CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

761082Orig1s000

NON-CLINICAL REVIEW(S)

DEPARTMENT OF HEALTH & HUMAN SERVICES
Food and Drug Administration
Pharmacology/Toxicology Memorandum
Center for Drug Evaluation and Research
Division of Pharm/Tox, OCHEN

Date: 3 Dec 2020
BLA 761082 (351K)
Sponsor: Kashiv Biosciences
Drug: Theragrastim (rhG-CSF)

Re: Resubmission, SN0049

Kashiv BioSciences provided a BLA resubmission after receiving a complete response letter from FDA in June 2019. Drs. Emily Place and Christopher Sheth, the pharmacology/toxicology reviewer and supervisor at that time, did not identify any deficiencies in the nonclinical program that would preclude approval. As such, this resubmission does not include further pharmacology/toxicology information and did not identify any new issue that would require nonclinical assessment. There remain no outstanding nonclinical issues that would preclude approval of this BLA.

Todd Bourcier, PhD
Director, Division of Pharm/Tox -OCHEN

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/

TODD M BOURCIER
12/03/2020 12:37:15 PM

MEMORANDUM

Date: April 12, 2018

From: Christopher Sheth, PhD
Pharmacology/Toxicology Supervisor
Division of Hematology Oncology Toxicology (DHOT)
Office of Hematology and Oncology Products (OHOP)

To: File for 351(k) BLA 761082 for Releuko (theragrastim)

Re: Approvability for Pharmacology and Toxicology

Adello Biologics, LLC. submitted this Biologic Licensing Application (BLA) under section 351(k) of the Public Health Service Act (PHS Act) for theragrastim (Releuko), a proposed biosimilar product to the reference product US-licensed Neupogen. Adello is seeking licensure of Releuko for the indications for US-licensed Neupogen that are available to them. US-licensed Neupogen product contains recombinant methionyl granulocyte colony stimulating factor (met-G-CSF). G-CSF is a lineage-specific colony stimulating factor. Binding of G-CSF to the G-CSF receptor on myeloid progenitor cells and mature neutrophils initiates transduction signals that lead to the proliferation and differentiation of neutrophil committed progenitor cells, increase of mature neutrophils in the blood, enhanced neutrophil function, and mobilization of CD34-positive hematopoietic stem cells. G-CSF is not species-specific and therefore the use of the rat in the nonclinical study to support this BLA was appropriate.

Adello conducted a 28-day repeat-dose toxicology studies in rats to evaluate the pharmacodynamics, toxicity, toxicokinetics, and local tolerance of theragrastim when compared to US-licensed Neupogen. No significant differences in pharmacodynamics, toxicity, and toxicokinetic parameters were noted in rats treated with either the proposed biosimilar product or US-licensed Neupogen.

Dr. Emily Place reviewed the nonclinical study. The nonclinical findings are summarized in the “Executive Summary” section of the BLA review. Based on the determination of similarity of Releuko to US-licensed Neupogen, the nonclinical sections of the labeling should be comparable to those in the labeling for US-licensed Neupogen.

Recommendation: I concur with Dr. Place’s conclusion that the submitted pharmacology and toxicology data support the similarity of Releuko to US-licensed Neupogen and approval of BLA 761082 for Releuko. From the perspective of the nonclinical discipline, there are no outstanding nonclinical issues that would preclude the approval of Releuko for the proposed indications.

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

CHRISTOPHER M SHETH
04/12/2018

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:	BLA 761082
Supporting document/s:	1
Applicant's letter date:	July 7, 2017
CDER stamp date:	July 10, 2017
Product:	Releuko (theragrastim)
Indication:	To decrease the incidence of infection in patients receiving myelosuppressive anti-cancer drugs
Applicant:	Adello Biologics, LLC.
Review Division:	Division of Hematology Oncology Toxicology (DHOT) for Division of Hematology Products (DHP)
Reviewer:	Emily J. Place, PhD MPH
Supervisor/Team Leader:	Christopher Sheth, PhD
Division Director:	John Leighton, PhD, DABT Ann Farrell, MD (DHP)
Project Manager:	Kris Kolibab, PhD

TABLE OF CONTENTS

Table of Tables

1 Executive Summary

1.1 Introduction

Adello Biologics, LLC. is requesting marketing approval for Releuko (theragrastim), as a proposed biosimilar product to the recombinant human granulocyte-colony stimulating factor (rhG-CSF) reference product, US-licensed Neupogen (also referred in this review as Neupogen). Neupogen was approved in the US in 1991 and the current label includes indications and usage information for: cancer patients receiving myelosuppressive chemotherapy; patients with acute myeloid leukemia receiving induction or consolidation chemotherapy; cancer patients receiving bone marrow transplant; patients undergoing peripheral blood progenitor cell collection and therapy; and patients with severe chronic neutropenia.

Adello's submitted a comparative 28-day rat toxicology study comparing their theragrastim product with US-licensed Neupogen, which assessed the pharmacodynamics (PD), toxicity, toxicokinetics (TK) and local tolerance of the products. The nonclinical toxicology study did not reveal any meaningful differences between the proposed biosimilar product and Neupogen. From the perspective of nonclinical pharmacology and toxicology, there are no residual uncertainties regarding the similarity of theragrastim to the reference product. There are no pharmacology/toxicology issues that would preclude approval of the BLA.

