adapted from IND review)

Group	Males - Δ% from control		Females - Δ% from control	
	Day 1 (24 hours postdose)	Day 22 (24 hours postdose)	Day 1 (24 hours postdose)	Day 22 (24 hours postdose)
RBC count				
1	N/A	N/A	N/A	N/A
2	-1.97	-6.69	-1.03	-7.43
3	-8.26	-30.07*	8.82	-5.86
4	-3.53	-25.92*	-1.70	-26.63*
5	-2.73	-13.11*	2.88	-7.56
6	-2.45	-17.86*	-2.17	-11.37
7	1.21	-21.11*	-3.59	-19.43*
HGB				
1	N/A	N/A	N/A	N/A
2	1.18	-4.10	-1.71	-11.11*
3	-0.51	-22.95*	6.91	-8.00
4	-1.52	-23.28*	-0.85	-25.93*
5	0.51	-10.00	1.54	-10.44*
6	-0.25	-15.33*	-2.99	-14.14*
7	3.37	-18.03*	-1.54	-20.03*
HCT				
1	N/A	N/A	N/A	N/A
2	1.80	-0.74	-2.17	-6.85
3	-2.44	-21.64*	6.64	-4.78
4	-1.13	-19.27*	0.16	-21.15*
5	1.90	-7.49	3.20	-7.20
6	0.64	-12.10*	-1.11	-9.73
7	3.19	-14.79*	-1.14	-14.97*
RDW				
1	N/A	N/A	N/A	N/A
2	4.72	15.73	-1.74	6.49
3	-3.35	41.23*	-4.57	15.66*
4	2.89	36.46*	-1.74	35.12*
5	-0.30	10.29	-5.51	2.40
6	4.79	32.15*	-0.51	17.07*
7	1.07	42.21*	-1.88	26.80*
RETI count				
1	N/A	N/A	N/A	N/A
2	67.10	34.02	-21.39	101.96
3	47.07	241.60*	14.43	83.82
4	41.37	148.36*	4.73	183.82*
5	4.23	13.52	-5.97	70.59

Group	Males - Δ% from control		Females - Δ% from control	
	Day 1 (24 hours postdose)	Day 22 (24 hours postdose)	Day 1 (24 hours postdose)	Day 22 (24 hours postdose)
6	36.32	108.40*	-5.10	119.85
7	6.84	95.90*	12.19	164.22*
WBC count				
1	N/A	N/A	N/A	N/A
2	248.77*	769.09*	306.05*	855.93*
3	244.74*	1053.23*	264.15*	1259.84*
4	312.81*	1373.74*	296.09*	1610.96*
5	194.74*	721.62	305.52*	838.03*
6	249.12*	930.61*	245.46*	1166.55*
7	196.67*	1322.63*	243.77*	378.08*
NEUT count				
1	N/A	N/A	N/A	N/A
2	460.60*	1462.31*	856.84*	2132.80*
3	470.94*	1989.28*	757.70*	3110.24*
4	608.52*	2633.03*	829.12*	3919.82*
5	408.81*	1388.87	848.43*	2078.61*
6	482.92*	1751.39*	703.79*	2862.93*
7	405.63*	2507.16*	702.45*	945.43
MONO count				
1	N/A	N/A	N/A	N/A
2	85.24	134.45*	388.69*	339.62*
3	138.63*	190.22*	221.57*	494.40*
4	135.68*	300.72*	233.50*	1322.31*
5	101.54*	185.53*	295.43*	303.73*
6	155.04*	180.52*	229.47*	738.93*
7	109.03*	413.69*	171.82*	79.05
BASO count				
1	N/A	N/A	N/A	N/A
2	541.76*	2556.59	519.23*	2786.13
3	519.71*	6891.09	511.75*	3607.23
4	652.35*	7518.60	458.97*	2168.21
5	260.00	1996.12	492.74*	2761.27
6	589.12*	5697.29	324.57	2050.29
7	383.53*	3398.45	348.29	908.09
LUC count				
1	N/A	N/A	N/A	N/A
2	163.88*	215.96	163.96	356.79
3	167.66*	380.92	98.28	352.38
4	179.70*	358.13	151.22	164.64
5	83.28	190.06	125.47	327.50
6	171.15*	387.45	218.88	498.21
7	137.91*	293.98	44.17	222.14

* p≤0.05 (ANOVA with Dunnett's/Dunn's test)

Coagulation

Unremarkable

Clinical chemistry

Unremarkable

Urinalysis

Unremarkable

Gross pathology

There were no gross observations at terminal necropsy.

At recovery necropsy, red discolored left lung lobes were observed in one male monkey treated with 750 µg/kg Neulasta; this finding correlated with histologically observable multifocal arterial thrombosis. Lungs from all other animals were examined histologically with no similar findings, therefore the relationship of this finding to Neulasta is unlikely.

Organ weights

The following organ weight alterations at terminal necropsy were considered treatment-related (see Table 8):

- The absolute spleen, spleen to body, and spleen to brain weights and ratios were increased in most groups, however this finding only achieved statistical significance in female monkeys dosed with 750 µg/kg CHS-1701 or Neulasta.
- The absolute thymus, thymus to body, and thymus to brain weights and ratios were decreased in most female monkeys, however this finding did not achieve statistical significance. Increased absolute thymus, thymus to body, and thymus to brain weights and ratios were observed in male monkeys dosed with 250 µg/kg CHS-1701, however this observation was not considered treatment-related due to the lack of dose-responsiveness and the isolated nature of the finding.
- The changes in spleen and thymus weights correlated with histologic observations of increased splenic red pulp mixed cell infiltrates and lymphoid depletion of the thymus, respectively.
- After the 4-week recovery period there were no treatment-related changes in organ weights indicating all changes were fully reversible.

Table 8: 4-week monkey study, statistically significant percent change in organ weights versus concurrent control

Group	Males - Δ% from control			Females - Δ% from control		
	Absolute weight	Relative to body weight	Relative to brain weight	Absolute weight	Relative to body weight	Relative to brain weight
Spleen						
1	N/A	N/A	N/A	N/A	N/A	N/A
2	15.71	26.84	8.16	12.07	6.26	19.69
3	41.48	49.27	42.24	25.32	11.45	25.38
4	53.92	70.10	50.41	89.61*	74.25*	91.03*
5	13.75	17.40	12.24	22.91	13.42	22.54
6	18.20	23.22	7.55	17.23	6.05	11.60
7	45.88	42.43	38.78	60.88	44.62	64.11*

Group	Males - Δ% from control			Females - Δ% from control		
	Absolute weight	Relative to body weight	Relative to brain weight	Absolute weight	Relative to body weight	Relative to brain weight
Thymus						
1	N/A	N/A	N/A	N/A	N/A	N/A
2	40.77	54.54	34.43	14.35	7.86	22.08
3	-2.97	3.32	-4.76	-46.40	-53.32	-46.37
4	32.93	47.18	32.97	-6.44	-16.41	-8.52
5	-14.49	-11.23	-14.65	-15.28	-24.18	-13.88
6	95.20*	99.44*	76.92*	-23.92	-32.88	-27.44
7	-42.01	-43.52	-45.05	-15.75	-29.37	-12.62

* p≤0.05 (ANOVA with Dunnett's/Dunn's test)

Histopathology

Adequate Battery: Yes

Peer Review: No

Histological Findings

The following treatment-related histological findings were observed at terminal necropsy on Day 29 (see Table 9).

- Minimal to mild increased cellularity in the bone marrow of the sternum and femur was observed; the incidence of this observation increased with dose. This observation was considered the result of increased hematopoiesis and was expected given the established pharmacology of pegfilgrastim.
- Minimal to mild increased mixed cell infiltrates were observed within the red pulp of the spleen; this observation was characterized by increased numbers of NEUT and MONO, and correlated with hematology findings.
- Minimal to mild mixed cell infiltrates were observed in the liver; the infiltrates were characterized by mixed WBCs and extramedullary hematopoiesis. Kupffer cells containing a golden-brown granular pigment were observed in some monkeys, however this finding was not dose-related.
- Minimal to moderate neutrophil infiltration and/or extramedullary hematopoiesis were observed in the axillary, mandibular, and mesenteric lymph nodes. The incidences of these findings were generally similar between dose groups.
- Minimal to mild mixed cell and/or mononuclear cell infiltrates were observed in the SC injection sites. The frequency of observations was similar at the two injection sites.
- Minimal lymphoid depletion of the thymus was observed, characterized by reduced cortex, and to a lesser extent, reduced medulla.
- Overall, the histological findings at terminal necropsy were comparable between CHS-1701- and Neulasta-treated monkeys.