1.2 Brief Discussion of Nonclinical Findings

General toxicology studies of theragrastim include a GLP-compliant repeat-dose study in rats with subcutaneous administration of theragrastim or US-licensed Neupogen once weekly for a total of 5 doses with a 2-week recovery period. Sprague-Dawley rats were administered vehicle, or 1.5, 11.5, 115, or 1150 µg/kg theragrastim or Neupogen by subcutaneous injection. One death occurred in the high dose theragrastim group prior the end of the study. Pharmacodynamic effects included increased white blood cell (WBC) count, increased % neutrophils correlating with decreased % lymphocytes in treated compared to control animals. Drug related toxicities shared between theragrastim and Neupogen included increased alkaline phosphatase (ALP) values (% change from control animals), and hematopoietic proliferation in the bone marrow and spleen. The nonclinical data submitted in support of IND 115333 were used to support BLA 761082 and demonstrate that from the perspective of pharmacology/toxicology, theragrastim is similar to Neupogen (i.e., similar safety, PD, and TK).

1.3 Recommendations

1.3.1 Approvability

From the Pharmacology/Toxicology perspective theragrastim may be approved for the proposed indications.

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

The nonclinical sections of the label will be comparable to the label of the reference product US-licensed Neupogen.

2 Drug Information

2.1 Drug

CAS Registry Number	121181-53-1
Proper Name	To be determined
Code Names used by Adello	Filagrastim
Chemical Name	N-(L-Methionyl) granulocyte colony-stimulating factor; recombinant human Granulocyte-Colony Stimulating Factor
Molecular Formula	Met-Thr-Pro-Leu-Gly-Pro-Ala-Ser-Ser-Leu- 10 Pro-Gln-Ser-Phe-Leu-Leu-Lys-**Cys**-Leu-Glu- 20 Gln-Val-Arg-Lys-Ile-Gln-Gly-Asp-Gly-Ala- 30 Ala-Leu-Gln-Glu-Lys-Leu-**Cys**-Ala-Thr-Tyr- 40 Lys-Leu-**Cys**-His-Pro-Glu-Glu-Leu-Val-Leu- 50 Leu-Gly-His-Ser-Leu-Gly-Ile-Pro-Trp-Ala- 60 Pro-Leu-Ser-Ser-**Cys**-Pro-Ser-Gln-Ala-Leu- 70 Gln-Leu-Ala-Gly-**Cys**-Leu-Ser-Gln-Leu-His- 80 Ser-Gly-Leu-Phe-Leu-Tyr-Gln-Gly-Leu-Leu- 90 Gln-Ala-Leu-Glu-Gly-Ile-Ser-Pro-Glu-Leu- 100 Gly-Pro-Thr-Leu-Asp-Thr-Leu-Gln-Leu-Asp- 110 Val-Ala-Asp-Phe-Ala-Thr-Thr-Ile-Trp-Gln- 120 Gln-Met-Glu-Glu-Leu-Gly-Met-Ala-Pro-Ala- 130 Leu-Gln-Pro-Thr-Gln-Gly-Ala-Met-Pro-Ala- 140 Phe-Ala-Ser-Ala-Phe-Gln-Arg-Arg-Ala-Gly- 150 Gly-Val-Leu-Val-Ala-Ser-His-Leu-Gln-Ser- 160 Phe-Leu-Glu-Val-Ser-Tyr-Arg-Val-Leu-Arg- 170 His-Leu-Ala-Gln-Pro- 175
Molecular Weight	18800 ± 1 Da
Biochemical Description	Theragrastim is a non-glycosylated form of recombinant human granulocyte colony-stimulating factor (rhG-CSF) with an additional N-terminal methionine added to force expression in E. coli. Filgrastim has one free Cys residue at position 17 and two intramolecular disulfide linkages, between Cys-37 and Cys-43, and between Cys-65 and Cys-75.
Pharmacologic Class	Recombinant granulocyte colony-stimulating factor (G-CSF)

2.2 Relevant INDs, NDAs, BLAs and DMFs

BLA 103353 (US-licensed Neupogen); IND 115333

2.3 Drug Formulation

Table 1 Theragrastim and Neupogen Drug Product Composition

Constituent	Vial		Syringe	
	300mcg/1.0mL	480 mcg/1.6mL	300 mcg/0.5mL	480 mcg/0.8mL
Filgrastim	300 mcg	480 mcg	300 mcg	480 mcg
Acetate	0.59 mg	0.94 mg	0.295 mg	0.472 mg
Sorbitol	50.0 mg	80.0 mg	25.0 mg	40.0 mg
Polysorbate 80	0.04 mg	0.064 mg	0.02 mg	0.032 mg
Sodium	0.035 mg	0.056 mg	0.0175 mg	0.028 mg
WFI	1.0 mL	1.6 mL	0.5 mL	0.8 mL

WFI = water for injection

(Excerpted from the submission)

2.4 Comments on Novel Excipients

None; the excipients used are all compendial.

2.5 Comments on Impurities/Degradants of Concern

None

2.6 Proposed Clinical Population and Dosing Regimen

Adello Biologics Inc. proposes clinical populations and dosing regimens that are consistent with current US-licensed Neupogen labeling. See approved US-licensed Neupogen label for more detailed information.

2.7 Regulatory Background

BLA 761082 was submitted on July 7, 2017 for the biologic product theragrastim under Section 351(k) of the Public Health Service Act (42 U.S.C. 262(k)). To fulfill the nonclinical requirements for a biosimilar BLA, Adello submitted a 28-day toxicity/TK study with 14-day recovery period that included assessment of the similarity of theragrastim to US-licensed in side-by-side comparisons in the rat.

3 Studies Submitted

3.1 Studies Reviewed

Number	Title	Location
13-00393-G1	A 28-day repeat subcutaneous dose toxicity study of Theragrastim in rats with a 14-day recovery period	4.2.3.2

3.2 Studies Not Reviewed

None

3.3 Previous Reviews Referenced

None

4 Pharmacology

4.1 Primary Pharmacology

Endogenous granulocyte-colony stimulating factor (G-CSF) is a lineage specific colony stimulating factor which is produced by monocytes, fibroblasts, and endothelial cells. GCSF regulates the production of neutrophils within the bone marrow and affects neutrophil progenitor proliferation, differentiation, and selected end-cell functional activation (including enhanced phagocytic ability, priming of the cellular metabolism associated with respiratory burst, antibody dependent killing, and the increased expression of some functions associated with cell surface antigens). Filgrastim regulates the production of neutrophils within the bone marrow and is proposed to be indicated in the treatment of neutropenic conditions. Per the nonclinical overview, an *in vitro* immunogenicity study (DC-T Cell Assay) was performed using healthy human PBMC donors in order to provide an evaluation of the human response to theragrastim and Neupogen administration. This study was not submitted with the IND.

A pharmacodynamics (PD) evaluation of neutrophil counts was conducted as part of the 28-day repeat-dose toxicology study submitted by Therapeutic Proteins International LLC., which compared theragrastim (batch GM12M0034) and Neupogen (batch 1033327) in normal rats (Study 13-00393-G1). These PD data are presented below.

Study title: A 28-day repeat subcutaneous dose toxicity study of theragrastim in rats with a 14-day recovery period

Study no.:	13-00393-G1
Study report location:	eCTD 5629165
Conducting laboratory and location:	(b) (4)
Date of study initiation:	February 6, 2013
GLP compliance:	Statement included and signed
QA statement:	Statement included and signed
Drug, lot #, and % purity:	Theragrastim, GM12M0034; purity not provided

In the 28-day repeat-dose toxicology study conducted in rats, vehicle and four different doses of theragrastim and Neupogen (1.15, 11.5, 115, or 1150 µg/kg) were administered. A day after dosing on days 2, 8, 15, and 22, a blood volume of 0.25 ml was collected from main study animals and neutrophil counts were measured. It was noted that two males and one female from each group accidentally received an extra dose on day 15 of the main study. In replacement of measuring neutrophils on day 15, these samples were instead measured on day 16 in addition to the other days noted. Relative neutrophil counts represent the percentage of white blood cells that are neutrophils.

Relative neutrophil counts were unaffected by low dose (1.15 µg/kg) theragrastim or Neupogen at any of the timepoints. At 11.5 µg/kg females receiving Neupogen had statistically significantly increased relative neutrophil counts on day 2, and both theragrastim and Neupogen at 11.5 µg/kg produced statistically significant increases in

relative neutrophil counts in males and females on days 15 and 22. Relative neutrophil counts were also statistically significantly increased in males and females dosed with 115 µg/kg or 1150 µg/kg theragrastim or Neupogen at all timepoints. Overall, the results demonstrate that the PD effects of theragrastim are comparable to those of Neupogen in normal rats.

Table 2 Relative neutrophil counts (males)

Group	Sex		Day numbers relative to Start Date			
			2**	8**	15**	22**
1 Vehicle Control	M	Mean	14.785	16.568	13.960	14.524
		S.D.	3.668	4.492	3.064	4.646
		N	13	13	13	13
2 Theragrastim Low Dose	M	Mean	15.338	16.688	19.288	15.888
		S.D.	4.945	5.479	5.728	2.750
		N	8	8	8	8
3 Theragrastim Low Mid Dose	M	Mean	18.688	23.200	36.063**	40.450**
		S.D.	4.007	9.199	9.874	7.768
		N	8	8	8	8
4 Theragrastim High Mid Dose	M	Mean	45.563**	55.438**	58.813**	59.288**
		S.D.	5.353	6.098	3.473	5.985
		N	8	8	8	8
5 Theragrastim High Dose	M	Mean	50.931**	59.862**	62.662**	61.792**
		S.D.	6.637	2.796	5.182	4.490
		N	13	13	13	13
6 Neupogen Low dose	M	Mean	15.700	14.663	19.338	16.164
		S.D.	3.108	1.621	5.443	5.499
		N	8	8	8	8
7 Neupogen Low Mid Dose	M	Mean	19.250	21.888	29.638**	30.775**
		S.D.	7.019	9.112	13.006	12.774
		N	8	8	8	8
8 Neupogen High Mid Dose	M	Mean	44.400**	54.363**	60.600**	58.700**
		S.D.	5.539	5.139	7.184	7.971
		N	8	8	8	8
9 Neupogen High Dose	M	Mean	52.585**	60.392**	61.392**	62.115**
		S.D.	6.051	6.688	5.801	6.094
		N	13	13	13	13
Statistics Test: 1 Way Analysis of Variance: * - 5% significance level; ** - 1% significance level Low Dose: 1.15 µg/mL; Low Mid Dose: 11.5 µg/mL; High Mid Dose: 115 µg/mL; High Dose: 1150 µg/mL						