Table 9: 4-week monkey study, histology findings at terminal necropsy

Observations	Severity	Dose group							
		Ctrl	Neulasta (µg/kg/dose)				CHS-1701 (µg/kg/dose)		
			0	75	250	750	75	250	750
			1	2	3	4	5	6	7
Bone Marrow, Sternum Increased Hematopoietic Cellularity	Minimal	0/6	2/6	0/6	1/6	4/6	0/6	0/6	
	Mild	0/6	3/6	6/6	5/6	1/6	6/6	6/6	
Bone Marrow, Femur Increased Hematopoietic Cellularity	Minimal	0/6	3/6	3/6	3/6	2/6	3/6	2/6	
	Mild	0/6	2/6	2/6	3/6	0/6	3/6	4/6	
Red Pulp, Spleen Mixed Cell Infiltrates and/or Extramedullary Hematopoiesis	Minimal	0/6	1/6	4/6	2/6	5/6	1/6	5/6	
	Mild	0/6	1/6	0/6	3/6	0/6	1/6	0/6	
Liver Mixed Cell Infiltrates	Minimal	0/6	2/6	2/6	2/6	1/6	2/6	4/6	
	Mild	0/6	0/6	3/6	2/6	0/6	2/6	1/6	
Liver Kupffer Cell Cytoplasmic Pigment	Minimal	0/6	2/6	1/6	2/6	3/6	1/6	0/6	
Axillary Lymph Node Neutrophil Infiltrate and/or Extramedullary Hematopoiesis	Minimal	0/6	3/6	3/6	2/6	1/6	2/6	2/6	
	Moderate	0/6	0/6	0/6	1/6	0/6	1/6	1/6	
Mandibular Lymph Node Neutrophil Infiltrate and/or Extramedullary Hematopoiesis	Minimal	1/6	1/6	4/6	1/6	1/6	3/6	3/6	
	Mild	0/6	0/6	0/6	1/6	0/6	2/6	2/6	
	Moderate	0/6	0/6	0/6	2/6	1/6	0/6	0/6	
Mesenteric Lymph Node Neutrophil Infiltrate and/or Extramedullary Hematopoiesis	Minimal	0/6	0/6	0/6	0/6	0/6	0/6	2/6	
Subcutaneous Injection Site No. 1 Mixed Cell and/or Mononuclear Cell Infiltrates	Minimal	0/6	0/6	2/6	1/6	0/6	1/6	2/6	
	Mild	0/6	0/6	0/6	0/6	1/6	0/6	0/6	
Subcutaneous Injection Site No. 2 Mixed Cell and/or Mononuclear Cell Infiltrates	Minimal	0/6	0/6	1/6	2/6	0/6	2/6	4/6	
	Mild	0/6	0/6	0/6	1/6	0/6	0/6	2/6	
Thymus Lymphoid Depletion	Minimal	0/6	0/6	2/6	0/6	2/6	1/6	3/6	

Male and female data were pooled because there were no distribution alterations between sexes.

The following treatment-related histological findings were observed at recovery necropsy on Day 57 (see Table 10).

- Increased hematopoietic cellularity in the bone marrow of the femur was observed; this finding was of minimal severity in contrast to the minimal to mild severity observed at the terminal necropsy.

- Minimal to mild increased mixed cell and/or neutrophil infiltrates were observed in the spleen, liver, and axillary and mandibular lymph nodes; in contrast, similar observations of minimal to moderate severity were observed at the terminal necropsy.
- Minimal to mild macrophage infiltrates were observed in the SC injection sites; this observation is indicative of ongoing clearance of injection site inflammation.
- Ungraded multifocal arterial thrombosis was observed in the left lung of a single monkey treated with 750 µg/kg Neulasta; the relationship of this finding to Neulasta is unlikely.
- Overall, the histological findings at recovery necropsy were less severe than at terminal necropsy, and were comparable between CHS-1701- and Neulasta-treated monkeys.

Table 10: 4-week monkey study, histology findings at recovery necropsy

Observations	Severity	Dose group							
		Ctrl	Neulasta (µg/kg/dose)				CHS-1701 (µg/kg/dose)		
		0	75	250	750	75	250	750	
		1	2	3	4	5	6	7	
Bone Marrow, Femur Increased Hematopoietic Cellularity	Minimal	0/4	1/4	N/A	3/4	1/4	N/A	2/4	
Red Pulp, Spleen Neutrophil Infiltrates	Minimal	0/4	2/4	N/A	1/4	0/4	N/A	0/4	
Liver Mixed Cell Infiltrates	Minimal	0/4	1/4	N/A	1/4	1/4	N/A	0/4	
	Mild	0/4	0/4	N/A	1/4	0/4	N/A	0/4	
Axillary Lymph Node Neutrophil Infiltrate and/or Extramedullary Hematopoiesis	Minimal	0/4	0/4	N/A	1/4	0/4	N/A	1/4	
Lung Thrombus; Arteriole	Ungraded	0/4	0/4	N/A	1/4	0/4	N/A	0/4	
Mandibular Lymph Node Neutrophil Infiltrate and/or Extramedullary Hematopoiesis	Minimal	1/4	0/4	N/A	0/4	0/4	N/A	1/4	
Subcutaneous Injection Site No. 1 Macrophage Infiltrates	Mild	0/4	0/4	N/A	0/4	1/4	N/A	0/4	
Subcutaneous Injection Site No. 2 Macrophage Infiltrates	Minimal	0/4	0/4	N/A	0/4	0/4	N/A	1/4	

Male and female data were pooled because there were no distribution alterations between sexes.