(Excerpted from the submission)

Table 3 Relative neutrophil counts (females)

Group	Sex		Day numbers relative to Start Date			
			2**	8**	15**	22**
1 Vehicle Control	F	Mean	14.795	16.237	16.209	14.272
		S.D.	5.343	5.418	6.209	3.670
		N	14	13	13	13
2 Theragrastim Low Dose	F	Mean	12.823	13.487	17.089	15.311
		S.D.	2.592	5.589	4.211	2.804
		N	9	9	9	9
3 Theragrastim Low Mid Dose	F	Mean	19.956	22.556	26.900*	36.044**
		S.D.	4.429	6.202	10.060	9.136
		N	9	9	9	9
4 Theragrastim High Mid Dose	F	Mean	45.111**	52.300**	55.489**	57.456**
		S.D.	11.580	7.931	8.943	8.047
		N	9	9	9	9
5 Theragrastim High Dose	F	Mean	55.808**	64.977**	64.923**	65.938**
		S.D.	8.896	6.891	5.520	4.697
		N	13	13	13	13
6 Neupogen Low dose	F	Mean	13.291	13.231	16.967	15.827
		S.D.	3.830	4.737	5.199	4.421
		N	9	9	9	9
7 Neupogen Low Mid Dose	F	Mean	22.811**	21.733	30.700**	37.100**
		S.D.	7.127	5.549	13.447	12.980
		N	9	9	9	9
8 Neupogen High Mid Dose	F	Mean	49.467**	54.722**	57.611**	60.667**
		S.D.	6.845	5.945	5.081	4.095
		N	9	9	9	9
9 Neupogen High Dose	F	Mean	50.800**	58.936**	61.350**	63.593**
		S.D.	12.041	11.618	5.845	4.297
		N	14	14	14	14

Statistics Test: 1 Way Analysis of Variance: * - 5% significance level; ** - 1% significance level
Low Dose: 1.15 µg/mL; Low Mid Dose: 11.5 µg/mL; High Mid Dose: 115 µg/mL; High Dose: 1150 µg/mL

(Excerpted from the submission)

4.2 Secondary Pharmacology

No studies were submitted for review.

4.3 Safety Pharmacology

Not applicable.

5 Toxicokinetics

5.1 TK

The toxicokinetics of theragrastim were evaluated in study and are summarized below with the individual toxicology study summary.

6 General Toxicology

6.2 Repeat-Dose Toxicity

Study title: A 28-day repeat subcutaneous dose toxicity study of theragrastim in rats with a 14-day recovery period

Study no.:	13-00393-G1
Study report location:	eCTD 5629165
Conducting laboratory and location:	(b) (4)
Date of study initiation:	February 6, 2013
GLP compliance:	Statement included and signed
QA statement:	Statement included and signed
Drug, lot #, and % purity:	Theragrastim, GM12M0034; purity not provided

Key Study Findings

- The toxicokinetics of theragrastim and Neupogen at dose levels between 1.5 and 1150 µg/kg are relatively similar in rats following single (day 1) and repeated (day 28) subcutaneous dosing.

- Theragrastim and Neupogen were associated with relatively similar dose-dependent increases in WBC counts, % neutrophils, and % change in absolute neutrophil counts (ANC) and a decrease in % lymphocytes.

- Theragrastim and Neupogen were associated with increased ALP levels, that reversed during the recovery period, at the 115 µg/kg and 1150 µg/kg dose levels.

- Target organs of toxicity were bone marrow, spleen and injection site.

Methods

Doses:	0, 1.15, 11.5, 115, 1150 µg/kg
Frequency of dosing:	Subcutaneously dorsally between shoulders
Route of administration:	Once weekly, 5 doses total (days 1, 7, 14, 21, and 28)
Dose volume:	4mL/kg
Formulation/Vehicle:	10 mM Sodium Acetate, 5% Sorbitol, 0.004% Tween 80
Species/Strain:	Sprague Dawley rats
Number/Sex/Group:	Main study: 10/sex/group Recovery: 5/sex/group for vehicle and high dose theragrastim and Neupogen
Age:	5 weeks old or older
Weight:	150.1-360.5 g
Satellite groups:	Toxicokinetics (TK): 3/sex/groups for vehicle control, 9/sex/group for theragrastim and Neupogen groups
Unique study design:	None mentioned
Deviation from study protocol:	Two males and one female from each group that received either vehicle, theragrastim or Neupogen were additionally dosed on day 15 of the study. TK animal # 159 (vehicle control male) was additionally dosed and animal # 151 (high dose Neupogen male) was not dosed on day 14.