Toxicokinetics

Plasma TK parameters were calculated after the first (Day 1) and fourth (Day 22) doses of CHS-1701 and Neulasta (see Table 11 and Figure 4).

- On Day 1, the mean $T_{1/2}$ of CHS-1701 and Neulasta ranged from 11.6-23.9 hrs and 13.5-24.6 hrs, respectively, and trended lower with increasing dose. The mean T_{max} of CHS-1701 and Neulasta ranged from 7.2-13.2 hrs and 7.2-15.6 hrs, respectively, and generally increased with increasing dose. In terms of exposure, the mean C_{max} for CHS-1701 and Neulasta increased in a dose-proportional manner, however greater than dose-proportional increase in mean AUC_{0-t} was observed. There were no apparent gender differences in the TK of CHS-1701 or Neulasta.
- On Day 22, the mean TK parameters for both CHS-1701 and Neulasta were more variable than on Day 1. The mean $T_{1/2}$ trended lower with increasing dose (similar to that observed on Day 1), however the variability in mean $T_{1/2}$ widened for both CHS-1701 and Neulasta to 5.3-26.1 hrs and 9.1-20.7 hrs, respectively. The mean T_{max} of CHS-1701 and Neulasta ranged from 6.0-14.4 hrs and 5.2-19.2 hrs, respectively, and generally increased with increasing dose, which was similar to that observed on Day 1. The largest variability and differences from Day 1 were observed for the exposure parameters C_{max} and AUC_{0-t} . In females at the 750 $\mu\text{g}/\text{kg}$ dose level, markedly lower C_{max} and AUC_{0-t} were observed in all CHS-1701-treated monkeys relative to Neulasta-treated monkeys. For this group, mean C_{max} and AUC_{0-t} were >40-fold and >80-fold lower (respectively) than the group mean for Neulasta-treated monkeys at the same dose level. In this group, ADAs were confirmed in 2/5 monkeys determined to have no or low systemic exposure.
- Although a formal statistical analysis was not conducted, the TK parameters for CHS-1701 and Neulasta were similar on Day 1, but the variability for both products on Day 22 prevented comparison.
- The Applicant attributed the high variability in TK parameters for CHS-1701 and Neulasta on Day 22 to ADA formation and/or target-mediated clearance.

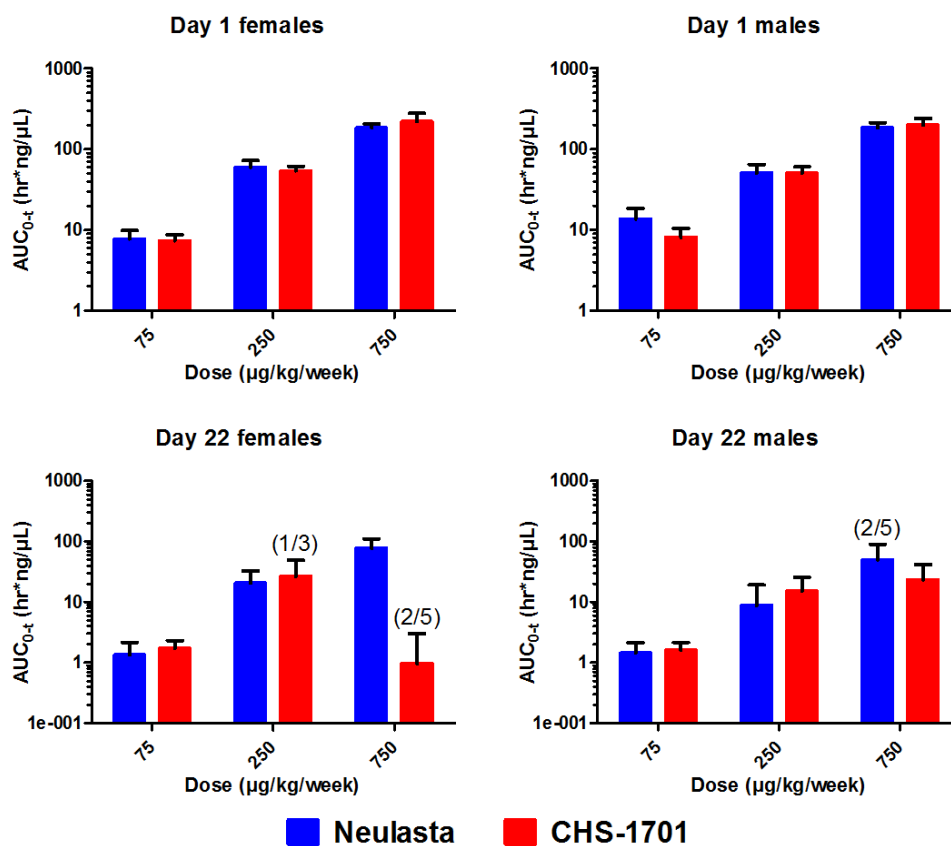
Table 11: 4-week monkey study, TK parameters for CHS-1701 and Neulasta