Study Design

Group	Number of Animals	Dose (µg/kg)	Dose Volume (mL/kg)	Day 29 Sacrifice	TK Animals	Day 43 Sacrifice (Recovery animals)	Formulation Concentration (ug/mL)
1 (Vehicle Control)	18 M / 18 F	N/A	4	10 M / 10 F	3 M / 3 F	5 M / 5 F	N/A
2 (Theragrastim™ Low Dose)	19 M / 19 F	1.15	4	10 M / 10 F	9 M / 9 F	N/A	0.2875
3 (Theragrastim™ Low Mid Dose)	19 M / 19 F	11.5	4	10 M / 10 F	9 M / 9 F	N/A	2.875
4 (Theragrastim™ High Mid Dose)	19 M / 19 F	115	4	10 M / 10 F	9 M / 9 F	N/A	28.75
5 (Theragrastim™ High Dose)	24 M / 24 F	1150	4	10 M / 10 F	9 M / 9 F	5 M / 5 F	2875
6 Neupogen® (Low dose)	19 M / 19 F	1.15	4	10 M / 10 F	9 M / 9 F	N/A	0.2875
7 Neupogen® Low Mid Dose)	19 M / 19 F	11.5	4	10 M / 10 F	9 M / 9 F	N/A	2.875
8 Neupogen® (High Mid Dose)	19 M / 19 F	115	4	10 M / 10 F	9 M / 9 F	N/A	28.75
9 Neupogen® (High Dose)	24 M / 24 F	1150	4	10 M / 10 F	9 M / 9 F	5 M / 5 F	2875

N/A = Not Applicable
M = Males
F = Females

(Excerpted from the submission)

Observations and Results

Table 4 Observations and Results

Mortality	Twice daily
Clinical signs	Twice daily
Body weights	Bi-weekly
Food consumption	Weekly
Ophthalmoscopy	Pretest and prior to terminal and recovery necropsy
Hematology*	Prior to necropsy**
Bone marrow	Prior to necropsy**
Immunogenicity	Prior to necropsy**
Clinical chemistry*	Prior to necropsy**
Coagulation	Prior to necropsy**
Urinalysis	Prior to necropsy**
Gross pathology	At necropsy**
Organ weights	At necropsy**
Histopathology	At necropsy**
Function observation battery/neurotoxicity	2 hours postdose on days 1 and 28
Toxicokinetics***	Days 1 and 28; predose and 0.5 hours, and 1, 2, 4, 6, 8, 12, 24, 48, 72, 96 and 120 hours after dosing

*Blood collected via the retro orbital plexus
**Necropsy conducted on day 29 (Main) and day 43 (Recovery) or within 12 hours of discovery
***Blood collected via jugular vein catheter

Mortality

- Two females were sacrificed early in the vehicle control group due to moribund condition on day 2 (animal #1105; retro orbital bleeding) and day 11 (animal #1102; retro orbital bleeding).
- One female from the high dose theragrastim group was found dead on day 12 of the dosing phase. Macroscopic findings included pale red and severely enlarged thymus, slightly enlarged liver, and discolored lacrimal gland.

Table 5 Unscheduled deaths

Drug	Dose (µg/kg)							
	Male				**Female**			
	1.5	**11.5**	**115**	**1150**	**1.5**	**11.5**	**115**	**1150**
Theragrastim mortality: main group	0	0	0	0	0	0	0	1 (4%)
Theragrastim mortality: TK group	0	1 (11%)	0	0	0	2 (22%)	0	0
Theragrastim Total	0	1	0	0	0	2	0	1
Neupogen mortality: main group		0	0	0	0	0	0	0
Neupogen mortality: TK group	0	0	1 (11%)	0	2 (22%)	1 (11%)	0	0
Neupogen Total	0	0	1	0	2	1	0	0

Clinical Signs

Unremarkable with the exception of two males treated with high dose Neupogen (1150 µg/kg) that were noted as slightly thin during the main study and recovery periods; this observation also correlated with body weight loss during the last two weeks of the study.

Body Weights

Unremarkable with the exception of two high dose Neupogen (1150 µg/kg) treated males that lost 15% of their body weights during the last two weeks of the study. They both gained weight during the recovery period.

Feed Consumption

Unremarkable

Ophthalmoscopy

Unremarkable

Hematology

Table 6 Hematology parameters

Theragrastim								
Hematology parameter	% change from control at sacrifice							
sex	males				females			
dose (mg/kg)	1.5	11.5	115	1150	1.5	11.5	115	1150
WBC								
day 29	-1.1	57.1	142.0	195.2	17.0	29.6	160.5	228.2
day 43				-31.6				35.4
MCH								
day 29	3.2	1.6	4.2	2.6	0.5	2.1	3.1	2.1
day 43				-0.5				3.6
neutrophils (%)								
day 29	24.8	81.0	220.5	244.8	21.0	102.2	369.7	425.1
day 43				6.4				-9.8
lymphocytes (%)								
day 29	-3.9	-15.2	-52.2	-58.1	-4.3	-18.3	-55.4	-61.2
day 43				-4.7				1.3
		-						
Neupogen								
Hematology parameter	% change from control at sacrifice							
sex	males				females			
dose (mg/kg)	1.5	11.5	115	1150	1.5	11.5	115	1150
WBC								
day 29	24.9	53.0	189.4	194.7	24.6	24.0	197.9	201.0
day 43				-5.3				22.2
MCH								
day 29	0.5	2.1	2.6	2.6	0.5	3.1	3.9	4.9
day 43				39.1				2.1
neutrophils (%)								
day 29	-2.6	74.5	226.6	236.1	0.3	104.5	324.9	406.8
day 43				-10.2				-21.1
lymphocytes (%)								
day 29	2.1	-16.6	-52.3	-56.1	-2.7	-17.7	-49.4	-61.9
day 43				2.7				5.0