Dose level	Test article	Sex	$T_{1/2}$ (hr)	T_{max} (hr)	C_{max} (ng/mL)	AUC_{0-t} (hr*ng/mL)
Day 1						
75 $\mu\text{g}/\text{kg}$	Neulasta	F	21.47	7.20	445.53	7804.31
		M	23.71	10.80	635.55	13703.82
	CHS-1701	F	19.76	7.20	372.19	7359.43
		M	19.50	7.20	366.33	8096.16
250 $\mu\text{g}/\text{kg}$	Neulasta	F	14.91	12.00	1806.91	59871.45
		M	24.59	8.00	1575.09	51220.29
	CHS-1701	F	23.86	12.00	1712.74	54315.91
		M	18.63	10.00	1228.91	50635.49
750 $\mu\text{g}/\text{kg}$	Neulasta	F	15.25	15.60	4286.75	184773.78
		M	13.53	14.40	4146.78	184351.00
	CHS-1701	F	11.58	12.00	5118.10	221676.59

Dose level	Test article	Sex	T _{1/2} (hr)	T _{max} (hr)	C _{max} (ng/mL)	AUC _{0-t} (hr*ng/mL)
		M	16.89	13.20	4532.55	199193.39
Day 22						
75 µg/kg	Neulasta	F	19.65	5.20	132.02	1346.27
		M	20.72	6.00	129.37	1440.63
	CHS-1701	F	26.08	6.00	183.16	1726.27
		M	18.69	6.00	180.19	1613.92
250 µg/kg	Neulasta	F	14.49	8.00	981.74	20520.43
		M	12.70	12.00	491.36	8863.84
	CHS-1701	F	12.00	8.00	1799.21	26681.81
		M	10.74	6.00	1138.35	15180.35
750 µg/kg	Neulasta	F	9.13	19.20	2644.98	78181.64
		M	10.55	15.00	1721.44	50240.47
	CHS-1701	F	5.32	14.40	57.21	957.94
		M	5.89	12.00	1080.27	23537.75

Shaded cells = notable anomalies in exposure

Figure 4: 4-week monkey study, mean pegfilgrastim exposure (AUC_{0-t})



(X/X): (Number confirmed ADA-positive / total number screened) on Day 28

A validated analytical procedure was used to detect the presence of ADAs. No ADAs were detected in monkeys treated with CHS-1701 or Neulasta on Days 1 or 15 (see Table 12 and Table 13). On Day 28, ADAs were detected in 2/26 (7.7%) monkeys treated with Neulasta and in 3/26 (11.5%) monkeys treated with CHS-1701. On Day 57, ADAs were detected in 1/8 (12.5%) monkeys treated with Neulasta and in 3/8 (37.5%) monkeys treated with CHS-1701. The relative immunogenicity of the two products cannot be accurately assessed given the limited number of monkeys studied. The presence of ADAs did not always correlate with lower circulating concentrations of CHS-1701 and Neulasta, suggesting multiple mechanisms affect the clearance of CHS-1701 and Neulasta.

Table 12: 4-week monkey study, incidence of ADAs in Neulasta treatment groups

Day	Males				Females			
	0 µg/kg	75 µg/kg	250 µg/kg	750 µg/kg	0 µg/kg	75 µg/kg	250 µg/kg	750 µg/kg
1	(0/5)	(0/5)	(0/3)	(0/5)	(0/5)	(0/5)	(0/3)	(0/5)
15	(0/5)	(0/5)	(0/3)	(0/5)	(0/5)	(0/5)	(0/3)	(0/5)
28	(0/5)	(0/5)	(0/3)	(2/5)	(0/5)	(0/5)	(0/3)	(0/5)
57	(0/2)	(0/2)	(0/0)	(1/2)	(0/2)	(0/2)	(0/0)	(0/2)

(Number confirmed/total number screened)

Table 13: 4-week monkey study, incidence of ADAs in CHS-1701 treatment groups

Day	Males				Females			
	0 µg/kg	75 µg/kg	250 µg/kg	750 µg/kg	0 µg/kg	75 µg/kg	250 µg/kg	750 µg/kg
1	(0/5)	(0/5)	(0/3)	(0/5)	(0/5)	(0/5)	(0/3)	(0/5)
15	(0/5)	(0/5)	(0/3)	(0/5)	(0/5)	(0/5)	(0/3)	(0/5)
28	(0/5)	(0/5)	(0/3)	(0/5)	(0/5)	(0/5)	(1/3)	(2/5)
57	(0/2)	(0/2)	(0/0)	(1/2)	(0/2)	(0/2)	(0/0)	(2/2)

(Number confirmed/total number screened)

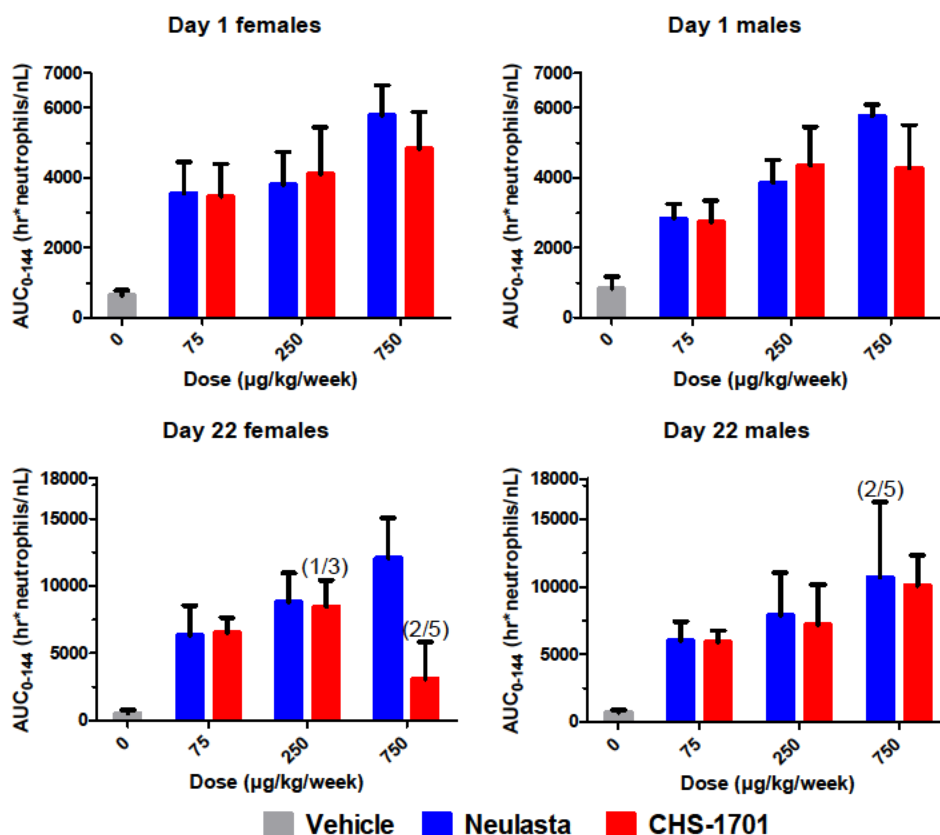
Special Evaluation

PD response was evaluated by monitoring ANC after the first (Day 1) and fourth (Day 22) doses of CHS-1701 or Neulasta (see Figure 5).

- Following the first dose of CHS-1701 or Neulasta, an increase in mean ANC was observed at all dose levels, however the increase was not dose-responsive in females and less than dose-proportional in males.
- Following the fourth dose of Neulasta a less than dose-proportional increase in mean ANC was observed, while no trend in mean ANC was observed for CHS-1701.
- Notably lower group mean ANC was observed in female monkeys treated with CHS-1701 at the 750 µg/kg dose level, mirroring the diminished pegfilgrastim exposure observed in this group.
- In a post-hoc similarity analysis of the geometric mean ratios for ANC AUC₀₋₁₄₄, the 90% confidence intervals for CHS-1701 and Neulasta did not fall

within the recognized similarity range of 80-125% (see Table 14); however, it should be noted that this study was not adequately powered to formally assess bioequivalence.

Figure 5: 4-week monkey study, mean ANC (AUC₀₋₁₄₄)



(X/X): (Number confirmed ADA-positive / total number screened) on Day 28

Table 14: 4-week monkey study, post-hoc similarity analysis of ANC for CHS-1701 and Neulasta

Dose (µg/kg)	Day	Parameter	Geometric mean ratio (%)	90% confidence intervals
75	1	AUC ₀₋₁₄₄	96.40	78.44, 118.47
	22	AUC ₀₋₁₄₄	104.76	83.80, 130.95
250	1	AUC ₀₋₁₄₄	108.60	84.07, 140.28
	22	AUC ₀₋₁₄₄	93.40	64.93, 134.35
750	1	AUC ₀₋₁₄₄	77.09	65.65, 90.52
	22	AUC ₀₋₁₄₄	51.71	26.00, 102.83

Dosing Solution Analysis

Dose formulations were evaluated by spectrophotometric absorption at 280 nm (A280). This method determines the total protein concentration and cannot distinguish between CHS-1701, Neulasta, or contaminating proteins. The Applicant indicates mean CHS-1701 and Neulasta dose formulations were 104% to 108% of the target theoretical concentration (and thus within the $\pm 10\%$ acceptance range), and control dose formulations had no significant amount of protein. The Reviewer disagrees this method is selective or specific enough to assess the identity, quality, or quantity of test or reference article.