Shaded values represent statistical significance, $p<0.05$

Table 7 Absolute Neutrophil Counts (ANC) - % change from control

Absolute Neutrophil Count- % change from control								
	males				females			
dose (mg/kg)	1.5	11.5	115	1150	1.5	11.5	115	1150
Thergrastim ANC 10^3/ul								
day 29	23.4	184.4	675.8	917.9	41.6	161.9	1123.7	1623.7
day 43				-27.3				22.6
Neupogen ANC 10^3/ul								
day 29	21.6	167.0	845.1	890.5	25.0	153.6	1166.0	1425.7
day 43				-13.6				-3.2

Clinical Chemistry

Table 8 Clinical chemistry parameters

Theragrastim								
Hematology parameter	% change from control at sacrifice							
sex	males				females			
dose (mg/kg)	1.5	11.5	115	1150	1.5	11.5	115	1150
ALT								
day 29	-16.2	16.2	-37.8	-21.6	-13.6	-6.8	4.5	-4.5
day 43				49.1				-13.1
ALP								
day 29	-11.4	3.6	25.4	61.9	-49.0	-39.4	3.7	15.0
day 43				29.7				-43.8
AST								
day 29	-21.8	2.6	-37.8	-23.6	4.2	5.1	32.7	11.3
day 43				26.6				-11.4
TRIG								
day 29	-21.2	-25.8	-30.3	-21.2	-5.9	8.5	3.2	-10.6
day 43				55.9				-7.4
Neupogen								
Hematology parameter	% change from control at sacrifice							
sex	males				females			
dose (mg/kg)	1.5	11.5	115	1150	1.5	11.5	115	1150
ALT								
day 29	-24.3	-33.8	-21.6	-17.6	-6.8	11.4	-2.3	-4.5
day 43				-6.2				-13.1
ALP								
day 29	-10.3	-0.2	40.3	60.0	13.5	16.0	88.9	135.4
day 43				3.1				-8.7
AST								
day 29	-24.2	-28.0	-13.4	-23.2	6.1	88.3	12.3	5.7
day 43				-10.8				-8.9
TRIG								
day 29	-33.3	-45.5	-31.8	-30.3	-4.7	4.4	-6.8	-8.5
day 43				49.3				-9.9

Shaded values represent statistical significance, p<0.05

Coagulation

Unremarkable

Gross Pathology

Table 9 Gross pathology findings in theragrastim treated animals

Macroscopic findings - Terminal		Male				Female			
No. animals		10	10	10	10	10	10	10	10
Theragrastim Dose (µg/kg)		1.5	11.5	115	1150	1.5	11.5	115	1150
Ileum	dilation	-	1	-	-	-	-	-	-
thymus	discoloration	-	-	-	1	-	-	-	-
Prostate	scabbing	-	-	-	1	-	-	-	-

(-): no finding noted

There were no gross pathology findings noted in vehicle control or Neupogen treated animals.

Organ Weights

Treatment related changes in spleen weights were observed in both males and females on day 29. A significant increase in spleen to body weight ratio was observed in both theragrastim and Neupogen administered males at the 115 µg/kg and 1150 µg/kg dose levels. Female rats had increased spleen to body weight ratios at 115 µg/kg of theragrastim and 1150 µg/kg of Neupogen. Spleen to body weight ratio was still significantly higher in the high dose (1150 µg/kg) Neupogen group during recovery.

Table 10 Gross pathology findings in theragrastim treated animals

Theragrastim								
organ weight: body weight	% change from control at sacrifice							
	males				females			
dose (mg/kg)	1.5	11.5	115	1150	1.5	11.5	115	1150
Spleen								
day 29	-1.8	9.7	30.2	30.7	-13.3	-11.4	-2.2	2.2
day 43				8.3				17.4
Thymus								
day 29	-16.1	-8.0	51.9	5.0	-8.5	-12.4	-4.4	234.7
day 43				12.8				16.7
Lung								
day 29	1.0	10.1	-54.1	11.9	4.1	9.7	11.2	7.2
day 43				-3.3				9.7
pituitary								
day 29	-19.0	-25.4	73.2	-12.4	-3.3	-6.4	-6.4	-7.0
day 43				16.9				-1.0
Neupogen								
	males				females			
dose (mg/kg)	1.5	11.5	115	1150	1.5	11.5	115	1150
Spleen								
day 29	12.8	8.7	27.1	40.8	-10.3	-0.7	-1.7	7.3
day 43				38.1				8.2
Thymus								
day 29	7.9	13.4	-2.6	20.4	3.7	-25.9	-9.2	-1.3
day 43				21.8				-1.6
Lung								
day 29	-3.0	-0.2	6.4	10.5	9.0	3.0	11.9	12.6
day 43				27.5				12.9
pituitary								
day 29	1.4	723.2	-9.9	67.0	-23.7	2.2	-5.0	-11.9
day 43				68.9				7.9