7 Genetic Toxicology

7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

Not applicable.

7.2 *In Vitro* Assays in Mammalian Cells

Not applicable.

7.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

Not applicable.

7.4 Other Genetic Toxicity Studies

Not applicable.

8 Carcinogenicity

Not applicable.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Not applicable.

9.2 Embryonic Fetal Development

Not applicable.

9.3 Prenatal and Postnatal Development

Not applicable.

10 Special Toxicology Studies

No studies were submitted for review.

11 Integrated Summary and Safety Evaluation

The Applicant conducted a stepwise approach to support the demonstration of similarity between pegfilgrastim-^{(b) (4)} and Neulasta; this approach consisted of a definitive

analytical similarity assessment and abbreviated nonclinical and clinical programs. As part of the definitive analytical similarity assessment two comparative in vitro pharmacology studies were conducted; these studies demonstrated pegfilgrastim- (b) (4) and Neulasta bound to the G-CSF receptor with similar affinity and had similar in vitro potency. It should be noted in vitro studies such as these may not be able to detect product differences with potential to impact in vivo TK and PD profiles.

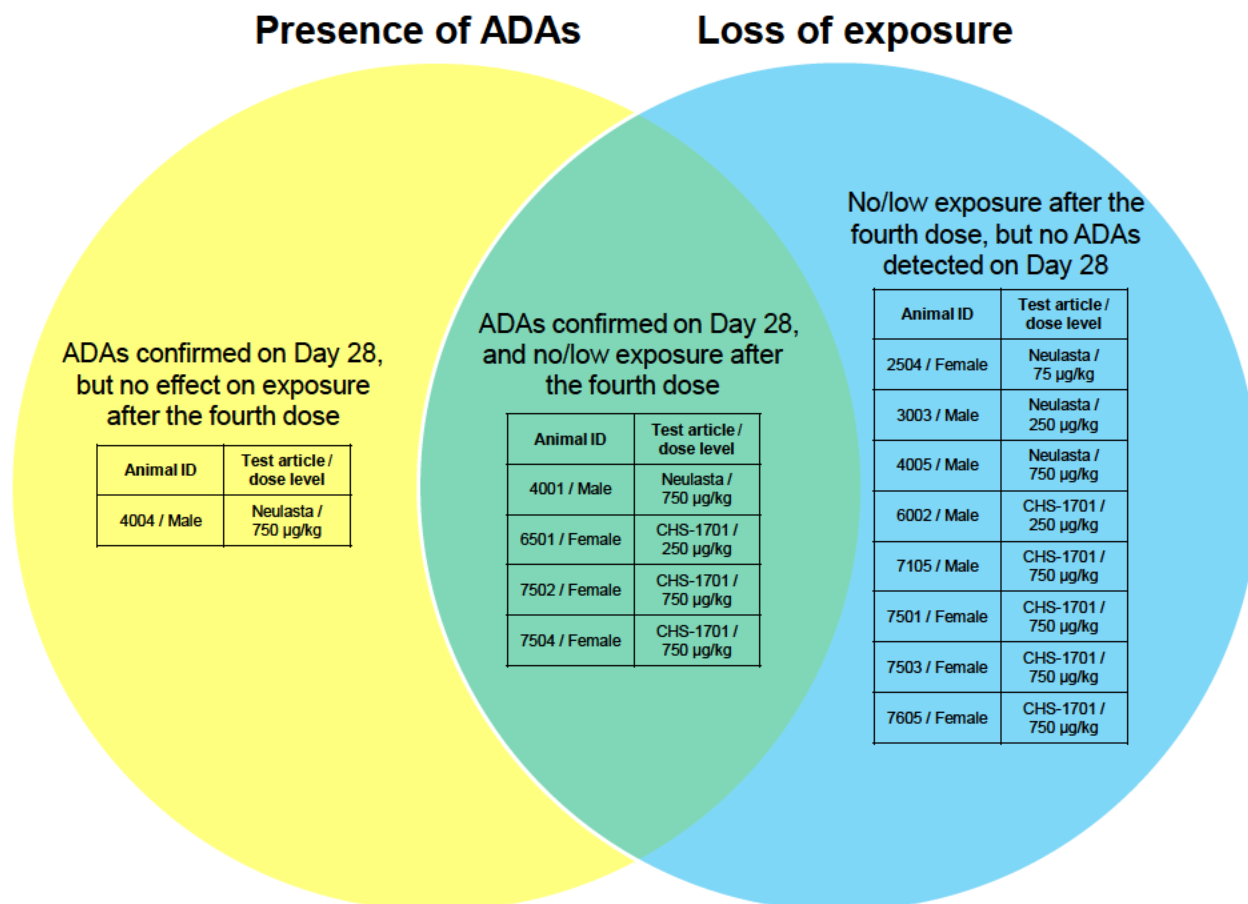
To further support the demonstration of similarity a 4-week comparative repeat-dose toxicity study was conducted in cynomolgus monkeys. The cynomolgus monkey was chosen for comparative toxicological assessment as pegfilgrastim is pharmacologically active in this species with TK and PD profiles comparable to that observed in humans. The low and high dose levels evaluated in the toxicity study were intended to approximate the human clinical dose and the high dose used in the toxicity studies to support the approval of Neulasta, respectively. Administration by the SC route is appropriate as this is the same route pegfilgrastim is administered to humans.

Target organs, histological findings, and most changes in hematology parameters were similar between pegfilgrastim- (b) (4) and Neulasta-treated monkeys, and were consistent with the well-characterized PD activity of pegfilgrastim. While the TK of pegfilgrastim- (b) (4) and Neulasta were similar after the first dose, the TK of both products were variable after the fourth dose. Systemic exposures of pegfilgrastim were markedly lower in isolated animals at the 250 and 750 µg/kg dose levels, as well as a single animal at the 75 µg/kg dose level. In addition, no or very low systemic exposure was observed in the entire group of female monkeys treated with pegfilgrastim- (b) (4) at the 750 µg/kg dose level. The exposure of pegfilgrastim is expected to decrease after repeated administration due to pharmacodynamics-mediated drug disposition⁴, however the differences between pegfilgrastim- (b) (4) and Neulasta-treated monkeys were unexpected. On Day 28, ADAs were detected in 2/26 (7.7%) monkeys treated with Neulasta and in 3/26 (11.5%) monkeys treated with pegfilgrastim- (b) (4). Diminished exposure of pegfilgrastim- (b) (4) and Neulasta did not always correlate with the presence of ADAs (see Figure 6), indicating target-mediated clearance and/or other uncharacterized mechanisms of clearance were functioning. Indeed, the elimination of pegfilgrastim in healthy volunteers is primarily attributed to target-mediated clearance⁵. The relative immunogenicity of the two products cannot be accurately assessed given the limited number of monkeys studied. Lower ANC correlated with low systemic pegfilgrastim exposure, however there were only modest differences between the ANC of affected and unaffected animals. In a similarity analysis for ANC response (AUC_{0-144}) the 90% confidence intervals for pegfilgrastim- (b) (4) and Neulasta did not fall within the recognized similarity range of 80-125%, however this study was not adequately powered to formally assess bioequivalence.

⁴ Yang, BB and A Kido, 2011, Pharmacokinetics and Pharmacodynamics of Pegfilgrastim, Clin Pharmacokinet, 50(5):295-306.

⁵ Roskos, LK, P Lum, P Lockbaum, G Schwab, and B Yang, 2006, Pharmacokinetic/Pharmacodynamic Modeling of Pegfilgrastim in Healthy Subjects, J Clin Pharmacol, 46:747-757.

Figure 6: 4-week monkey study, correlation between presence of ADAs and diminished pegfilgrastim exposure



Conclusion

Despite sharing similar toxicity profiles pegfilgrastim-^{(b) (4)} and Neulasta demonstrated dissimilar TK profiles, and to a lesser degree, PD profiles. The root of residual uncertainty between pegfilgrastim-^{(b) (4)} and Neulasta primarily lays in the loss of exposure in females administered pegfilgrastim-^{(b) (4)} at the 750 µg/kg dose level. The Applicant suggested the diminished exposure was the result of target-mediated clearance and/or formation of ADAs, however it is unclear why Neulasta-treated monkeys were not equally affected by these mechanisms. Given the limited number of monkeys per group the nonclinical team acknowledges the loss of exposure in this group may have been a chance occurrence.

The nonclinical team recognizes the TK and PD profiles of pegfilgrastim-^{(b) (4)} and Neulasta are similar at the 75 µg/kg dose level, and that the 75 µg/kg dose level represents a clinically meaningful dose. However, the lack of exposure to pegfilgrastim-^{(b) (4)} in females at the 750 µg/kg dose level indicates exposure was not maintained throughout the study, drawing into question the other conclusions of the study.

From the perspective of nonclinical pharmacology and toxicology, there is residual uncertainty regarding the similarity of pegfilgrastim-^{(b) (4)} to Neulasta. However, the nonclinical team acknowledges biosimilarity should be evaluated using a totality-of-the-evidence approach, and agrees pegfilgrastim-^{(b) (4)} may be approved for the proposed indication.

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/s/

MICHAEL L MANNING
05/05/2017

CHRISTOPHER M SHETH
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