Shaded values represent statistical significance, $p<0.05$

Histopathology

Adequate Battery: Yes

Peer Review: No

Histological Findings

Table 11 Histological findings in main study group animals

Treatment related microscopic findings											
				Males/Females				Males/Females			
				Theragrastim				Neupogen			
dose (µg/kg)			0	1.5	11.5	115	1150	1.5	11.5	115	1150
Number of animals examined			10	10	10	10	10	10	10	10	10
Organ	Finding	Grade									
Administration site	inflammation	1	9/6	-	-	-	10/9	-	-	-	10/8
		2	1/2	-	-	-	-	-	-	0/1	-
Uterus	dilation, endometrium, focal	-	-	-	-	-	0/2	-	-	-	-
Bone marrow, femur	hematopoetic cell proliferation, granulocytic		-	1/0	-	-	-	-	-	-	-
	hematopoetic cell proliferation, granulocytic, difuse	-	0/0	-	10/10	10/10	10/8	-	10/10	10/10	10/9
spleen	hematopoetic cell proliferation		-	-	1/0	-	-	-	-	0/1	-
spleen	hematopoetic cell proliferation, difuse		4/3	3/0	5/0	8/1	10/5	4/0	5/0	7/1	5/8

Grade 1 = minimal; Grade 2 = mild; Grade 3 = moderate; Grade 4 = severe
(-): No toxicologically significant findings

Table 12 Histological findings in recovery animals

Treatment related microscopic findings-recovery											
				Males/Females				Males/Females			
				Theragrastim				Neupogen			
dose (µg/kg)			0	1.5	11.5	115	1150	1.5	11.5	115	1150
Number of animals examined			10	10	10	10	10	10	10	10	10
Organ	Finding	Grade									
Uterus	dilation, endometrium, focal	-	-	-	-	-	0/1	-	-	-	0/1

(-): No toxicologically significant findings

Special Evaluation

Immunogenicity
Serum samples were analyzed for anti-human G-CSF antibody levels by ELISA. The secondary antibody in this ELISA recognizes IgG, IgM and IgA and therefore, isotype specificity cannot be determined.

Of the 203 total samples analyzed, twenty-four samples were found as potentially positive, of which four theragrastim (2 dosed at 115µg/mL; 2 dosed at 1150µg/mL) and five Neupogen (1 dosed at 115µg/mL; 4 dosed at 1150µg/mL) samples were confirmed positive. Positivity was confirmed using a 10 µg/mL solution of theragrastim as an inhibitor.

Functional Observation battery
Neurological tests were performed 2 hours post dose on days 1 and 28.
Results were unremarkable with the exception of one statistically significant finding of increased forelimb grip strength on day 28 for males treated with 11.5 and 1150 µg/kg of theragrastim compared to the vehicle control group.

Toxicokinetics

Blood volume of approximately 0.5 mL was collected from 3 TK animals per sex for theragrastim or Neupogen groups via jugular vein, with the exception of the last scheduled sample collection which was collected by cardiac puncture.

Table 13 Summary Toxicokinetics parameters in animals

Day	Group	Dose (mg/kg/dose)	Analyte: Filgrastim			
			C_{max} (ng/mL)	$AUC_{0-\infty}$ (h*ng/mL)	T_{max} (hr)	$t_{1/2}$ (hr)
1	2 (T)	1.15	42081 (9029)	4625 (2234)	2.8 (2.8)	9.8 (14.3)
	3 (T)	11.5	6340 (4478)	53629 (15653)	18.5 (38.0)	46.2 (64.7)
	4 (T)	115	222035 (51163)	1852564 (1134230)	2.0 (1.10)	3.2 (1.1)
	5 (T)	1150	3411310 (480857)	24752957 (4175311)	1.7 (0.52)	6.0 (5.8)
	6 (N)	1.15	526 (87)	2742 (770)	2.8 (2.8)	7.8 (12.0)
	7 (N)	11.5	7707 (1470)	44937 (10372)	2.2 (1.0)	3.6 (1.5)
	8 (N)	115	220004 (51794)	1447312 (262526)	2.2 (1.0)	3.0 (1.4)
	9 (N)	1150	4218013 (643382)	33450123 (10506555)	2.2 (1.0)	4.2 (1.3)
28	2 (T)	1.15	297 (89)	NC	1.8 (2.0)	NC
	3 (T)	11.5	157342 (20724)	93199 (133333)	4.0 (4.3)	6.4 (0.1)
	4 (T)	115	258105 (128489)	1813404 (1165575)	8.3 (2.9)	18.4 (22.3)
	5 (T)	1150	4040878 (1166602)	31965783 (23771702)	6.3 (8.8)	19.1 (28.3)
	6 (N)	1.15	2165 (526)	8254193 (1628378)	3.8 (2.4)	145.1 (226.9)
	7 (N)	11.5	3098 (1725)	22467 (9466)	2.3 (1.9)	32.3 (36.3)
	8 (N)	115	96559 (36673)	558596 (101814)	4.8 (2.7)	6.4 (3.3)
	9 (N)	1150	3185683 (1091239)	23591824 (2828418)	4.3 (2.0)	8.0 (11.5)

NC: Not calculated due to incomplete data.

(Excerpted from the submission)

T: Theragrastim
N: Neupogen

An information request was sent to Therapeutic Proteins International LLC on August 27, 2014 requesting the sponsor to explain the variations and large range of PK values in the toxicology report (# 13-0393-G1). These values do not seem to correlate with pharmacodynamic (PD) effects or the formation of anti-drug antibodies (ADA). For example, the AUC of Neupogen on day 28 at the dose of 11.5 µg/kg is 22.5 µg.hr/mL, which is 375 fold less than the AUC at the dose of 1.15 µg/kg. Despite this decrease in the AUC, the PD effects were significantly increased.

We received an official response on August 29, 2014. The sponsor indicated that the mechanism of action, receptors and intended effect of PEGylated and non-PEGylated G-CSF are the same. They cited a paper by Scholz et al., 2009[1]. They explained that the lack of concordance is due to increased degradation of higher amounts of receptors in the bone marrow or because of increased neutrophil elastase as a consequence of an increased amount of neutrophils.

Given the large variability between toxicokinetic parameters measured, there might an issue with the detection assay. However, since the toxicology findings, PD, immunogenicity findings and the amount of protein measured (dosing solution analysis) are similar for the two products, this is not a hold issue.

Summary

- There was a dose-dependent response in C_{max} and AUC across dose groups, the trend was not linear or dose proportional.
- T_{max} varied from 1.5-3.5 hours across dose groups and sex
- T_{max} for theragrastim treated male rats at a dose of 11.5µg/kg was ~33.5 hours.
- There are large variations in both AUC and half-life that do not correlate with the formation of anti-drug antibodies (see Immunogenicity) or PD response.

Dosing Solution Analysis

Dose formulations were analyzed using both a validated High Performance Liquid Chromatography (HPLC) method (Agilent 1100 Series HPLC) and ELISA method (Quantikine® ELISA: Human G-CSF Immunoassay). The amount of protein measured is comparable between theragrastim and Neupogen solutions.

Selected dose formulation samples were analyzed for levels of recombinant Human G-CSF by HPLC. The samples included theragrastim and Neupogen both at and 287.5 µg/mL and 28.75 µg/mL.

Table 14 Dose formulation analysis by HPLC

Summary of Dose Analyses

Run Date	Theragrastim		Neupogen	
	High Mid Dose (28.75 µg/mL)	High Dose (287.5 µg/mL)	High Mid Dose (28.75 µg/mL)	High Dose (287.5 µg/mL)
March 21, 2013	23.11	302.14	23.54	305.06
April 1, 2013	29.32	307.70	27.83	302.30

(Excerpted from the submission)

Selected dose formulation samples were analyzed for levels of recombinant Human G-CSF by ELISA. The samples included theragrastim and Neupogen both at 2.88 µg/mL,

[1] Scholz M, Engel C, Apt D, Sankar SL, Goldstein E, Loeffler M. Pharmacokinetic and pharmacodynamic modelling of the novel human granulocyte colony-stimulating factor derivative Maxy-G34 and pegfilgrastim in rats. *Cell Proliferation*. 2009; 42(6): 823-37.

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

EMILY J PLACE
04/12/2018

CHRISTOPHER M SHETH
04/12/2018
I concur

MEMORANDUM

Date: March 23,2018

To: File for BLA 761082 - Supporting Document 1

From: Emily Place, PhD MPH
Pharmacology-Toxicology Reviewer
Division of Hematology Oncology Toxicology (DHOT)
Office of Hematology and Oncology Products (OHOP)

Through: Christopher Sheth, PhD
Pharmacology-Toxicology Supervisor
Division of Hematology Oncology Toxicology (DHOT)
Office of Hematology and Oncology Products (OHOP)

Subject: BLA 761082
Applicant: Adello Biologics
Drug: Releuko/theragrastim

Re: Pharmacology/toxicology assessment of the mistaken use of [redacted] in the manufacturing suites at Adello Biologics

At a recent internal meeting reagrding the BLA 761082, the product quality team noted that upon a facility inspection it was discovered that there was a deviation from standard operating procedures (SOP); specifically, that standard operating procedure (SOP) [redacted] was not followed as required. [redacted] The Applicant provided a final impact assessment that contained the details of the deviation and planned corrective actions.

The FDA's pharmacology/toxicology BLA review team has evaluated the final impact assessment report regarding the deviation. In our evaluation of the assessment, we examined the worst-case scenario and compared that to the toxicology database for the compound and limits set forth in relevant guidance documents. Pharmacology/toxicology considers that the maximum amount of [redacted] that could have ended up coming into contact with any drug substance or drug product to be extremely low. We calculated that amount to be [redacted] times lower than the lethal dose to 50% of exposed animals. Thus, from our perspective there is negligible risk for any of the acute toxicities of [redacted] to manifest in anyone receiving potentially contaminated drug. We also assessed the exposure in terms of the components being genotoxic impurities, and looked to ICH M7 for the acceptable intakes. [redacted] Using a conservative approach, the maximum exposure to [redacted] would be [redacted] µg, which is 24-fold lower than the acceptable daily intake. In summary, pharmacology/toxicology concludes that the deviation noted above does not pose a

safety concern to subjects who may be exposed to drug substance or drug product lots coming from these manufacturing suites.

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

CHRISTOPHER M SHETH
03/23/2018